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Fishery Bulletin

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Articles

Evaluation of the In Situ Ichthyoplankton Imaging System (ISIIS): comparison with the traditional (bongo net) sampler

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Abstract—Plankton and larval fish sampling programs often are limited by a balance between sampling frequency (for precision) and costs. Advancements in sampling techniques hold the potential to add considerable efficiency and, therefore, add sampling frequency to improve precision. We compare a newly developed plankton imaging system, In Situ Ichthyoplankton Imaging System (ISIIS), with a bongo sampler, which is a traditional plankton sampling gear developed in the 1960s. Comparative sampling was conducted along 2 transects ~30–40 km long. Over 2 days, we completed 36 ISIIS tow-yo undulations and 11 bongo oblique tows, each from the surface to within 10 m of the seafloor. Overall, the 2 gears detected comparable numbers of larval fishes, representing similar taxonomic compositions, although larvae captured with the bongo were capable of being identified to lower taxonomic levels, especially larvae in the small (<5 mm), preflexion stages. Size distributions of the sampled larval fishes differed considerably between these 2 sampling methods, with the size range and mean size of larval fishes larger with ISIIS than with the bongo sampler. The high frequency and fine spatial scale of ISIIS allow it to add considerable sampling precision (i.e., more vertical sections) to plankton surveys. Improvements in the ISIIS technology (including greater depth of field and image resolution) should also increase taxonomic resolution and decrease processing time. When coupled with appropriate net sampling (for the purpose of collecting and verifying the identification of biological samples), the use of ISIIS could improve overall survey design and simultaneously provide detailed, process-oriented information for fisheries scientists and oceanographers.

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Regular surveys of early life stages of fishes provide a wealth of information for fisheries managers and fishery oceanographers. Indices of larval abundance are used quantitatively as fishery-independent measures of population abundance in stock assessments (Scott et al., 1993; Gledhill and Lyczkowski-Shultz, 2000; Simmonds, 2009). Larval fish abundance also is used qualitatively, as evidence for change in stock status (Smith and Morse, 1993; Lo et al., 2010; Richardson et al., 2010). Spawning areas and times are inferred from early-life-stage abundance and distribution, and they contribute to the definition of essential fish habitat (Brodziak, 2005; Levin and Stunz, 2005) and stock identification (Begg et al., 1999; Hare, 2005). Larval fish surveys combined with process-oriented research also help forecasting capability of year-class strength (e.g., Megrey et al., 1996; Lough and O'Brien, 2012).

Although larval fish studies make substantial contributions to the assessment of fish stocks, 3 factors currently limit their applicability. First, larval fishes are relatively rare within the plankton and estimates of variance in larval abundance can be large, limiting the power of statistical comparisons of abundance between years or locations (Cyr et al., 1992). Second, larval fishes are patchily distributed (e.g., Davis et al., 1990; Cowen et al., 1993; Pepin, 2004) but not randomly distributed; patches often are associated with fronts, thermoclines, or specific water masses (Cowen et al., 1993; Kingsford and Suthers, 1994). Most larval surveys, however, are conducted along fixed grids or as random stratified designs; significant differences in larval abundance between sampling times may simply reflect a varying intersection of sampling with dynamic larval habitat. Third, the cost of ichthyoplankton surveys is an important consideration and most programs are cost-limited in terms of ship time or the number of samples that can be processed (Tanaka, 1973; Lo et al., 2001; Simmonds, 2009).

In the United States, there are numerous federally supported ichthyoplankton programs that provide

data for fisheries management. All these efforts are limited by the 3 factors described above: rarity, patchiness, and cost. The In Situ Ichthyoplankton Imaging System (ISIIS; Cowen and Guigand, 2008) has the potential to minimize all 3 limitations, and, if successful, would provide the stock assessment toolbox with robust and timely fishery-independent measures of spawning distribution and stock size based on early-life-stage information. The overall goal of this study, therefore, was to evaluate the effectiveness of ISIIS for quantifying fish larvae and thus show the potential benefits of its integration into larval surveys, with the ultimate goal of improving stock assessments.

Specifically, we compare ISIIS with a traditional bongo sampler, which is composed of a frame supporting paired nets with mouth openings on either side of and in front of the towing wire (Posgay and Marak, 1980). The bongo has been used in ichthyoplankton programs throughout the United States since its development in the late 1960s: in the shelf ecosystem of the northeastern United States since 1971 (Richardson et al., 2010), in the Gulf of Mexico since 1982 (Lyczkowski-Shultz and Hanisko, 2007), and in the northeast Pacific Ocean since 1972 (Matarese et al., 2003). Here we present a comparison of larval fish abundance and size distribution based on results from the ISIIS and bongo sampler.

Methods

This study was conducted 54 km south of Woods Hole, Massachusetts, (Fig. 1), on 23–24 October, 2008, on the NOAA Ship *Delaware II*. The cruise immediately followed the passage of a low-pressure system, which brought strong winds to the study area; these winds diminished throughout the duration of the cruise. Sampling was completed along 2 parallel transects, which were 41.4 and 27.7 km in length and separated by ~6 km. To complete the comparison, the prototype ISIIS-1 (herein referred to as ISIIS) was towed along a transect; then the ship returned to the beginning of the transect, and net samples were made with the bongo over the same transect. Sampling along each transect encompassed both day and night periods, but no attempt was made to compare day and night differences in larval abundance or vertical distribution. Morse (1989) compared day:night catches in the region and found no significant differences for most of the taxa captured in this study. He did find some day:night bias at larger transect lengths, but, in our study, both the bongo net and ISIIS sampled during day and night, and therefore we assume this length bias was randomly distributed between the gears.

Sampling gear

The imaging output from ISIIS is unique in that it provides a continual image for the entire tow duration, with a pixel resolution of ~68 µm. Such fine resolution enables detection of particles as small as a 100 µm (e.g., diatoms), although the ability to clearly resolve particles is typically in the range of 700 µm (i.e., small copepods and larvaceans) and larger sizes (e.g., larval fishes, chaetognaths, and ctenophores). One distinctive feature of ISIIS is its large depth of field (~30 cm for

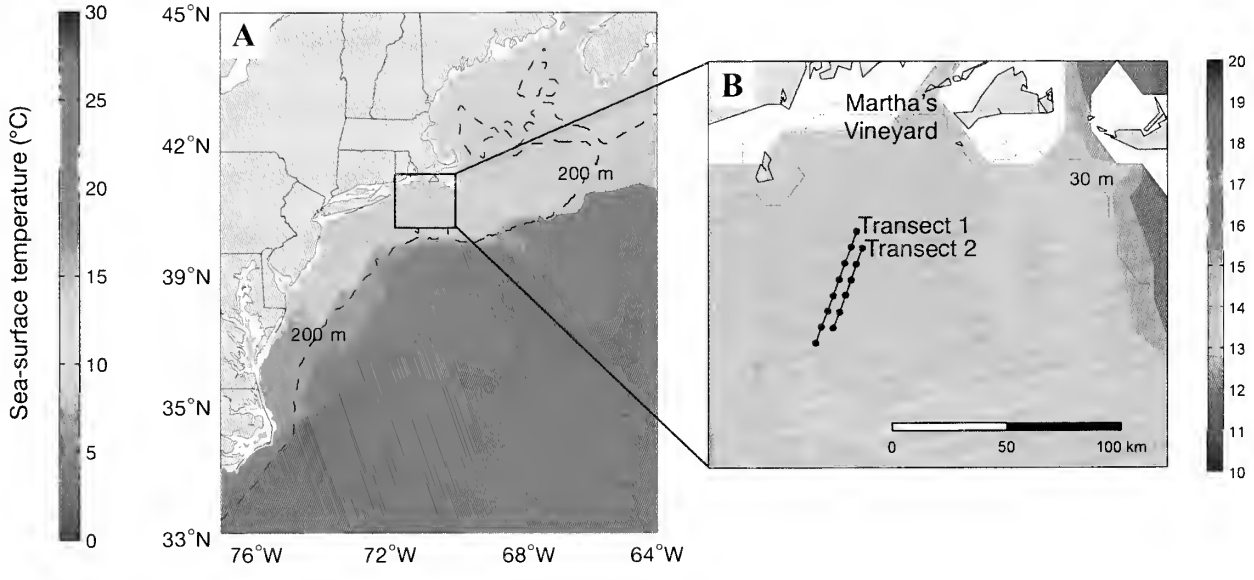

Figure 1

Eight-day average (20–27 October 2008) sea-surface temperature (SST, °C) of northeastern U.S. continental shelf from Cape Hatteras, North Carolina, to Nova Scotia, Canada. (**A**) The sampling location offshore of Martha's Vineyard, Massachusetts. (**B**) The inset shows the 2 In Situ Ichthyoplankton Imaging System (ISIIS) transects and the bongo collection locations marked by black dots along the same transects. Note the change in SST scale between the 2 panels.

mesozooplankton), which enables the concentration of even relatively rare mesoplankters, such as larval fishes and gelatinous zooplankton, to be quantified (Cowen and Guigand, 2008; McClatchie et al., 2012). Using the image analysis software that we have developed (Tsechpenakis et al., 2007, 2008), we could essentially quantify the plankton field for every centimeter of our tow, and we could match these data centimeter by centimeter with the corresponding environmental data collected by the onboard sensors (pressure [depth], temperature, salinity, and fluorometry). Consequently, ISIIS can evaluate from very fine-scale (centimeters) to submesoscale features. ISIIS sensors for this study were those for temperature (SBE 3[1] Sea-Bird Electronics, Inc., Bellevue, WA) and conductivity (SBE 4) and a fluorometer (ECO FLRT, WET Labs, Philomath, OR).

A 61-cm bongo sampler was used and fitted with 505- and 333-μm mesh nets (Posgay and Marak, 1980). A flowmeter (General Oceanics, Miami, FL) was attached in the center of each mouth opening to quantify the volume of water filtered by the net. A conductivity, temperature, depth (CTD) instrument (SeaCAT SBE 19) was attached to the tow wire above the bongo net. The CTD was used in real time to monitor the depth of the bongo net during deployment.

Sampling approach

For this study, ISIIS was towed at a speed of 2.5 m s^{-1} in a tow-yo (vertically undulating) fashion between the surface and a target depth of 10 m above the seafloor, thereby following changes in seafloor depth. The ISIIS was towed in an undulating manner by paying cable in and out from the winch, and therefore continual winch operation was required. (Since this study, a self-undulating version of ISIIS has been designed and the need for continual winch operation has been eliminated). Each undulation (surface to depth to surface) took ~10 min, resulting in a distance covered of 1.5 km, which also equates to the distance between downcasts (or upcasts). While being towed, ISIIS records environmental data (temperature, salinity, fluorescence) and imagery continually, sending the data up the fiber-optic cable for onboard recording. The continual imagery is parsed into single images of 13×13 cm at a rate of 17.3 images s^{-1}. Thus, ISIIS generates ~64,000 images h^{-1}, and for this study, an estimated total of ~478,000 images over ~7.68 h of total recording time.

Because the focus of this study was specifically larval fishes, processing of images specifically targeted larval fishes, thereby eliminating the need to capture and classify all imaged particles (e.g., copepods, larvaceans, medusae, and ctenophores). Consequently, all images were manually reviewed for larval fishes. This process is relatively rapid, although ~3 months were required to complete this task because of the large number of images. Future development of ISIIS will include automated image processing; however, the current manual processing requires viewing each image. When a larval fish was present, that portion of the image was extracted and saved to a file. All fish images were then reviewed for identification to the lowest taxonomic level possible and measured with ImageJ (National Institute of Health public domain Java-based image-analysis program available at http://rsbweb.nih.gov/ij/). Environmental data from ISIIS were interpolated across each transect with a cubic interpolation function in Matlab (vers. 7.11.0.584 [R2010b], The MathWorks, Inc., Natick, MA). The depth and environmental variables associated with each fish larva were obtained by matching time stamps from image and environmental data.

The bongo tows were conducted in standard fashion by following Jossi and Marak (1983). For each tow, the wire was paid-out at a rate of 50 m min^{-1} to a depth of 10 m above the seafloor, then the wire was retrieved to the surface obliquely at 20 m min^{-1}, while the ship moved at 0.75–1.0 m s^{-1}. At completion of each tow, the nets were washed down and the contents rinsed onto a 333-μm sieve. The sample was preserved in 5% buffered formalin. Samples were then sorted for larval fishes under a dissecting microscope and identified to the lowest taxonomic level following Fahay (2007). The 333-μm mesh bongo samples were used for comparisons of the bongo and ISIIS methods since this mesh size is the one that has been used for more than 20 years by the Northeast Fisheries Science Center for ichthyoplankton surveys.

To compare larval fish concentrations, each bongo tow and each ISIIS undulation were treated as replicates. There are potential statistical problems with this assumption, but to date, the decorrelation length scale in ichthyoplankton distributions in the study region has not been calculated. This assumption will be examined in future studies with ISIIS. The larval fish concentrations were transformed by the natural log, and a Shapiro test was performed to test for normality of larval fish concentrations within each gear type. Where the null hypothesis of normality was accepted, a Welch's *t*-test was used to compare larval fish concentrations between transects within gear and then between gear across both transects. Comparisons were made for total larvae, family-level larvae, and species-level larvae both within and between gears for abundance and size differences. In these tests, the nonparametric Kruskal-Wallis test was used because concentrations at the family level were zero-inflated, making transformations to a normal distribution impossible. All counts per tow (or undulation) were standardized to volume sampled (number of fish larvae per cubic meter).

All larvae collected in the bongo net were measured to the nearest 0.1 mm for notochord (preflexion) or standard length under a dissecting microscope with

[1] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

an ocular micrometer. Larvae observed in ISIIS images were measured digitally with ImageJ software after each image was calibrated to standard pixel size. Fishes were measured for notochord or standard length (the position of the posterior end of the hypural plate was estimated if the pigmentation on a fish was too dense for the internal caudal fin structure to be visible). A subset (6 out of 409) of the fish images was discarded because orientation of the fish precluded accurate measurement. Despite our effort to remove such images from measurement, some fish sizes likely were underestimated when the observer was not able to discern the offset that may have occurred where the orientation was not exactly parallel to field of view. Lengths of all larvae were compared between the 2 gears and the 2 transects. To avoid pseudoreplication, the average length of all larvae, family-level larvae, and species-level larvae from a bongo tow or ISIIS undulation was used for comparison. Size distributions were all highly skewed, and therefore a Kruskal-Wallis test was used to compare sizes within and between gear types. Statistical analyses were performed in R software, vers. 2.14 (R Development Core Team, 2011) with the package "plyr" (Wickham, 2011) as well as visualization techniques with the package "ggplot2" (Wickham, 2009).

Results

Along 2 transects, we completed 24 and 12 ISIIS undulations and 6 and 5 bongo tows, respectively. ISIIS sampled an estimated 297 m^3 h^{-1} (or an average of 63 m^3 per tow-yo (i.e., down and up undulation), for a total sampled volume of 2281 m^3. The actual volume sampled was lower than the maximum possible because of a slight misalignment in the mirrors that occluded

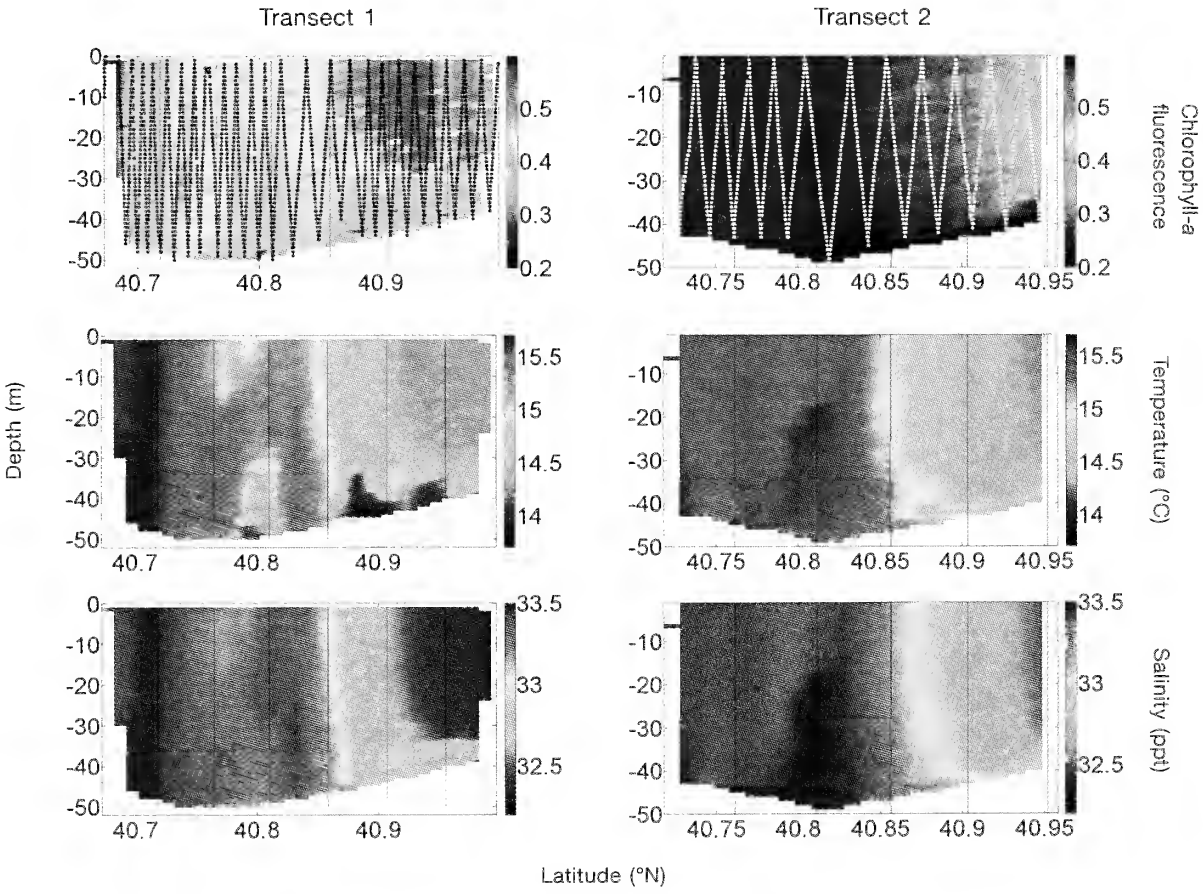

Figure 2

Fluorescence (voltage), temperature (°C), and salinity (ppt) measured from ISIIS along the western (transect 1) and eastern (transect 2) transects during 23–24 October 2008. Dotted lines in the fluorometry panels represent the undulations of the In the Situ Ichthyoplankton Imaging System (ISIIS). The vertical solid lines represent the approximate tow positions for the bongo sampler which was deployed along the length of the same transect once the ISIIS tow was completed.

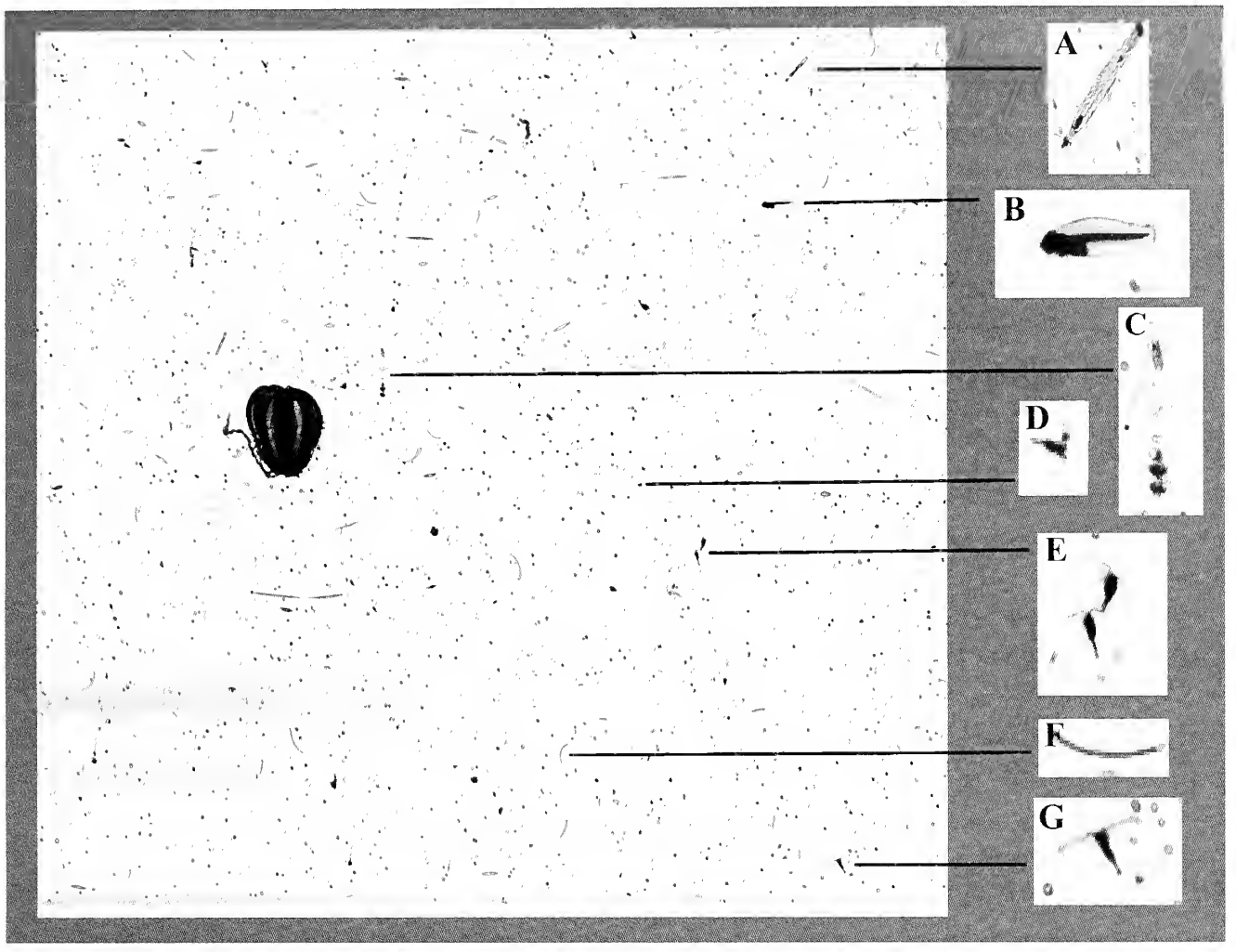

Figure 3

Example of a full-frame image collected with the In Situ Ichthyoplankton Imaging System (ISIIS). Larval fish (small [~4 mm], *Paralichthys dentatus*) and other plankters (especially copepods) are evident throughout. The small circular and elongate particles are diatoms (centric and pinnate) and diatom chains, which can be detected but are too small to clearly resolve. Also seen is a ~1.5-cm ctenophore with tentacles retracted. Several small aggregates (marine snow) are evident in the full-frame image. Overall, the full frame provides a good indication of the plankton field encountered by the observed larval fish. Surface is to the top of the image. Select plankters are shown to the right of the full frame in higher magnification (from top to bottom): (A) chaetognath (note that an improved image has been substituted for demonstration purpose only), (**B**) preflexion stage larval fish, (**C**) marine snow, (**D**) small copepod, (**E**) 2 copepods, (**F**) diatom chain (rotated to fit figure), and (**G**) copepod.

about 15% of the imaging field (i.e., the image field of view was 11 cm versus 13 cm). In comparison, the typical bongo sampled 137 m^3 per oblique tow, for a total volume sampled of 1506 m^3. The maximum depth of tows was 49 m for ISIIS tows and 52 m for the bongo tows.

The water column along both transects was defined by limited vertical stratification, especially in its upper 35 m (Fig. 2). A slight decrease in chlorophyll concentration below a depth of ~35 m in the inshore portion of the easterly transect was apparent and also was observed with a change in temperature and salinity; still, the differences were small. In contrast, considerable horizontal variation (south to north) was observed in hydrography along both transects with temperature lower, salinity lower, and chlorophyll fluorescence higher in the inshore (northern) portions than in the offshore (southern) portions (Fig. 2).

The productivity of the water column was evident in ISIIS imagery as a preponderance of diatoms visible throughout most images (Fig 3). Also imaged were a variety of invertebrate plankters, ranging from copepods and larvaceans to ctenophores and medusae to invertebrate larval types, such as echinoderm pluteus. Because most imagery was dominated by the smaller plankton (diatoms, copepods, and larvaceans;

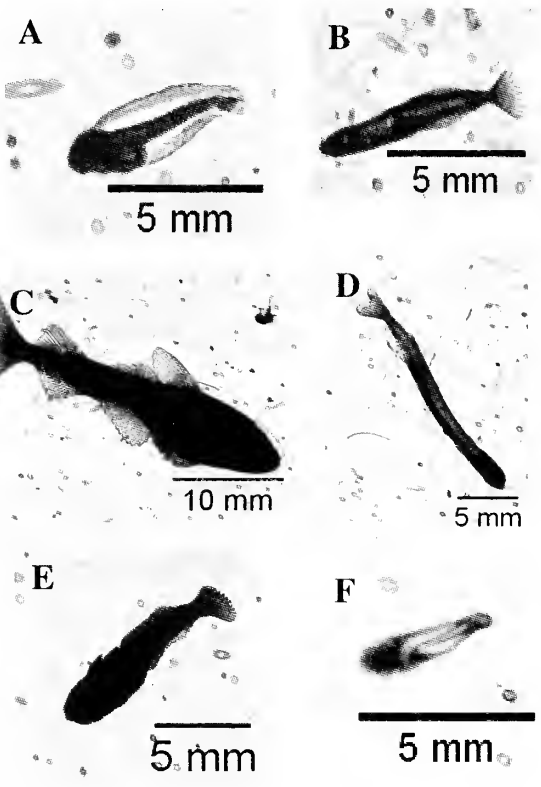

Figure 4

Examples of close-up, in situ images of different larval fish taxa imaged with the In Situ Ichthyoplankton Imaging System (ISIIS). (**A**) *Paralichthys dentatus* (4 mm); (**B**) Gobiidae (8 mm); (**C**) Gadidae (32 mm); (**D**) Clupeidae (21 mm); (**E**) *Merluccius* spp. (14 mm); (**F**) unknown (preflexion stage) (3.2 mm).

see Fig. 3), and larval fishes were relatively rare, the imagery provided a relative measure of abundance of different plankters. In most cases when fish larvae were encountered, the imagery was sufficient to discern characteristics valuable for identification at the family or genus level (e.g., shape, number and location of fins, overall body shape, fish size, and, in some cases, certain skeletal features; see Fig. 4).

The 2 sampling methods allowed us to detect comparable quantities of larval fishes. ISIIS imaged a total of 409 larvae, and the bongo tows collected a total of 359 larvae. When standardized for the volume of water actually sampled, ISIIS estimated ~0.18 fish larvae (±0.015 standard error of the mean [SE] m^{-3}), a value that was not significantly different from the estimate from the bongo tows (0.24 ±0.037 SE m^{-3}; P=0.074). Similarly, within gears, there were no differences in larval fish concentrations between transects.

The estimates of larval abundance, however, were made on the basis of the 2 gears sampling different portions of the water column. The bongo net sampled all depths equally as it was towed from depth to the surface, but ISIIS spent less time at depths >40 m than at depths near the surface (Fig. 5A). This sampling effect is evident in the difference in measured fish abundance by depth (Fig. 5B), where the apparent pattern was for a continual increase in fish abundance with depth from the surface down to 40 m and then a decrease in abundance by depth beyond 40 m. This decrease was directly coincident with the drop-off in sampling time with depth by ISIIS. When an adjusted abundance was estimated by computing depth-specific concentrations (Fig. 5C), then with the assumption of equal sampling effort per depth as with the bongo tows, an adjusted mean ISIIS fish concentration was 0.22 fish larvae m^{-3}, which is very close to the bongo estimate.

The taxonomic diversity collected by each gear also was similar; both collected larval fishes representing the same 7 families (Table 1), although bongo samples were typically identifiable to lower levels (genus and species) than those in ISIIS samples. Images of fish larvae from ISIIS were identifiable to at least the genus level for ~35% of larvae (143 out of 409). On the other hand, larvae were unidentifiable in 60 fish images and most of these unidentifiable fishes were in the early preflexion stages (~15%); in contrast, all bongo tow larvae were identified at least to the family level. Comparison of the relative proportions of taxa between the 2 sampling methods indicates that they were similar. There were a few notable exceptions: ISIIS underestimated paralichthyids and scopthalmids and estimated relatively greater proportions of phycids and ophidiids than the bongo sampler. The total number of larvae sampled was similar, but it is not known if the "unknown" category would have evened these discrepancies or added further differences among certain taxa.

Size distributions of larvae differed considerably between the 2 sampling methods. ISIIS imaged a larger size range and larger mean size of fish larvae than the bongo sampler (Fig. 6, Table 2). This sampling gear pattern was evident across several individual taxa, notably the gadiform fishes, Phycidae and Gadidae, with the latter mean size from ISIIS samples being more than 3 times the mean size of this family from bongo samples (Table 2). There was also a significant difference between gear types with respect to size of Paralichthyidae, although this very small difference (0.103 mm) may not be biologically meaningful and likely was significant only because of the rank nature of the Kruskal-Wallis test. There was a significant difference in overall larval size between transects for the ISIIS samples, but there was no significant difference in overall larval size for the bongo tows between the 2 transects or for any taxonomic group between transect within gear type (Fig. 6, Table 2). Therefore, most of the differences in size were attributed to sampling gear.

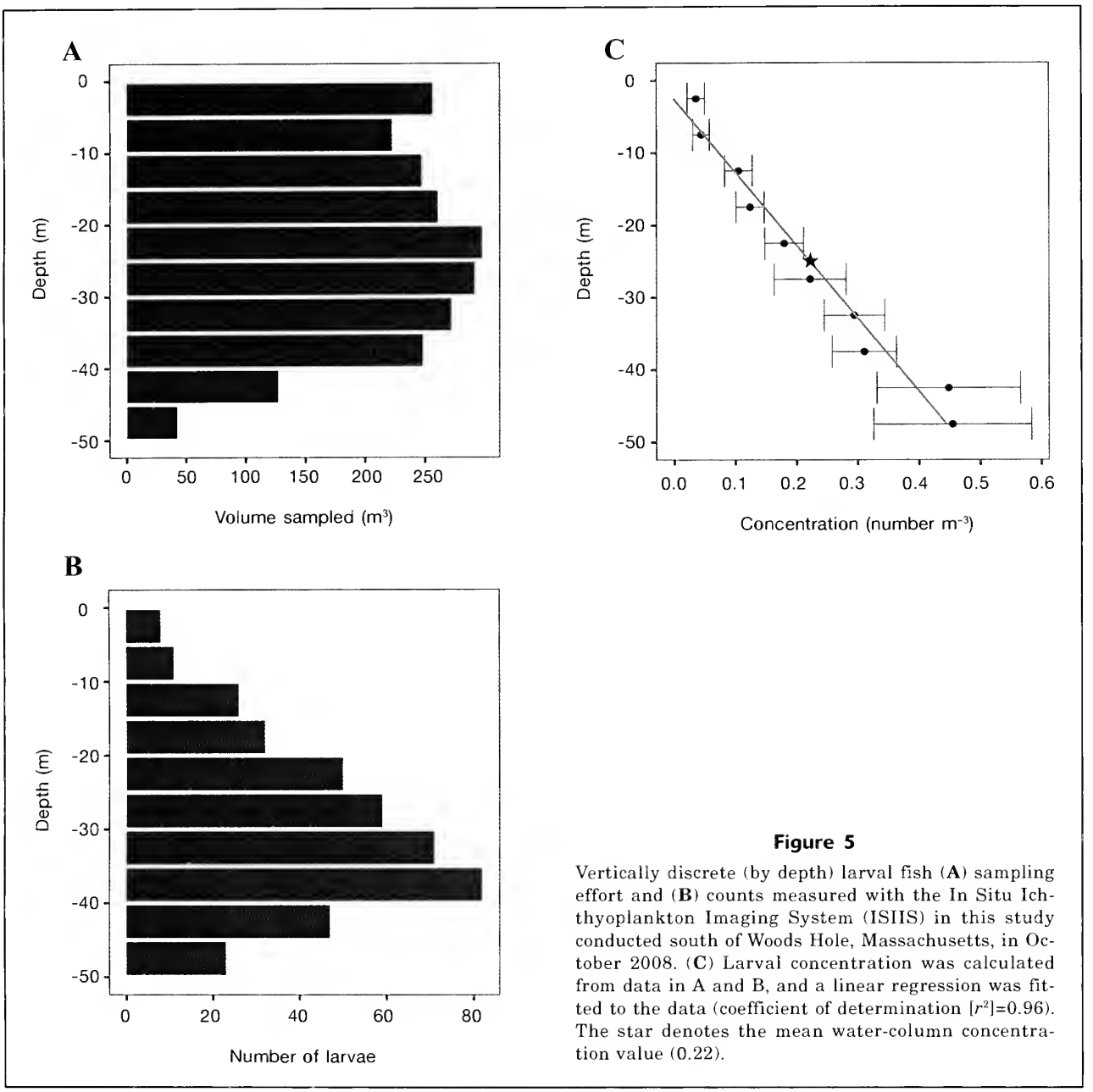

Figure 5

Vertically discrete (by depth) larval fish (**A**) sampling effort and (**B**) counts measured with the In Situ Ichthyoplankton Imaging System (ISIIS) in this study conducted south of Woods Hole, Massachusetts, in October 2008. (**C**) Larval concentration was calculated from data in A and B, and a linear regression was fitted to the data (coefficient of determination [r^2]=0.96). The star denotes the mean water-column concentration value (0.22).

Discussion

Design of larval fish surveys requires a balance of ship time, sample-processing time, and adequate sampling effort for resolution of the spatial (and temporal) variation to provide a robust measure of spatial distribution and abundance of this life-history stage. In essence, survey design is a cost-benefit issue. Greater sampling frequency will improve precision of estimates (e.g., Cyr et al., 1992), but it does so at a cost of greater ship time and laboratory sample processing. Consequently, surveys are limited, in part, by the sampling tool of choice (and its inherent limitations and benefits).

Results indicate that data collected with this prototype version of ISIIS are comparable to data collected with a bongo sampler. Measurements of larval concentrations were similar, although identifications of larvae fish were possible with ISIIS only at a coarser level of taxonomic resolution compared to that with the bongo sampler. In waters with relatively low species diversity of ichthyoplankton, like the shelf of the northeastern United States, the taxonomic resolution possible with ISIIS is adequate for conducting an array of studies, particularly when data are verified with net samples. However, in species-rich waters, the taxonomic resolution possible with ISIIS may limit the applications of

Table 1

(**Upper**): Comparison of taxonomic resolution between bongo and In Situ Ichthyoplankton Imaging System (ISIIS) samples collected south of Woods Hole, Massachusetts, in October 2008 as part of this study. Data are presented as "total," which is the combined lowest level of identification across all taxa; "family," which is a comparison just at the family level (where all taxa are subsumed into relevant family taxa), and "species," where only identifications to species level are presented. (**Lower**): Summary comparison between the bongo sampler and ISIIS gears for number and proportion of identifications at family, genus, and species levels, as well as number and proportion of unknowns.

Taxa	Identification level					
	Total (lowest)		Family level		Species level	
	Bongo	ISIIS	Bongo	ISIIS	Bongo	ISIIS
Clupeidae	1	3	2	3		
Brevoortia tyrannus	1	0			1	0
Gadidae	3	13	3	13		
Merlucciidae	0	0	48	44		
Merluccius bilinearis	48	44			48	44
Phycidae	0	83	48	104		
Urophycis spp.	48	21			48	21
Ophidiidae	0	29	7	34		
Lepophidium profundorum	7	5			7	5
Gobiidae	3	6	3	6		
Paralichthyidae	1	62	217	135		
Citharichthys arctifrons	14	0			14	0
Etropus spp.	10	8			10	8
Paralichthys oblongus	4	0			4	0
Paralichthys dentatus	188	65			188	65
Scopthalmidae	31	10	31	10		
Unknown	0	60	0	60		
Total larvae	359	409				

	Numbers		Proportion	
	Bongo	ISIIS	Bongo	ISIIS
Family	39	206	0.11	0.50
Genus	58	29	0.16	0.07
Species	262	114	0.73	0.28
Unknown	0	60	0.00	0.15
Total	359	409	1	1

the technology. The version of ISIIS used in this study was an early prototype (Cowen and Guigand, 2008); considerable advancements have been made in the image sharpness and depth of field since the field work reported here, and these changes should improve identification of individual fishes, especially of smaller taxa.

Larval lengths were different for ISIIS and the bongo sampler. The bongo sampler collected smaller larvae, indicating limitations with our ISIIS image-processing procedures for recording larval fishes <5 mm (and obvious diagnostic morphological features on small larvae). On the other hand, ISIIS imaged larger larvae, indicating that avoidance of the ISIIS by larger larvae was reduced. With the potential of an increase in image resolution to advance identification of smaller larvae (e.g. the improved image of a chaetognath in Fig. 3, upper right), the overall size range sampled by ISIIS could be a significant improvement over the range of the bongo sampler that has been used by the NEFSC for the past 30-plus years. If there is an effort to merge abundance time series between the bongo and ISIIS, careful calibration studies would be required to account for variances, including length-based, diel, and regional differences in detectability. These types of calibration studies also are necessary to combine data across different mesh sizes of the bongo sampler (see Johnson and Morse, 1994; Richardson et al., 2010).

Our results indicate that ISIIS could be a valuable addition to the survey sampling toolbox because it suc-

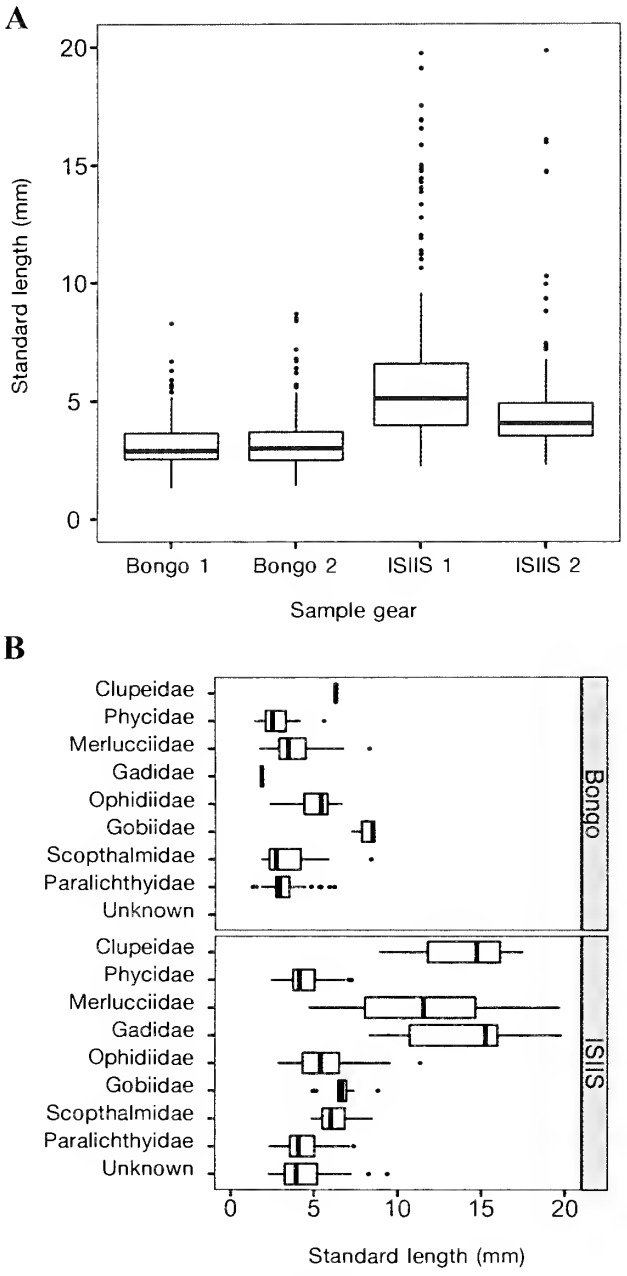

Figure 6

(**A**) Summary statistics (box plot) of larval size distribution by sampling gear and transect (1 and 2). The vertical bars of the box plot represent the range, the box represents the 1st (lower) and 3rd (upper) quartile, and the central (horizontal) line is the median of the distribution of observations. Perceived outliers are denoted as separate points beyond the range. Sampling was conducted with the In Situ Ichthyoplankton Imaging System (ISIIS) and a bongo sampler south of Woods Hole, Massachusetts, in October 2008. (**B**) Taxon-specific comparison of fish lengths between bongo sampler (top) and ISIIS (bottom). Note: the box plots are rotated 90°relative to A; however, basic features are the same as in A.

cessfully has estimated larval fish concentration, and, in an environment of relatively low diversity, as in this study, resolved the taxonomic composition of the larval ichthyofauna. Under such conditions, the rapid sampling speed of ISIIS could be used to increase spatial and temporal resolution of ichthyoplankton patchiness, without the need for additional ship days. For example, rapid undulation of ISIIS resulted in 24 vertical forays through the water column being repeated every 1.7 km along the 41.4-km transect in just 4.6 h. In comparison, 6 bongo tows were completed along the same transect in ~6 h for a spatial resolution of 6.9 km. Therefore, ISIIS can provide 3–4 times the spatial resolution of a bongo sampler over a comparable (or shorter) time frame. Other benefits of ISIIS include its ability to resolve very fine-scale patchiness because its sampling rate is both continuous and rapid. Consequently, depending on how it is towed, ISIIS can be used to assess detailed vertical distributional data, a feat that is not possible with a bongo sampler, or even with opening and closing net systems, without very extensive (and expensive) sampling efforts. Further, simultaneous sampling by other environmental sensors provides detailed concurrent image and physical data. Information about nearest-neighbor scaling and fish larval distribution in relation to their predators and prey, as well as environmental conditions, would be possible because of the fine-scale, in situ information available in the ISIIS imagery. Such sampling with ISIIS would allow targeted, process-oriented studies, even while general survey designs are being employed.

Still, the results of this study indicate several specific functional aspects that need to be considered or addressed for ISIIS to be a highly effective sampling tool for survey and process-oriented studies. First, ISIIS detected fewer smaller larvae than did the bongo sampler. Further, the small larvae detected with ISIIS were largely classified as unknown. These results indicate that the image resolution of ISIIS should be improved to increase the detectability and identification of small larvae, although preflexion larvae will likely always be problematic because of their limited morphological distinctiveness. An increase in detectability will require an increase in the depth of field such that particles that pass between the viewing ports are *all* in focus, thereby eliminating regions of out-of-focus particles that potentially can obscure the remaining image. The current version of ISIIS (ISIIS-2) has been successful at extending the depth of field from ~30 cm to the full 50-cm space between viewing ports, adding to the volume sampled and the overall clarity of imagery (Cowen and Guigand, unpubl. data).

The second issue is the need for rapid, accurate image processing. The large number of images produced makes computer-aided image analysis a requirement for large-scale application of this instrument. We were able to use manual assessment of the images taken in the current study (by focusing only on fish larvae), but further analysis of these data or more extensive surveys

Table 2

Kruskal-Wallis test for comparison of size difference (in mm) by transect and sampling gear for all fishes combined, as well as for the 3 most dominant fish families, from this study where 2 gear types were used: bongo sampler and the In Situ Ichthyoplankton Imaging System (ISIIS), to sample fish larvae south of Woods Hole, Massachusetts, in October 2008. (**Upper**): comparison within gear between transects. (**Lower**): comparison between gears. Asterisks (*) denote significant differences.

	Bongo Transect 1	Bongo Transect 2	*P*	ISIIS Transect 1	ISIIS Transect 2	*P*
Mean size—all larvae	3.514	4.397	0.275	7.223	4.959	0.001*
Paralichthyidae mean size	3.809	5.044	0.547	4.672	4.122	0.061
Phycidae mean size	2.335	2.980	0.221	4.617	4.295	0.199
Merlucciidae mean size	3.998	3.550	0.783	13.701	12.037	0.496

	Bongo	ISIIS	*P*
Mean size—all larvae	3.858	6.468	1.67E-12*
Paralichthyidae mean size	4.385	4.488	0.0001*
Phycidae mean size	2.622	4.486	5.41E-05*
Merlucciidae mean size	3.795	13.398	3.806E-06*

with ISIIS will require automated computer analysis. Several different options may be available for addressing some of these needs (e.g., Davis et al., 2004; Hu and Davis, 2005; Luo et al., 2005;; Culverhouse et al., 2006; Benfield et al., 2007; Zhao et al., 2010), although these alternatives have not been tested with repetitive processing of millions of images. Consequently, we are currently developing and testing algorithms suitable for segmenting and classifying individual organisms from full image files. These algorithms must be capable of processing data at high speeds (or with multiprocessor computers) and must be able to handle large data sets (e.g., Tsechpenakis et al., 2008). With such analysis capabilities, the typical time between research cruise and ultimate data analysis could be reduced greatly.

Conclusion

Although ISIIS can be a powerful tool for resolving fine to mesoscale patchiness in both vertical and horizontal distributions of plankton, it is limited by the fact that it is a nondestructive sampler (i.e., it does not collect specimens). ISIIS will not replace nets for all studies. There is still a strong need for sample collection, whether for identification verification (for larvae or eggs) or for more specific studies, such as projects on food habits, growth, and genetics, that require specimens. In addition, many nets, including bongo nets, can be used by a greater variety of vessels and in a wider range of weather conditions than the ISIIS instrument package. When these different tools are combined, however, ISIIS could be used to establish the vertical and spatial setting of fish larvae. This information could be used to identify locations for targeted net samples. This melding of samplers also would lead to more efficient requirements for ship time and processing time (i.e., less time spent with nets and on processing the survey samples from areas where the targeted specimens are rare or absent). Therefore, ISIIS (and the technology it represents) is a valuable addition to both process-oriented studies and routine surveys. This technology can contribute both to the understanding of the relation between larval fishes and their biological and physical oceanographic habitat and to the quantification of larval fish abundance and distribution for use in stock and ecosystem assessments.

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New insights into the diets of harbor seals *(Phoca vitulina)* in the Salish Sea revealed by analysis of fatty acid signatures

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Abstract—Harbor seals (*Phoca vitulina*) are an abundant predator along the west coast of North America, and there is considerable interest in their diet composition, especially in regard to predation on valued fish stocks. Available information on harbor seal diets, primarily derived from scat analysis, suggests that adult salmon (*Oncorhynchus* spp.), Pacific Herring (*Clupea pallasii*), and gadids predominate. Because diet assessments based on scat analysis may be biased, we investigated diet composition through quantitative analysis of fatty acid signatures. Blubber samples from 49 harbor seals captured in western North America from haul-outs within the area of the San Juan Islands and southern Strait of Georgia in the Salish Sea were analyzed for fatty acid composition, along with 269 fish and squid specimens representing 27 potential prey classes. Diet estimates varied spatially, demographically, and among individual harbor seals. Findings confirmed the prevalence of previously identified prey species in harbor seal diets, but other species also contributed significantly. In particular, Black (*Sebastes melanops*) and Yellowtail (*S. flavidus*) Rockfish were estimated to compose up to 50% of some individual seal diets. Specialization and high predation rates on Black and Yellowtail Rockfish by a subset of harbor seals may play a role in the population dynamics of these regional rockfish stocks that is greater than previously realized.

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The harbor seal (*Phoca vitulina*) is the most abundant pinniped species in the protected coastal waters of Washington State and British Columbia, Canada (Jeffries et al., 2003). This species is a generalist piscivorous predator, at or near the apex of marine food webs. Such large and mobile endothermic predators require high caloric intake to support growth, reproduction, and foraging activity (e.g., Williams et al., 2004). Given their abundance and trophic position, harbor seals undoubtedly make up an influential component of their marine ecosystems (Sergio et al., 2006; Heithaus et al., 2008; Schmitz et al., 2010).

Numerous fish stocks of historic commercial importance are depressed or have declined significantly in the Salish Sea of western North America, including Pacific Herring (*Clupea pallasii*), Chinook Salmon (*Oncorhynchus tshawytscha*) in Puget Sound, Steelhead Trout (*O. mykiss*), Pacific Hake (*Merluccius productus*), Walleye Pollock (*Theragra chalcogramma*), and many species of rockfish (*Sebastes* spp.) (Federal Register, 2007). Under the Endangered Species Act, the Puget Sound and Georgia Basin distinct population segments of Yelloweye (*S. ruberrimus*) and Canary (*S. pinniger*) Rockfish recently were listed as threatened, and Bocaccio (*S. paucispinis*) was listed as endangered (Federal Register, 2010). Three additional rockfish species—Brown Rockfish (*S. auriculatus*), Copper Rockfish (*S. caurinus*), and Quillback Rockfish (*S. maliger*)—now are considered federal species of concern, and the remaining 7 species found in the Salish Sea are listed as species of concern by the State of Washington (M. Lance, personal commun.). Continued declines in fish abundance and the failure of depleted populations to recover have elevated concerns among fishing crews, managers, and conservationists (Musick et al., 2001; Williams et al., 2010).

The concurrence of abundant harbor seals and depressed fish populations has stimulated debate about the degree to which harbor seals may regulate prey abundance (Orr et al., 2004). Numerous factors may have contributed to the declines in fish

abundance, although overexploitation has likely played a prominent role (e.g., Levin et al., 2006). Predation may have contributed to historic declines or may be inhibiting recovery, because the abundance of Salish Sea pinnipeds has been increasing and is thought to be near carrying capacity (Jeffries et al., 2003). Although pinnipeds have the potential to deplete local fish stocks or hinder management actions that would promote the recovery of depleted stocks (Harwood and Croxall, 1988; Bowen et al., 1993; Fu et al., 2001; Bjørge et al., 2002; Boyd, 2002; MacKenzie et al., 2011), there is no direct evidence to that effect in the Salish Sea. Consequently, an improved understanding of the role of pinniped predation in regulation of prey abundance would enhance our knowledge of marine ecosystem dynamics and potentially inform the effective management of fish stocks.

The diets of harbor seals in this region are thought to be composed primarily of adult salmon (*Oncorhynchus* spp.), Pacific herring, and gadids (Scheffer and Slipp, 1944; Olesiuk, 1993; Tollit et al., 1997; Browne et al., 2002; Wright et al., 2007; Thomas et al., 2011; Lance et al., 2012). However, seals are considered opportunistic predators that target locally abundant prey and switch between prey species in response to changes in prey abundance—a type-III functional response (Holling, 1959; Middlemas et al., 2006). Such predatory behavior, in combination with local and seasonal diversity in the availability of prey (Stasko et al., 1976; Willson and Womble, 2006; Therriault et al., 2009; Thomas et al., 2011), implies harbor seal diet composition will vary both spatially and temporally, and thus complicate accurate diet assessment.

Prior investigations of harbor seal diets in the Pacific Northwest have relied primarily on observational studies, stomach content analyses, and especially scat analyses (Scheffer and Slipp, 1944; Everitt et al., 1981; Brown and Mate, 1983; Olesiuk, 1993; Zamon, 2001; Orr et al., 2004; Wright et al., 2007; Thomas et al., 2011; Lance et al., 2012). Such methods provide important insights into predatory behavior and document the presence of particular prey species in predator diets; however, several well-known factors can limit their utility in quantitative investigations of diet (Phillips and Harvey, 2009; Klare et al., 2011). For example, scat analyses frequently are compromised by unequal probabilities of detecting prey classes, as well as by difficulty in derivation of quantitative estimates of diet composition from frequency-of-occurrence data. In addition, results pertain only to a short period of time, ranging from the last predatory event in observational studies to 1–2 days in scat-based investigations (Harvey, 1989; Cottrell and Trites, 2002; Tollit et al., 2004; Trites and Joy, 2005; Hauser et al., 2008; Phillips and Harvey, 2009).

Quantitative fatty acid signature analysis (QFASA; Iverson et al., 2004) has important advantages over other methods of diet assessment. Perhaps, most important, the method produces statistical estimates of diet composition and measures of precision. The number of fatty acids that can be biosynthesized by animals is limited (Ackman, 1989); therefore, the presence of some compounds can be attributed to diet alone. This fact, in combination with the large number of fatty acid compounds present in adipose tissue, particularly in marine ecosystems, enables QFASA to estimate the contribution of a large number of prey classes to diets, limited primarily by the diversity of fatty acids among prey classes. In addition, although most methods of diet assessment provide information only on recent consumption, sampling of adipose deposits may provide insights into diets over a period of weeks to months (Iverson et al., 2004; Budge et al., 2006). QFASA requires the development of comprehensive data on the fatty acid composition of potential prey, work that may be costly or otherwise difficult. Although predators must be captured and handled, only a small incision is required for sampling and predators can be quickly released. Overall, QFASA presents predators with limited negative consequences and can produce diet composition estimates that largely avoid potential biases characteristic of other methods.

We used QFASA to investigate the diets of harbor seals captured from haul-out sites among the San Juan Islands of Washington State and the southern Gulf Islands of British Columbia; both island groups are within the Salish Sea. Blubber samples were collected from captured harbor seals and representative specimens of known or potential prey species also were collected. Samples from both predators and potential prey were analyzed to determine their fatty acid composition, and diet compositions of sampled harbor seals were estimated with QFASA mixture modeling. The resulting estimates provide new insights into harbor seal predation on depressed fish populations and reveal dietary heterogeneity on spatial, demographic, and individual scales.

Materials and methods

Study area

The San Juan Islands and the southern Gulf Islands lie in the transboundary waters of Washington State and British Columbia between the Strait of Georgia, Strait of Juan de Fuca, and Puget Sound (Fig. 1). This area is characterized by hundreds of large and small islands, rocky intertidal reefs, protected bays and estuaries, and rich marine life. Harbor seals use more than 150 haul-out locations in the study area, including intertidal sandbars and numerous small islands and rocky reefs distributed throughout the region. Harbor seals are abundant throughout the Salish Sea (Jeffries et al., 2003).

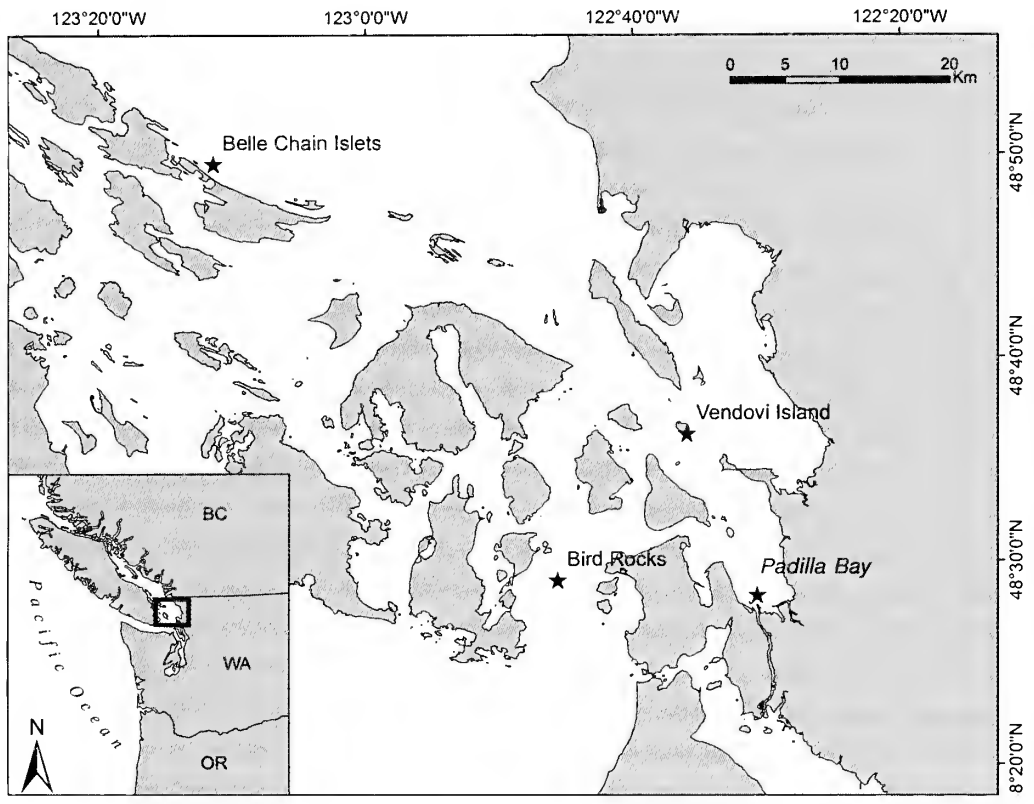

Figure 1

Map of the San Juan Island region, where samples were collected for our investigation of the diet composition of harbor seals (*Phoca vitulina*) in the Salish Sea. Harbor seals were captured in the vicinity of Padilla Bay, Bird Rocks, Vendovi Island, and the Belle Chain Islets.

Sampling of predator and prey

Harbor seals were captured from April 2007 to March 2008 at 3 sites in the San Juan Islands of Washington State and at a fourth site in the adjacent Gulf Islands in British Columbia (Fig. 1). Padilla Bay (48°28.37′N, 122°30.88′W) is characterized by estuarine-mudflat habitat, Vendovi Island (48°67.10′N, 122°61.10′W) consists of rocky reef habitat located in close proximity to Bellingham, Samish, and Padilla Bays, and Bird Rocks (48°29.16′N, 122°45.61′W) comprises rocky reef habitat in Rosario Strait. The fourth site was the Belle Chain Islets, a rocky reef in the southeastern Gulf Islands of British Columbia (48°49.67′N, 123°11.56′W) with habitat similar to that of Bird Rocks.

Forty-nine blubber samples were collected from harbor seals according to standard techniques (Iverson et al., 1997; Walton et al., 2000; Walton and Pomeroy, 2003) under Marine Mammal Protection Act Research Permit 782-1702-00. Seals were captured in salmon landing nets and physically restrained during processing following the method of Jeffries et al. (1993). The sampling location on the left side of the pelvic region was shaved with a razor, rinsed with isopropyl alcohol, scrubbed with Betadine, and rinsed again with isopropyl alcohol. A complete cross section of blubber from skin to muscle was collected with a sterile, 6-mm biopsy punch. A full cross-section sample provides the most complete information regarding diet because phocid blubber is not homogenous throughout its depth and the inner layer responds most quickly to diet shifts (Iverson et al., 1997). The biopsy site was then filled with antiseptic cream and left open to drain. Each sample was placed immediately in chloroform with 0.01% butylated hydroxytoluene to inhibit oxidation in glass vials with Teflon lids, placed on ice while in the field, and subsequently stored frozen at –80°C until analysis. Seal samples were associated with these covariates: sampling location, sex, and season (Table 1). Seasons were defined as spring (March to May), fall (October to November), and winter (December to February).

We sampled fish and cephalopod species known to be consumed by harbor seals in the San Juan Islands region on the basis of previous fecal analyses (Lance et al., 2012). Some adult salmon samples were obtained from seafood processors and staff of the NOAA Northwest Fisheries Science Center. Other prey were captured from throughout the study area between June

Table 1

Number of harbor seal samples, by location, sex, and season, used in our investigation of diet composition of harbor seals (*Phoca vitulina*) in the Salish Sea through quantitative fatty acid signature analysis.

	Female			Male		
Location	Spring	Fall	Winter	Spring	Fall	Winter
Belle Chain	4	0	0	6	0	0
Bird Rocks	1	0	2	5	4	2
Padilla Bay	14	1	0	3	0	0
Vendovi Island	0	2	1	0	4	0

and December, 2008, with a variety of gear, including hook and line, longline, and trawl. Samples were obtained from 269 specimens representing these 20 species: Black (*Sebastes melanops*), Yellowtail (*S. flavidus*), Copper, and Puget Sound (*S. emphaeus*) Rockfish; Chinook, Chum (*Oncorhynchus keta*), Coho (*O. kisutch*), Sockeye (*O. nerka*), and Pink (*O. gorbuscha*) Salmon; Pacific Herring, Walleye Pollock; Pacific Sand Lance (*Ammodytes hexapterus*); Northern Anchovy (*Engraulis mordax*); Shiner Perch (*Cymatogaster aggregata*); Plainfin Midshipman (*Porichthys notatus*); Spiny Dogfish (*Squalus acanthias*); Opalescent Inshore Squid (*Loligo opalescens*); Kelp Greenling (*Hexagrammos decagrammus*); Pacific Staghorn Sculpin (*Leptocottus armatus*); and Starry Flounder (*Platichthys stellatus*). Specimens were identified with Hart (1973) for fish species and Roper et al. (1984) for squid. Because some species were represented by individuals with differences in size and total fat content (for example, immature and mature species of salmon), 27 prey classes were defined (Table 2).

Prey specimens were placed in airtight plastic bags and stored at –80°C as soon as possible after collection. In the laboratory, each specimen was given a unique sample number, partially thawed, weighed and measured (standard, fork, and total lengths), and homogenized with a medium or large mechanical blender, depending on fish size. The smallest prey animals were homogenized with a mortar and pestle because the blender was ineffective. Stomach contents were not removed from prey specimens, to mimic ingestion by predators (Budge et al., 2002). Approximately 5–10 g of homogenate was placed in labeled scintillation vials with Teflon lids and stored in a –80°C freezer. Samples were express shipped in a cooler on dry ice to the Applied Sciences, Engineering, and Technology (ASET) Laboratory at the University of Alaska Anchorage.

Fatty acid extraction and selection

All samples were processed at the ASET Laboratory through the use of a method for microscale recovery of total lipids with the Dionex ASE 200[1] automated solvent extraction system (Thermo Fisher Scientific, Waltham, MA), which provides lipids for the determination of 80 unique fatty acids (Dodds et al., 2005). The total body mass, percent fat composition, and fat mass of prey specimens were obtained for 27 prey classes (Table 2). Total mass data were not available for mature Chinook, Sockeye, and Pink Salmon obtained from the Northwest Fisheries Science Center; therefore, an approximate mean mass for these prey classes (e.g., Quinn, 2005) was used in calculation of fat mass. Given the large range of mass among prey classes (Table 2), the results were insensitive to our use of these approximate values.

Extracted lipids were dissolved in hexane to a concentration of 100 mg/mL, hydrolyzed by a base-catalyzed reaction with potassium hydroxide, and then esterified to form fatty acid methyl esters (FAMEs) by reaction with boron trifluoride in methanol. Each sample was spiked with a C21:0 internal standard (25 µg/mL) and separated on a Hewlett-Packard 5890 gas chromatograph (GC) with a flame ionization detector (FID) (Hewlett-Packard Co., Palo Alto, California) by using a 60-m J&W DB-23 column (Agilent Technologies, Inc., Santa Clara, CA) with a 0.25-mm inside diameter and 0.25-µm cyanopropyl polysiloxane film. Signal data were collected and analyzed with Agilent GC Chemstation software.

Supelco 37-Component FAME Mix (catalog no. 47885-U; Sigma-Aldrich Co., St. Louis, MI) was used as a continuing calibration verification (CCV) to verify both the retention times and recovery values. This CCV also contained 25 µg/mL of a C21:0 internal standard, which is required to meet a tolerance of no greater than ±20% of actual value. Analyte identity was verified further by mass spectrometry through the use of a Varian CP3800 GC (Agilent Technologies, Inc.) and a Varian Saturn 2200 ion trap mass spectrometer

[1] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the U.S. Government.

Table 2

The number of prey animals from which fatty acid signature data were obtained (n) and the prey class (class) into which each prey type was assigned after evaluation of discriminant analysis and mean fat mass in our investigation of the diet composition of harbor seals (*Phoca vitulina*) in the Salish Sea through quantitative fatty acid signature analysis. Prey classes are defined as B&YR (Black [*Sebastes melanops*] and Yellowtail [*S. flavidus*] Rockfish), CR (Copper Rockfish [*S. caurinus*]), PSR (Puget Sound Rockfish [*S. emphaeus*]), Chin (mature Chinook Salmon [*Oncorhynchus tshawytscha*]), Chum (mature Chum Salmon [*O. keta*]), Coho (mature Coho Salmon [*O. kisutch*]), Sock (mature Sockeye salmon [*O. nerka*]), Pink (mature pink salmon [*O. gorbuscha*]), Sal-M (medium-sized Chinook and Coho Salmon), Sal-S (small Chinook, Chum, Sockeye, and Pink Salmon), Pol (Walleye Pollock [*Theragra chalcogramma*]), Her (Pacific Herring [*Clupea pallasii*] at least 2 years old), YH&SL (Pacific Herring less than 2 years old and Pacific Sand Lance [*Ammodytes hexapterus*]), NA (Northern Anchovy [*Engraulis mordax*]), SP (Shiner Perch [*Cymatogaster aggregata*]), PM (Plainfin Midshipman [*Porichthys notatus*]), SD (Spiny Dogfish [*Squalus acanthias*]), OIS (Opalescent Inshore Squid [*Loligo opalescens*]), G&S&F (Kelp Greenling [*Hexagrammos decagrammus*], Pacific Staghorn Sculpin [*Leptocottus armatus*], and Starry Flounder [*Platichthys stellatus*]). For each prey type, the sample size (n), mean (mean), and standard deviation (SD) of total mass, percent fat composition, and total fat mass are shown. Mass data were not available for mature Chinook, Sockeye, or Pink Salmon, and an approximate mean mass was used for the computation of fat mass.

			Mass (g)			Percent fat			Fat mass (g)		
Prey type	n	Class	n	Mean	SD	n	Mean	SD	n	Mean	SD
Black Rockfish	5	B&YR	5	293.8	48.3	5	6.5%	0.4%	5	19.3	4.0
Yellowtail Rockfish	5	B&YR	5	152.8	28.2	5	5.7%	1.5%	5	8.8	2.6
Copper Rockfish	12	CR	12	201.3	195.7	12	2.4%	0.4%	12	4.7	4.5
Puget Sound Rockfish	14	PSR	14	53.9	8.9	5	2.2%	0.3%	5	1.1	0.4
Chinook, mature	10	Chin	0	10000.0	NA	10	12.2%	2.3%	10	1218.8	233.3
Chum, mature	10	Chum	10	4955.9	784.6	10	15.1%	7.8%	10	789.7	455.6
Coho, mature	10	Coho	10	3765.4	660.8	10	5.5%	2.8%	10	208.2	125.0
Sockeye, mature	10	Sock	0	2500.0	NA	10	12.4%	1.8%	10	309.4	45.4
Pink, mature	10	Pink	0	2000.0	NA	10	5.3%	2.1%	10	105.6	43.0
Chinook, medium	5	Sal-M	5	133.5	70.3	5	3.0%	1.3%	5	4.8	3.1
Coho, medium	4	Sal-M	4	193.0	28.6	4	2.9%	0.5%	4	5.7	1.7
Chinook, small	11	Sal-S	12	20.9	8.0	12	1.3%	0.3%	12	0.3	0.2
Chum, small	12	Sal-S	12	62.8	24.6	12	2.3%	1.1%	12	1.6	1.5
Sockeye, small	12	Sal-S	12	15.5	2.5	12	1.5%	0.2%	12	0.2	0.1
Pink, small	12	Sal-S	12	47.2	13.6	12	2.4%	0.8%	12	1.2	0.7
Pollock	13	Pol	13	29.4	78.6	13	1.8%	0.4%	13	0.5	1.2
Pacific Herring ≥2 yr	12	Her	12	37.5	4.2	12	11.7%	3.4%	12	4.4	1.6
Pacific Herring <2 yr	12	YH&SL	12	5.8	0.8	12	3.5%	1.3%	12	0.2	0.1
Pacific Sand Lance	12	YH&SL	12	1.9	0.3	12	3.3%	0.8%	12	0.1	0.0
Northern Anchovy	11	NA	11	18.8	1.7	11	12.2%	3.4%	11	2.3	0.7
Shiner Perch	12	SP	12	21.0	5.8	12	6.9%	2.4%	12	1.5	1.0
Plainfin Midshipman	9	PM	9	61.7	13.4	9	3.4%	0.7%	9	2.1	0.6
Spiny Dogfish	4	SD	4	1712.5	383.8	4	9.0%	3.6%	4	160.5	83.5
Opalescent Inshore Squid	12	OIS	12	7.1	1.9	12	3.0%	0.4%	12	0.2	0.1
Kelp Greenling	7	G&S&F	7	179.7	396.3	7	1.5%	0.4%	7	3.0	6.8
Pacific Staghorn Sculpin	12	G&S&F	12	21.0	10.1	11	1.5%	0.6%	11	3.4	5.7
Starry Flounder	11	G&S&F	11	220.2	410.1	11	1.5%	0.6%	11	3.4	5.7

with a scan range of 50–400 mass-to-charge ratios (m/z). Additionally, a National Institute of Standards and Technology 1946 international standard was used to externally verify the method and the quality of recoveries.

The ASET Laboratory implements several protocols to improve data quality that are not routinely implemented in analyses of fatty acid data. Rather than normalize the peak data of each sample to C18:0, the laboratory adds an internal standard to all samples, method blanks, and CCVs. This protocol is beneficial because it provides a data point of known quantity to each resulting set, including blanks, allowing the significance of low-recovery peak data to be verified. In addition, because normalization to a recovered compound incorrectly entails the assumption that all compounds respond equally in the FID, use of an internal standard avoids errors that might otherwise result from that assumption (Dodds et al., 2005). The laboratory also verifies the identity of each peak by using a GC mass spectrometer (GC-MS)—verification that is necessary to eliminate misclassification of non-fatty acid

byproducts from the derivatization process. Finally, the laboratory performs periodic standard calibrations of the spectrometer at varying levels of concentration to determine the limit-of-detection for each compound.

Several criteria were used to evaluate the suitability of each fatty acid compound for inclusion in mixture modeling. At a minimum, each compound had to pass GC-MS verification, have a minimal variance for the majority of samples collected (<20% relative standard deviation), and average at least 1% of the total fatty acid contained in each sample. The compounds needed to be predominately from a dietary source, as delineated in Iverson et al. (2004). Compounds 18:2n-6 and 18:3n-3 were automatically included as neither compound is biosynthesized by seals. These selection criteria led to a suite of 22 fatty acid compounds to be used in mixture modeling: C16:2n-6, C16:2n-4, C16:4n-1, C18:1n-9, C18:1n-7, C18:2n-6, C18:3n-6, C18:3n-4, C18:3n-3, C18:4n-3, C20:1n-11, C20:1n-9, C20:1n-7, C20:2n-6, C20:3n-6, C20:4n-6, C20:3n-3, C20:4n-3, C20:5n-3, C22:6n-3, C21:5n-3, and C22:5n-6. Data are available at the Biological and Chemical Oceanography Data Management Office of the National Science Foundation (http://osprey.bcodmo.org/project.cfm?flag=viewr&id=224&sortby=project).

Estimating diet composition

Obtaining unique estimates of diet composition with mixture models requires the number of prey classes to be no greater than the number of fatty acids (e.g., Phillips, 2001). Furthermore, combining prey classes reduces the dimensionality of the parameter space and can increase estimation precision. Linear discriminant functions were used to identify prey classes with potential to be merged, with R software, vers. 2.10.1 (R Development Core Team, 2009) and function lda of package MASS (Venables and Ripley, 2002). The accuracy of classifying individual prey into correct prey classes was estimated with discriminant functions and cross validation. Data from each prey specimen were removed temporarily, discriminant functions were estimated from the remaining data, and the estimated functions were used to classify the excluded specimen to a prey class. Prey classes with the largest misclassification rates were candidates to be merged, provided that the mean adipose masses of the 2 classes were similar.

Methods of QFASA mixture modeling closely followed those of Iverson et al. (2004) and Beck et al. (2007), methods that have been applied to the research of numerous marine species, including harbor seals (Nordstrom et al., 2008), gray seals (*Halichoerus grypus*; Iverson et al., 2004; Beck et al., 2007; Tucker et al., 2008; Lundstrom et al., 2010), harp seals (*Pagophilus groenlandicus*; Iverson et al., 2004), northern fur seals (*Callorhinus ursinus*; Hofmeyr et al., 2010), Steller sea lions (*Eumetopias jubatus*; Hoberecht, 2006), polar bears (*Ursus maritimus*; Thiemann et al., 2008), and various species of seabirds (Williams et al., 2009). A mixture model based on the Kullback-Liebler (KL) distance measure (Iverson et al., 2004) was used to estimate the diet composition of each seal. The calibration coefficients for harbor seals reported by Nordstrom et al. (2008) were used to convert prey fatty acid signatures (FAS) to the scale of predator FAS, and the distance measure was evaluated on the predator scale; note that Iverson et al. (2004) converted predator FAS to the prey scale. Estimation variance for each seal was estimated with 1000 bootstrap replications of the prey FAS data. The resulting estimates of diet composition (fat unadjusted, the p_k of Iverson et al., 2004), also were transformed to account for adipose mass per prey, expressing diet composition in terms of the number of animals consumed (fat adjusted, the a_k of Iverson et al., 2004).

Multivariate analysis of variance (function manova in R; R Development Core Team, 2009) was used to explore diet composition estimates for structure associated with the following covariates: sampling location, season (spring, fall, winter), and sex. The initial model contained these 3 main effects and all 2-way interactions, and nonsignificant terms were sequentially eliminated from the model. A significance level (α) of 0.01 was used for all tests. The mean diet composition for a class of predators (e.g., males or females) was computed as the sample average of their individual diet composition estimates. The variance of mean diet composition was assessed with the estimator of Beck et al. (2007). Mixture proportions and variances were estimated with a custom computer program written in Fortran (Metcalf et al., 2004) and compiled with the Intel Visual Fortran Compiler Professional Edition, vers. 11.1 (Intel Corp., Santa Clara, CA).

Results

Estimating diet composition

Given the suite of 22 fatty acid compounds used to form FAS, the 27 original prey classes needed to be reduced to no more than 22 prey classes for mixture model estimates to be unique (Phillips, 2001). Among the 27 original prey types, Black and Yellowtail Rockfish; medium-size Chinook and Coho Salmon; small Chinook, Chum, Sockeye, and Pink Salmon; young Pacific Herring aged 0 to 1 and Pacific Sand Lance; and Kelp Greenling, Pacific Staghorn Sculpin, and Starry Flounder were combined to reduce discriminant analysis misclassification among prey classes (Table 2). The resulting prey data set contained 19 prey classes, for which 251 of 269 prey animals (93.3%) were assigned to the correct prey class.

The mean diet composition of all 49 seals, both adjusted and unadjusted for differential fat mass among prey, was estimated with FAS for 22 fatty acid compounds and data for 19 prey classes. The species esti-

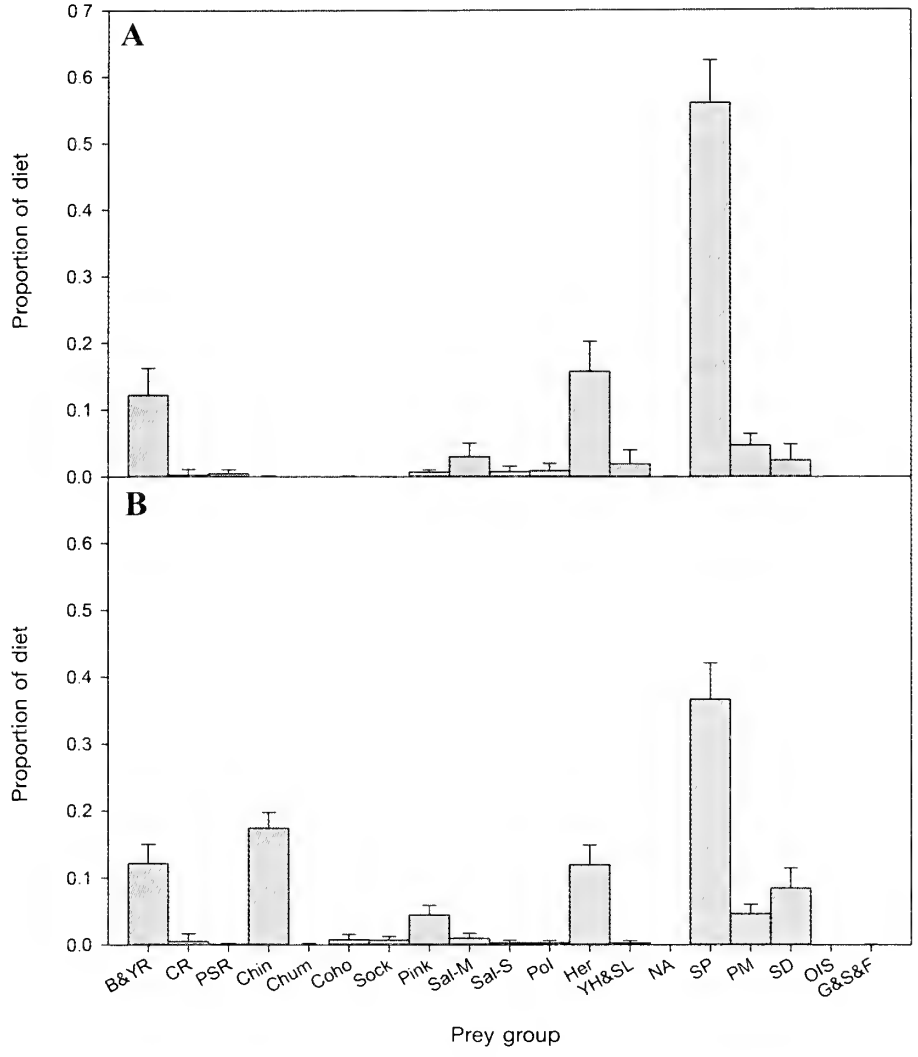

Figure 2

Mean diet composition estimates: **(A)** adjusted and **(B)** unadjusted for differential fat mass among prey classes, for all harbor seals (*Phoca vitulina*) combined in our investigation of the diet composition of harbor seals in the Salish Sea. Error bars are ±1 standard error of the estimate. Prey classes are defined as B&YR (Black [*Sebastes melanops*] and Yellowtail [*S. flavidus*] Rockfish), CR (Copper Rockfish [*S. caurinus*]), PSR (Puget Sound Rockfish [*S. emphaeus*]), Chin (mature Chinook Salmon [*Oncorhynchus tshawytscha*]), Chum (mature Chum Salmon [*O. keta*]), Coho (mature Coho Salmon [*O. kisutch*]), Sock (mature Sockeye Salmon [*O. nerka*]), Pink (mature Pink Salmon [*O. gorbuscha*]), Sal-M (medium-size Chinook and Coho Salmon), Sal-S (small Chinook, Chum, Sockeye, and Pink Salmon), Pol (Walleye Pollock [*Theragra chalcogramma*]), Her (Pacific Herring [*Clupea pallasii*] at least 2 years old), YH&SL (Pacific Herring less than 2 years old and Pacific Sand Lance [*Ammodytes hexapterus*]), NA (Northern Anchovy [*Engraulis mordax*]), SP (Shiner Perch [*Cymatogaster aggregata*]), PM (Plainfin Midshipman [*Porichthys notatus*]), SD (Spiny Dogfish [*Squalus acanthias*]), OIS (Opalescent Inshore Squid [*Loligo opalescens*]), G&S&F (Kelp Greenling [*Hexagrammos decagrammus*], Pacific Staghorn Sculpin [*Leptocottus armatus*], and Starry Flounder [*Platichthys stellatus*]).

mated to contribute most to harbor seal diets included Black and Yellowtail Rockfish, Chinook Salmon, adult Pacific Herring, and Shiner Perch (Fig. 2). Large differences in fat mass among prey classes led to substantial differences in the 2 estimates. Most noticeably, the high fat content of mature salmon species (Table 2) reduced the contribution of adult Chinook Salmon in the estimates adjusted for fat mass, suggesting that few individual Chinook Salmon need to be consumed for them to contribute significantly to the fat composition of harbor seals.

Multivariate analysis of variance results revealed substantial heterogeneity among estimated diets of individual seals by sampling location ($P<0.001$) and sex ($P<0.001$), although the interaction was not statistically significant ($P=0.111$). For that reason, the 49 seals were independently stratified by sampling location and sex and the mean diet composition, unadjusted for differential fat mass, was estimated for the seals in each stratum. Season was eliminated from the model because it was not a statistically important covariate (see *Discussion* section). Seals sampled in the vicinity of Belle Chain and Bird Rocks, both of which are characterized by rocky, high-current habitat, had the most diverse diets, with important contributions from Black and Yellowtail Rockfish, adult salmon species, Pacific Herring, Shiner Perch, and Spiny Dogfish (Fig. 3). Conversely, seals sampled from Padilla Bay, which consists of shallow estuarine habitat, had diets that were, on average, dominated by Shiner Perch. Harbor seals sampled near Vendovi Island, which has rocky habitat with nearby access to several bays, appeared to have an intermediate diet.

Male harbor seals were estimated to consume larger quantities of Black and Yellowtail Rockfish, Pacific Herring, and Spiny Dogfish than females, for which Shiner Perch appeared to be more important (Fig. 4). Diet estimates for individual seals reflected additional between-seal heterogeneity that was not explained by the covariates. For example, although Black and Yellowtail rockfish were estimated to be more important to males than females overall, males were not consistent in their reliance on rockfish species. Of the 24 males sampled, 10 had an estimated

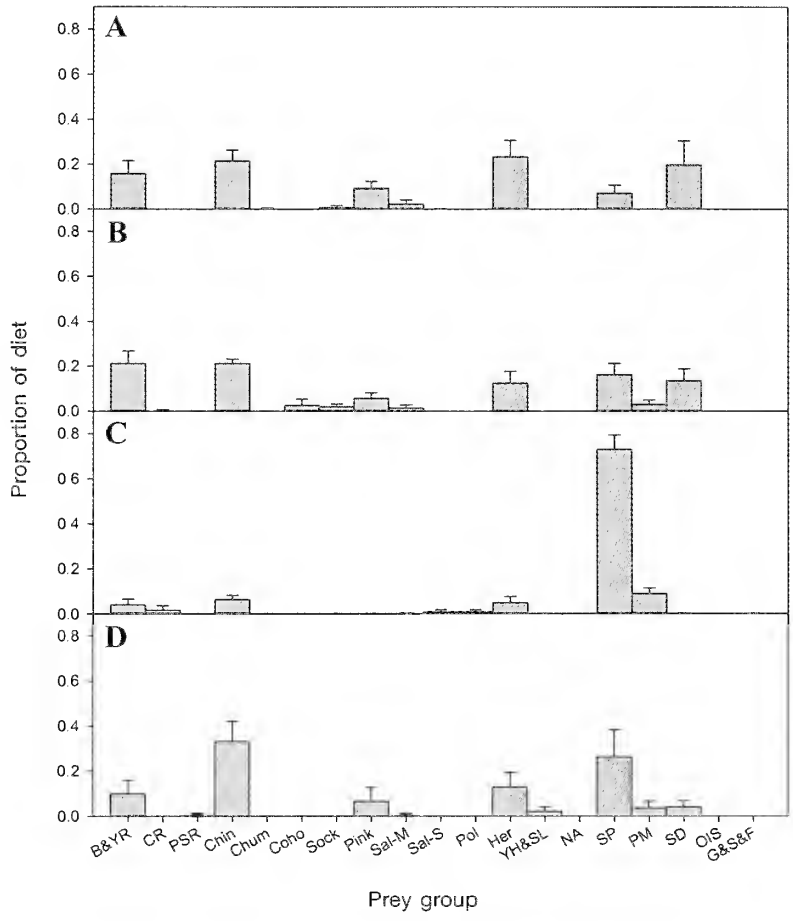

Figure 3

Estimates of mean diet composition for harbor seals (*Phoca vitulina*) in the Salish Sea, unadjusted for differential fat mass among prey classes, by sampling location: **(A)** Belle Chain Islets, **(B)** Bird Rocks, **(C)** Padilla Bay, and **(D)** Vendovi Island. Error bars are ±1 standard error of the estimate. Prey classes are defined as B&YR (Black [*Sebastes melanops*] and Yellowtail [*S. flavidus*] Rockfish), CR (Copper Rockfish [*S. caurinus*]), PSR (Puget Sound Rockfish [*S. emphaeus*]), Chin (mature Chinook Salmon [*Oncorhynchus tshawytscha*]), Chum (mature Chum Salmon [*O. keta*]), Coho (mature Coho Salmon [*O. kisutch*]), Sock (mature Sockeye Salmon [*O. nerka*]), Pink (mature Pink Salmon [*O. gorbuscha*]), Sal-M (medium-size Chinook and Coho Salmon), Sal-S (small Chinook, Chum, Sockeye, and Ppink Salmon), Pol (Walleye Pollock [*Theragra chalcogramma*]), Her (Pacific Herring [*Clupea pallasii*] at least 2 years old), YH&SL (Pacific Herring less than 2 years old and Pacific Sand Lance [*Ammodytes hexapterus*]), NA (Northern Anchovy [*Engraulis mordax*]), SP (Shiner Perch [*Cymatogaster aggregata*]), PM (Plainfin Midshipman [*Porichthys notatus*]), SD (Spiny Dogfish [*Squalus acanthias*]), OIS (Opalescent Inshore Squid [*Loligo opalescens*]), G&S&F (Kelp Greenling [*Hexagrammos decagrammus*], Pacific Staghorn Sculpin [*Leptocottus armatus*], and Starry Flounder [*Platichthys stellatus*]).

diet composition of 0.0% for Black and Yellowtail Rockfish, and estimates for the remaining 14 males ranged from 8.2% to 51.4% and averaged 31.8%. Although females were more consistent in their reliance on Shiner Perch, the estimated contribution of Black and Yellowtail Rockfish exceeded 25% for 3 individuals. There were no discernible patterns in the capture location or date with respect to the magnitude of rockfish estimates for either males or females, a result that is consistent with the nonsignificant interaction between location and gender in the linear model. One female seal was captured twice, at Padilla Bay in spring 2007 and at Vendovi Island in winter 2008. The diet composition of this female was estimated to be ~90% Shiner Perch and ~9% Chinook Salmon, with negligible contributions from other prey classes, on both occasions.

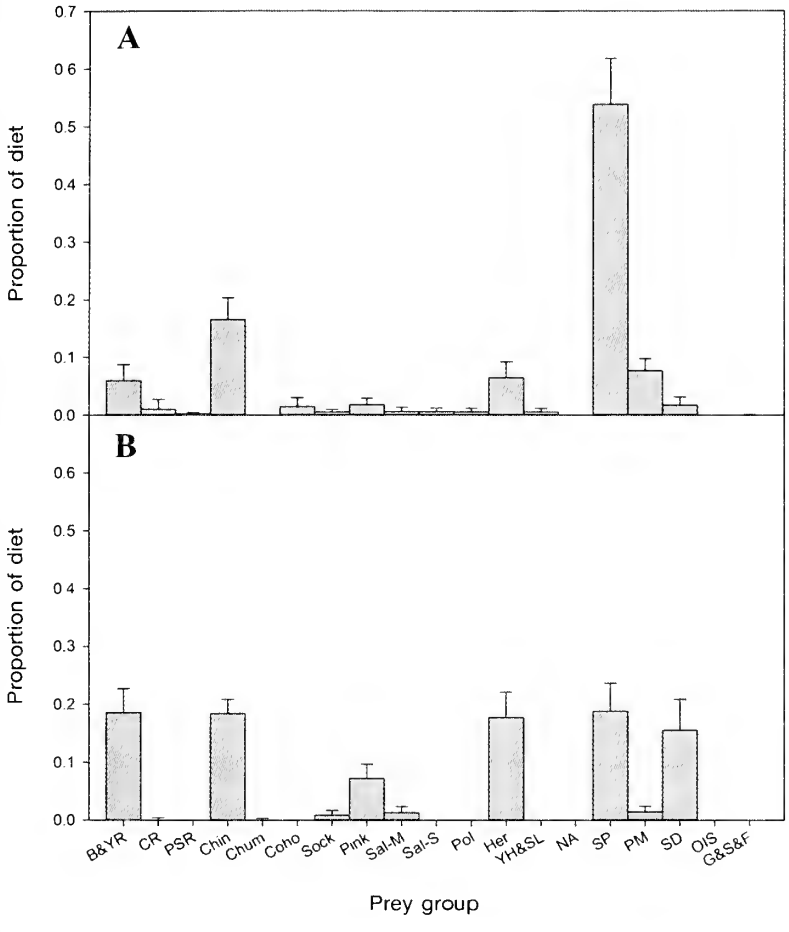

Figure 4

Mean diet composition estimates for harbor seals (*Phoca vitulina*) in the Salish Sea, unadjusted for differential fat mass among prey classes, by sex: **(A)** females and **(B)** males. Error bars are ±1 standard error of the estimate. Prey classes are defined as B&YR (Black [*Sebastes melanops*] and Yellowtail [*S. flavidus*] Rockfish), CR (Copper Rockfish [*S. caurinus*]), PSR (Puget Sound Rockfish [*S. emphaeus*]), Chin (mature Chinook Salmon [*Oncorhynchus tshawytscha*]), Chum (mature Chum Salmon [*O. keta*]), Coho (mature Coho Salmon [*O. kisutch*]), Sock (mature Sockeye Salmon [*O. nerka*]), Pink (mature Pink Salmon [*O. gorbuscha*]), Sal-M (medium-size Chinook and Coho Salmon), Sal-S (small Chinook, Chum, sockeye, and Pink Salmon), Pol (Walleye Pollock [*Theragra chalcogramma*]), Her (Pacific Herring [*Clupea pallasii*] at least 2 years old), YH&SL (Pacific Herring less than 2 years old and Pacific Sand Lance [*Ammodytes hexapterus*]), NA (Northern Anchovy [*Engraulis mordax*]), SP (Shiner Perch [*Cymatogaster aggregata*]), PM (Plainfin Midshipman [*Porichthys notatus*]), SD (Spiny Dogfish [*Squalus acanthias*]), OIS (Opalescent Inshore Squid [*Loligo opalescens*]), G&S&F (Kelp Greenling [*Hexagrammos decagrammus*], Pacific Staghorn Sculpin [*Leptocottus armatus*], and Starry Flounder [*Platichthys stellatus*]).

Discussion

Our findings re-affirm the importance of several commercially important fish species to harbor seal diets, particularly salmon species, Pacific Herring, and Shiner Perch, reported by prior investigators (Scheffer and Slipp, 1944; Everitt et al., 1981; Brown and Mate, 1983; Olesiuk, 1993; Zamon, 2001; Orr et al., 2004; Wright et al., 2007; Thomas et al., 2011; Lance et al., 2012). However, our results also reveal that rockfish species contribute more substantially to harbor seal diets than has been recognized previously, exceeding 10% of the average diet of all harbor seals combined. Given that QFASA estimates are thought to describe diets integrated over a period of weeks to months (Iverson et al., 2004; Budge et al., 2006), estimates of this magnitude may reflect substantial periodic (and, perhaps, sustained) predation on species of rockfish. Although quantitative estimates of rockfish abundance are unavailable, rockfish populations are considered depressed and, given the regional abundance of harbor seals (Jeffries et al., 2003), the predation rates indicated by these findings may be sufficiently high to influence their population dynamics, on a local or, perhaps, regional scale. Consequently, management plans to enhance rockfish abundance may need to give greater consideration to the potential influence of pinniped

predation. Additional research to verify and refine our estimates of diet composition, and to begin quantifying rockfish population dynamics and the influence of pinniped predation through incorporation of information on harbor seal consumption rates (Howard, 2009; Howard et al., 2013) is warranted.

Although rockfish species appear to constitute a more foundational prey resource for harbor seals than was recognized previously, harbor seal diets do not appear to be homogeneous, a finding that is consistent with the results of observational studies of predatory behavior (Suryan and Harvey, 1998; Tollit et al., 1998; London, 2006; Wright et al., 2007; Hardee, 2008; Thomas et al., 2011; Peterson et al., 2012). Substantial spatial heterogeneity in diet composition was detected among seals from the 4 sampling locations. For example, the mean diet of seals sampled near Padilla Bay was dominated by Shiner Perch, a common species in bays and estuaries throughout the west coast of North America (Hart, 1973). Seals sampled from the other locations, which are characterized by deeper and more open waters and greater rocky relief, tended to rely more on species of rockfish and salmon and Pacific Herring. Spatial patterns of habitat suitability undoubtedly underlie the relative abundance of prey in local areas—a dynamic that is subsequently reflected in seal diets. Heterogeneity among sexes also was observed; a more diverse diet and greater use of rockfish species and Spiny Dogfish were observed for male seals than for females. Sex-based heterogeneity in diet was not expected, given the slight sexual dimorphism in harbor seals, but it may reflect a number of factors, including intersexual competition for food resources, foraging behavior, predatory efficiency, and differences in reproductive investment. For example, reproductively active females tend to make shorter foraging trips during early lactation (Boness et al., 1994)—behavior that may reduce their access to some prey classes.

Although the sampling location and sex covariates explained primary patterns among estimates of seal diet composition, substantial unexplained heterogeneity was observed in the estimates. In particular, Black and Yellowtail Rockfish were among the most important prey species for a number of individual seals, especially males, but they were absent from the diets of other seals. Whether differences between individual seals could be explained by unmeasured covariates or are attributable to individual preference or specialization is unknown. In either case, this heterogeneity with respect to rockfish predation is an intriguing aspect of the results of this study.

Our estimates of mean diet composition are not thought to provide an accurate assessment of harbor seal diets on an annual basis. Most seals were sampled in the spring (Table 1), and no seals were sampled from late May through late October. One would expect season to be an important covariate that could explain differences in diets, especially given the large changes in the relative abundance of prey during the spring spawning migration of Pacific Herring and the summer availability of migrating adult salmon species (Stasko et al., 1976; Willson and Womble, 2006; Therriault et al., 2009; Thomas et al., 2011). We surmise that such temporal heterogeneity exists, but that evidence of these seasonally available prey species in harbor seal blubber was diminished by late October. The lack of summer seal samples may partially explain the difference between these results and assessments of harbor seal diet based on scats, in which salmon species and Pacific Herring are prevalent (Luxa, 2008; Lance et al., 2012). A complete assessment of seasonal variation in harbor seal diets would require a somewhat expanded investigation, in which the distribution of sampling effort would be designed to investigate potential changes in diet expected on the basis of seasonally predictable shifts in the availability of prey species. The expected deposition and turnover rates of fatty acid compounds in adipose tissue (Nordstrom et al., 2008) also would contribute importantly to an optimized sample design. On the basis of the results of this investigation, an expanded effort to more fully explore spatial, temporal, and demographic patterns in harbor seal diets likely would be successful.

Two estimates of mean diet composition, one unadjusted and one adjusted for differential fat mass of prey, were provided for all seals combined (Fig. 2). However, no adjustment for differential fat mass was made for the estimates stratified by location and sex. The large differences in fat composition among the prey classes (Table 2) and, to a lesser extent, the lack of total mass data for mature Chinook, Sockeye, and Pink Salmon, all of which have high fat content, somewhat reduce our confidence in the fat-adjusted estimates. The estimates unadjusted for differential fat mass are informative ecologically, providing information on the likely sources of adipose tissue ingested by harbor seals. Fat-adjusted estimates may be of greater interest from the perspective of prey population demographics because rescaling the estimates with mean fat per prey converts the units to the relative numbers (proportions) of prey animals consumed. Given an estimate of the number of fish consumed per unit of time, the fat-adjusted estimates would facilitate the investigation of predation rates by prey class.

Although QFASA is a powerful method for investigation of predator diets, it is important to recognize potential problems with its use. With respect to marine mammals, logistical constraints and permit requirements may limit sample sizes and preclude comprehensive investigations of free-ranging populations. From a statistical perspective, it is important to acknowledge that estimates of diet composition are conditioned on the calibration coefficients, the suitability of which in any particular application cannot be verified. In the instance of this investigation, the calibration coefficients were estimated during a controlled feeding study of captive harbor seals (Nordstrom et al., 2008), the species of interest. Even so, the degree to which the coef-

ficients are applicable to wild seals with a more diverse diet is unknown, and use of previously published coefficients is a potential source of bias. To conduct an independent feeding trial in association with every field investigation obviously is infeasible and therefore reliance on published calibration coefficients may be unavoidable. However, some investigators have noted that diet composition estimates are sensitive to the values of calibration coefficients (Meynier et al., 2010), and such sensitivity may also be the case for the suite of fatty acid compounds used in mixture modeling. Achievement of adequate sample sizes of all potential prey species, including representatives of the same species at various life history stages and seasons, such as immature and mature species of salmon, is obviously an important precursor to implementation of QFASA. Although such considerations do not negate the utility of QFASA as a tool to estimate diet composition, researchers need to be cognizant of these issues, and therefore the development of analytical procedures to assess sensitivity may be helpful.

Conclusions

Several fish stocks of historic commercial importance within the Salish Sea are considered to be depressed and their recovery is a high management priority. Whether abundant pinniped populations may be impeding management actions intended to stimulate recovery is an open question in this region. Our findings confirmed the importance of salmon species and Pacific Herring in harbor seal diets, but they also revealed that other species, including rockfish species, may contribute more substantially to harbor seal diets than had been realized previously. Although estimates of harbor seal diet composition varied spatially, demographically, and among individual seals, species of rockfish were estimated to compose a large proportion of the diets of several individual seals. These results, in combination with the current high abundance of harbor seals, indicate that predation may be an important ecological factor in the regulation of the local and regional abundance of rockfish populations—a possibility that warrants additional investigation.

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Fish consumption by harbor seals *(Phoca vitulina)* in the San Juan Islands, Washington

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Abstract—The harbor seal (*Phoca vitulina*) is a large-bodied and abundant predator in the Salish Sea ecosystem, and its population has recovered since the 1970s after passage of the Marine Mammal Protection Act and the cessation of bounties. Little is known about how this large predator population may affect the recovery of fish stocks in the Salish Sea, where candidate marine protected areas are being proposed. We used a bioenergetics model to calculate baseline consumption rates in the San Juan Islands, Washington. Salmonids (*Oncorhynchus* spp.) and herring (Clupeidae) were the 2 most energetically important prey groups for biomass consumed by harbor seals. Estimated consumption of salmonids was 783 (±380 standard deviation [SD]) metric tons (t) in the breeding season and 675 (±388 SD t in the nonbreeding season. Estimated consumption of herring was 646 (±303 SD) t in the breeding season and 2151 (±706 SD) t in the nonbreeding season. Rockfish, a depressed fish stock currently in need of population recovery, composed one of the minor prey groups consumed by harbor seals (84 [±26 SD] t in the nonbreeding season). The variables of seal body mass and proportion of prey in seal diet explained >80% of the total variation in model outputs. Prey groups, such as rockfish, that are targeted for recovery may still be affected by even low levels of predation. This study highlights the importance of salmonids and herring for the seal population and provides a framework for refining consumption estimates and their confidence intervals with future data.

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Overfishing and habitat change have affected fish populations heavily in the inland waters of the Pacific Northwest. Many formerly abundant fish species are now species of conservation concern, including groundfish stocks, such as rockfish species (*Sebastes* spp.) and Pacific Hake (*Merluccius productus*), forage fish stocks such as Pacific Herring (*Clupea pallasii*), and several salmonid species (*Oncorhynchus* spp.) (Musick et al., 2000; Mills and Rawson, 2004). Most recently, 3 rockfish species (*S. ruberrimus, S. pinniger, S. paucispinis*) were listed under the Endangered Species Act as threatened or endangered in Puget Sound, Washington State (Federal Register, 2010).

The decline of all these populations, which perform a critical function in regional food webs (Simenstad et al., 1979; Schindler et al., 2003) and have commercial and recreational value, has created a need for recovery strategies at the ecosystem level. Fish recovery efforts currently rely on traditional fisheries management approaches, such as reduction of fishing pressure and creation of no-take refuges or marine reserves, and on habitat restoration (Allison et al., 1998; Roni et al., 2002). Marine reserves in particular are more likely to be successful for species, such as rockfish, that have small home ranges and high site fidelity (Love et al., 2002), and reserves are important management tools for recovery of rockfish in the Pacific (Murray et al., 1999). More reserves have been proposed recently for the San Juan Islands,[1] an island group that is part of the Salish Sea marine ecosystem that spans U.S. and Canadian waters (Fig. 1). For pelagic species, such as salmonids and forage fishes, recovery efforts call for habitat protection and mitigation of water-pollution issues, among other factors, as management tools (Fluharty, 2000; Schindler et al., 2003).

The restoration of predators in marine ecosystems can reestablish trophic relations and restructure habi-

[1] McConnell, M. L., and P. A. Dinnel. 2002. Rocky reef bottomfish recovery in Skagit County. Phase II final report: assessment of eight potential marine reserve sites & final site recommendations. Skagit County Marine Resources Committee, Mount Vernon, WA, 43 p. [Available from http://www.nwstraits.org/Archives/Library.aspx.]

tat with usually positive results (Shears and Babcock, 2002; Shears et al., 2006); however, predators also can cause declines in the size distributions and abundance of prey species inside marine reserves (Sala and Zabala, 1996; Fanshawe et al., 2003). Large-bodied and abundant predators can contribute significantly to fish mortality, especially when prey species are already low in abundance, and may theoretically influence prey population recovery (Mohn and Bowen, 1996; Bundy, 2001; DeMaster et al., 2001; Fu et al., 2001; Trzcinski et al., 2006). Therefore, there is a need to understand the prey requirements of predators that consume fish species of conservation concern to evaluate if such requirements conflict with regional management goals.

In the Salish Sea, the harbor seal (*Phoca vitulina*) is an abundant, generalist marine predator whose population has steadily increased since gaining protected status in the 1970s. The harbor seal population in Washington State experienced logistic growth from the 1970s to the 1990s, increased 7- to 10-fold in size in different regions, and now appears to be at carrying capacity (Jeffries et al., 2003). Estimates of the regional population in the San Juan Islands and eastern bays in the early 1970s were approximately 1000 animals; currently, there are approximately 8000.[2] The age structure of the harbor seal population in British Columbia was documented in Bigg (1969), on the basis of seals collected and aged in the 1960s. After exponential population increases, this population was heavily weighted toward juvenile age classes by the 1980s (Olesiuk, 1993). Given the population increase in all regions of the Salish Sea, the current age structure of the harbor seal population in the San Juan Islands is unknown.

As with other harbor seal populations in the eastern Pacific, harbor seals in the San Juan Islands take advantage of the large influx of adult salmonids in late summer and fall and increase the diversity of their diet at other times of the year when salmonids are less available (Hauser et al., 2008; Lance et al., 2012). Salmonids, Pacific Herring, Pacific Sand Lance (*Ammodytes hexapterus*), Northern Anchovy (*Engraulis mordax*), Walleye Pollock (*Theragra chalcogramma*), and estuarine species, such as Shiner Perch (*Cymatogaster aggregata*), also form significant proportions of their diet in the San Juan Islands and nearby estuarine ecosystems (Lance et al., 2012).

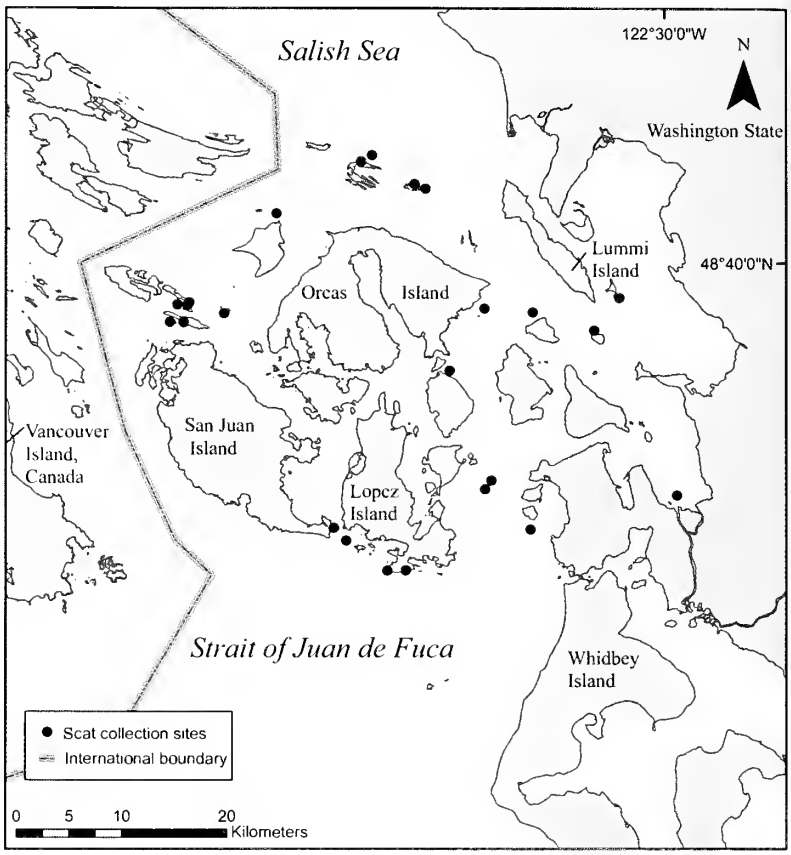

Figure 1

Map of the study area, the San Juan Islands and eastern bays, where seal scat collections were made for a bioenergetics model to examine the quantity of fish consumption by the harbor seal (*Phoca vitulina*) population during 2007–08. Black circles indicate harbor seal scat collection sites.

To calculate population-level consumption of fish species of conservation concern and other common harbor seal prey in the San Juan Islands, a bioenergetics model was used to determine energetic requirements. The model incorporated seasonal changes in seal diet and life history parameters during breeding and nonbreeding seasons. We also used simulated data and sensitivity analyses to address uncertainty in the overall model and in 2 specific components that may have a strong influence on predicted consumption of prey: 1) uncertainty in age structure of the harbor seal population and 2) seasonal changes in energy intake (e.g., fasting during breeding season).

Methods

Area and timeframe of study

The region of the San Juan Islands and eastern bays is an area where many fish species of conservation concern occur and also an area where the majority of the harbor seal population resides in the inland waters

[2] Washington Department of Fish & Wildlife. Unpubl. data. Washington Department of Fish & Wildlife, 7801 Phillips Road SW, Lakewood, WA 98498.

of Washington State. The San Juan Islands (48°35′N, 122°55′W) are characterized by tidally influenced rocky reefs and isolated rocks surrounded by deep water where harbor seals often congregate at haul-outs (locations where seals come ashore). The adjacent eastern bays, in contrast, consist of large, soft-bottomed, shallow bays (48°33′N, 122°30′W) (Fig. 1).

The consumption model was constructed for a single annual cycle for the harbor seal population during 2007–08. The model included 2 seasons: breeding (15 June–15 September) and nonbreeding (16 September–14 June) determined on the basis of seal pupping phenology in the San Juan Islands (Huber et al., 2001; Patterson and Acevedo-Gutiérrez, 2008). The 2 seasons were delineated to reflect known behavioral shifts (more time spent ashore to nurse pups, shallow-water breeding displays by males) related to pupping and breeding activities and subsequent changes in energetic expenditures (Coltman et al., 1998; Bowen et al., 1999).

The model was programmed in R software, vers. 2.7.1 (R Development Core Team, 2008) and used regional activity, abundance, and diet data, as well as physiological data from the literature. Model parameters were grouped into 3 categories: bioenergetics, population, and diet (Lavigne et al., 1982; Winship et al., 2002) (Table 1).

Model structure

Bioenergetics Energetic requirements were calculated with a bioenergetics approach that described the energy budget of an individual seal, which is a function of body size, activity budgets, growth, and reproductive costs. Sex- and age-specific gross energy requirements were calculated with Equation 5 in Boyd (2002):

$$EG_i = \frac{\left[\sum_{f=1}^{f=n_{\text{activities}}} \left(\gamma_f q_{f,i}\right) 86400\right] + g_i}{\sum^{d_i}}, \quad (1)$$

where EG_i = energy requirements in a particular stage i of the annual cycle;
γ_f = the power (watts) generated under activity f within stage i of the annual cycle;
$q_{f,i}$ = proportion of time spent in activity f;
g_i = the cost of growth in stage i of the annual cycle; and
d_i = the digestive efficiencies of food being eaten.

The model had 6 sex-and-age classes: 1) adult females (>6 years), 2) adult males (>8 years), 3) subadult females (1–6 years), 4) subadult males (1–8 years), 5) female pups (<1 year), and 6) male pups (<1 year). The subadult to adult division was made at the age(s) harbor seals reach their predicted maximum weight (approximately 66 kg and 89 kg for females and males, respectively) on the basis of the growth curve in Olesiuk (1993). Daily growth increments for each sex-and-age class were calculated from the same growth curve. Activity budgets were estimated from free-living harbor seals tagged with data recorders that recorded 3 behavioral periods: haul-out, diving, and shallow-water activity (Table 1).

Population abundance and age structure Aerial population surveys of harbor seals have been conducted annually by the Washington Department of Fish & Wildlife with fixed-wing aircraft to estimate the number of animals hauled-out during the lowest tide of the day since 1978 (Jeffries et al., 2003). Results from these surveys were used to estimate the abundance of harbor seals in the study area in 2007–08. The breeding season (July) correction factor of 1.53 (to account for seals not hauled-out at the time of the survey) was used to estimate the size of the breeding season population (Huber et al., 2001). Age-dependent mortality rates in Olesiuk (1993) were used to estimate the age structure (number of seals in each sex and age class) of the harbor seal population:

$$N_{s(x+t)} = N_{s(x)} e^{-rt}, \quad (2)$$

where $N_{S(x)}$ = number of seals in sex class S and age class x;
$-r$ = the age-dependent mortality rate; and
t = time interval between age classes.

The breeding season population vector was adjusted by iteration to sum to the total population estimate from aerial surveys. Seal abundance in the nonbreeding season was calculated by estimating the numbers still alive in each sex and age class, by using the same age-dependent mortality rates calculated per day (instead of annually) and by multiplying the number of days in the breeding cycle.

Population energetic requirements were calculated by multiplying individual requirements by the population abundance vectors to estimate energetic requirements for each sex and age class. Reproductive costs were then calculated for the entire population on the basis of values from the literature for gestation and lactation costs and fertility rates (Bigg, 1969; Olesiuk, 1993).

Digestive efficiency Data from the literature were used to translate net energy requirements of the harbor seal population into gross energy requirements and prey consumption by first taking into account assimilation efficiency and the heat increment of feeding (the increase in metabolism or heat produced during digestion) for harbor seals. We used the minimum and maximum values reported in the literature to account for differences in digestive efficiencies related to protein and fat content of prey (Markussen et al., 1994; Trumble et al., 2003).

Table 1

Data sets used in the consumption model in a study of the harbor seal (*Phoca vitulina*) population in the San Juan Islands and eastern bays during breeding and nonbreeding seasons, 2007–08. Model parameter symbols refer to Equations 1–3 in text. All energy units were converted to watts. H=haul-out; d=dive; s=surface. NA= not applicable.

Parameter category	Model parameter	Source[1]—data set	Probability distribution	Equation and value			
Bioenergetics	γ_b	3,15—basal metabolism	–	$\gamma_b = 1.93\ w^{0.87}$			
	γ_d γ_s	2,18—active metabolism (dive[2], surface)	–	$\gamma_d = 0.016\ w^{0.76}$ $\gamma_s = 1.9 * 3.39\ w^{0.76}$			
				Breeding season		Nonbreeding season	
	q	12,20—activity budgets (%time) h=haul-out d=dive s=surface	uniform	Females	h 10.6–32.5 d 14.9–63.6 s 100.0 – sum(h+d)	Females	h 13.6–20.6 d 46.1–81.0 s 100.0 – sum(h+d)
				Males	h 9.3–22.0 d 17.1–78.3 s 100.0 – sum(h+d)	Males	h 21.3–33.1 d 51.0–72.7 s 100.0 – sum(h+d)
				Pups	h 57.9–62.5 d 3.4–7.4 s 100.0 – sum(h+d)		
	w	1,7—seal mass at sex and age	lognormal	Female: 24–66 kg Male: 24–89 kg			
	g	4–5, 7—daily growth	gamma	0.015–0.018 kg d^{-1} * 321			
		6—cost of reproduction: lactation	–	$N_{S(x)} * f * 24.2$ MJ $* w^{0.75}$			
		6—cost of reproduction: gestation	–	93 MJ pup^{-1}			
Population	$N_{S(x)}$	19—abundance	uniform	Basic age structure: (no. of seals) Adult female (1188–1338) Adult male (271–314) Subadult female (2307–2892) Subadult male (2461–3346) Pup female (757–817) Pup male (757–817)			
	f	1,7—fertility rates	triangular	0–91% of age class			
	$-r$	1,7—mortality rates to estimate age structure	–	–0.17 to –0.29 y^{-1}			
	t	Number of days in breeding season	–	93 d			
	d_a	17—assimilation efficiency	uniform	87.4–93.2%			
	d_h	8—heat increment of feeding	uniform	1.8–13.7%			
Diet		9–11,13–14,16—energetic density of prey	uniform	2700–11,000 j g^{-1}			
		Biomass reconstruction of proportion of prey in diet	uniform	Breeding season (% of biomass) Salmonids 10.0–80.0 Herring 20.0–80.0 Walleye Pollock 1.0–3.0 Shiner Perch 1.0–2.0 Rockfish NA		Nonbreeding season (% of biomass) Salmonids 1.0–25.0 Herring 60.0–70.0 Walleye Pollock 1.0–2.0 Shiner Perch 1.0–2.0 Rockfish 1.0–2.0	

[1] (1: Bigg, 1969; 2: Kleiber, 1975; 3: Lavigne et al., 1986; 4: Innes et al., 1987; 5: Markussen et al., 1990; 6: Bowen et al., 1992; 7: Olesiuk, 1993; 8: Markussen et al., 1994; 9: Perez, 1994; 10: Van Pelt et al., 1997; 11: Paul et al., 1998; 12: Bowen et al., 1999;13: Payne et al., 1999; 14: Anthony et al., 2000; 15: Hoelzel, 2002; 16: Roby et al., 2003; 17: Trumble et al., 2003; 18: Sparling and Fedak, 2004; 19: Hardee, 2008; 20: Howard, 2009).

[2] Converted from mL O^2 consumed min^{-1} to watts by using a conversion factor of 20.1.

Diet

Collection of scat samples Scat samples were collected at 23 sites that represented regional variation in habitat in the San Juan Islands from 2005 to 2008 as part of a larger harbor seal diet study conducted in the northern Puget Sound (Fig. 1) (Lance et al., 2012). Samples collected during seal breeding and nonbreeding seasons in 2007–08 were used in our study. Detailed scat sample processing, collection information, and analysis of frequency occurrence of prey items in harbor seal diet are summarized in Lance et al. (2012). Briefly, samples for the diet study were collected from harbor seal haul-out locations during daytime low tides, placed in plastic bags, and then frozen until they were processed. Scat samples were processed following Lance et al.[3] and Orr et al. (2003). Otoliths were measured and graded according to the methods of Tollit et al. (2007). On otoliths that were graded as good (no or minimal erosion) and fair (small amount of erosion), the width and length were measured with an ocular micrometer. For our study, scat samples were pooled by seal breeding and nonbreeding seasons for further analyses.

Reconstruction of wet biomass To choose appropriate input values for diet in the model, a wet biomass reconstruction technique (Laake et al., 2002) was used to estimate the proportion by wet weight of prey items in harbor seal diet. This technique focuses on energetic content of seal diet, rather than on frequency of items in diet, by accounting for the number and size of prey consumed in a diet sample. The proportion of wet biomass of a prey item (π_i) in harbor seal diet was calculated by (Laake et al., 2002):

$$\pi_i = \frac{n_i w_i}{\sum_{i=1}^{w} n_i w_i}, \quad (3)$$

where n_i = the corrected number of items of prey item i; and
w_i = the average weight (in grams) of all prey items i.

The corrected number of "items" (n_i, number of individuals in the sample) was calculated by applying a species-specific (or closest proxy) correction factor to account for otolith loss during digestion. We used otoliths to enumerate all species except Shiner Perch, for which we used the number of pharyngeal plates to derive a more reliable passage rate. We lacked otolith-loss correction factors for herring (Clupeidae) and Walleye Pollock; therefore, we considered the correction factors for Pacific Sardine (*Sardinops sagax*) and Pacific Hake in Phillips and Harvey (2009), respectively, to be reasonable proxies because these species are similar in size and structure (M. M. Lance, personal commun.). We used a Pink Salmon (*Oncorhynchus gorbuscha*) otolith-loss correction factor for all salmonids, a Shortbelly Rockfish (*Sebastes jordani*) correction factor for all rockfish species, and species-specific correction factors for Shiner Perch and Pacific Staghorn Sculpin (*Leptocottus armatus*) (Harvey, 1989; Phillips and Harvey, 2009).

Length correction factors were applied to measurements from otoliths scored as being in good or fair condition to account for otolith erosion during digestion. Corrected otolith lengths then were used to calculate the fish size with species-specific length-weight regressions (Harvey et al., 2000). When we lacked species-specific correction factors or length-weight regressions, we used estimated body sizes of prey items.

Otoliths of juvenile and adult salmonids were distinguished on the basis of otolith and bone sizes. Otoliths that were graded in good enough condition to measure and reconstruct salmonid size were uncommon in scat samples; therefore, for salmonid adults that were not identified to species, we used an approximate average size (1589 g) for Pink Salmon, the species most commonly consumed by harbor seals (Lance et al., 2012). An average estimated size of 35 g was used for all salmonid juveniles. We also lacked otolith-length correction factors for herring and Walleye Pollock; therefore, we used Pacific Sardine and Pacific Hake as proxies. The remaining length correction factors that we used were a Shortbelly Rockfish correction factor for all rockfish species, and species-specific correction factors for Shiner Perch and Pacific Staghorn Sculpin.

It should be noted that reconstruction was not possible for all species in the diet samples because of the diversity of harbor seal diet and lack of appropriate correction factors as noted previously and in Table 2. Given the complexity of harbor seal diet and lack of reconstruction techniques for several species, we reconstructed the proportion in the sample only for prey species of conservation concern or for prey species whose frequency of occurrence was ≥5.0 in the broader study of harbor seal diet (Lance et al., 2012). Our goal was to set a reasonable range of values for model input in addition to describing diet composition; therefore, we make here a distinction between diet sample results and the parameter values used in the model to calculate consumption. When there was great uncertainty in percent contribution by wet weight to harbor seal diet because of the use of proxy correction factors or omission of some species from biomass reconstruction, confidence intervals were increased (see *Model uncertainty and parameter estimation* section).

Consumption rates

We calculated consumption (as biomass) for 5 key prey species or groups that are species of conservation concern or most common in harbor seal diet: her-

[3] Lance, M. M., Orr A. J., Riemer S. D., Weise M. J., and Laake J. L. 2001. Pinniped food habits and prey identification techniques protocol. AFSC Processed Report 2001-04, 41 p. Alaska Fisheries Science Center, Seattle, WA. [Available from http://access.afsc.noaa.gov/pubs/search.cfm.]

Table 2

Wet biomass construction results for the most common (frequency of occurrence ≥5.0) prey species or groups in diet of harbor seals (*Phoca vitulina*) during breeding and nonbreeding seasons, 2007–08.[1] All prey with frequency of occurrence ≥5.0 are listed to illustrate which common species or groups were not reconstructed because of data limitations; rockfish species were not common but were included because of their conservation status. NA=no prey size reconstruction equation was available or prey were not identified to species level; NP=not present in samples; est.=estimated size when no measurable otoliths were present or prey not identified to species level.

		Biomass reconstruction estimates for prey of breeding seals			Biomass reconstruction estimates for prey of nonbreeding seals		
Prey family or group	Prey species	No. of otoliths measured (*n*)	Avg. reconstructed weight (g)	Avg.[1] wet biomass (% range)	No. of otoliths measured (*n*)	Avg. reconstructed weight (g)	Avg. wet biomass (% range)
Gadidae	Unidentified gadid species	NA	NA	NA	NA	NA	NA
	Walleye Pollock	52	57.0	2.77 (0.05–5.32)	27	18.3	0.29 (0.04–0.78)
Clupeidae	Herring[2]	136	63.0	79.70 (60.45–98.95)	188	80.0	81.82 (61.40–97.38)
	American Shad (*Alosa sapidissima*)	NA	NA	NA	NA	NA	NA
Salmonidae	Juvenile Chinook Salmon	0	est. 35.0	0.17 (0.15–0.19)	0	est. 35.0	0.16 (0.00–0.47)
	Juvenile salmon species	0	est. 35.0	0.20 (0.11–0.29)	0	est. 35.0	0.19 (0.00–0.68)
	Adult Chinook Salmon	NP	NP	NP	0	est. 10000.0	1.49 (0.00–7.42)
	Adult salmon species	0	est. 1589.0	14.74 (0.00–29.47)	0	est. 1589.0	6.80 (0.00–23.60)
Cottidae	Pacific Staghorn Sculpin	12	257.0	1.77 (0.00–3.55)	44	188.6	1.45 (0.00–20.63)
Ammodytidae	Pacific Sand Lance[3]	38	4.5	0.25 (0.05–0.50)	76	5.8	1.52 (0.24–5.35)
Embiotocidae	Shiner Perch	11	38.6	0.50 (0.06–0.90)	29	28.0	2.32 (0.09–6.56)
Rajidae	Skate species	NA	NA	NA	NA	NA	NA
Scorpaenidae	Juvenile rockfish species	2	189.6	0.39 (0.00–0.80)	0	NA	NA
	Adult rockfish species	NP	NP	NP	0	NA	NA

[1]Average and ranges reported are between sampling months.
[2]Includes all unidentified clupeids.
[3]No otolith length or otolith loss correction factor was available; these estimates should be treated with caution.

ring, salmonids, rockfish, Walleye Pollock, and Shiner Perch. Gross energy requirements were translated to consumption rates by applying the energetic density of prey to the proportion by wet weight of prey items in seal diet (Perez, 1994; Van Pelt et al., 1997; Paul et al., 1998; Payne et al., 1999; Anthony et al., 2000; Roby et al., 2003). After biomass reconstruction, all species of adult and juvenile salmonids were combined into a "salmonid" complex. A "herring" complex represented *Clupea pallasii* and unidentified clupeid species. There are 2 other clupeid species in the study area, but, because of their rareness, we assumed most species were *C. pallasii* (M.M. Lance, personal commun.). When prey were placed into broader taxonomic groups, we used the minimum and maximum values for energetic density reported for all prey sizes and ages in the literature to represent the prey group.

Model uncertainty and parameter estimation

Model variables described in Table 1 were randomly chosen during 1000 simulations from probability distributions to estimate uncertainty in all model outputs. Where estimation of distribution parameters was not straightforward (e.g., lognormal), a maximum likelihood technique with the MASS package in R was used; this technique estimates the joint likelihood for distribution parameter values, given the seal body mass values for each sex-and-age class (Venables and Ripley, 2002). We also made the following changes to diet results to adjust the uniform distribution parameters for percentage by wet weight of prey in diet. If we had set the minimum and maximum values for a uniform distribution for proportion in diet exactly as found in diet samples, it would have been uninformative (i.e., a range of 0–100 often occurred but would imply no prior knowledge of diet composition; Table 2). Therefore, zero values from diet samples were discarded and minimum values for herring and salmonids were set as calculated from the remaining diet samples. For Shiner Perch and Walleye Pollock, zero values also were discarded. The minimum possible value was assumed to be 1%, and the maximum value was set near the average calculated from diet samples. Harbor seal diet is diverse; therefore at least 20–30% of harbor seal diet was assumed to be made up of other species, and the maximum value possible for any prey species was set at 70–80% (the maximum value for nonbreeding season was set slightly lower because of increased diversity of diet). All model outputs are reported as means (±standard deviation).

Sensitivity analyses also were used to identify parameters with the most influence on model outputs by systematically allowing one parameter at a time to be chosen randomly while other variables were fixed at their mean value(s). In this manner, any variation in the model outputs should be the direct result of variation in the parameter of interest (Shelton et al., 1997; Stenson et al., 1997; Winship et al., 2002). The percentage of variance explained by a single variable was calculated as the variance of model outputs when single random variables were used and divided by the total variance when all variables were randomly chosen.

To estimate the effect of age structure on total prey consumption, we used different ratios of adults to subadults in 3 alternate model scenarios. We increased the number of adults in the population by 25%, 50%, and 100% and kept the total population size stable.

During the breeding season, adult harbor seals fast or reduce consumption (Bowen et al., 1992; Coltman et al., 1998); therefore, there may be a discrepancy between predicted energy requirements and timing of consumption during an annual cycle. Rather than use direct consumption, we addressed the effect of this discrepancy with a correction factor that accounted for energy obtained from burning body fat stores in the breeding season. We estimated the amount of energy consumed, stored as body fat, and later metabolized by adult seals with the same estimates of digestive efficiency and energy density of prey that were used in the overall consumption model.

Results

Fish consumption

There were 196 and 361 scat samples collected during the breeding and nonbreeding seasons, respectively. In these samples, 23 and 29 prey taxa were identified during the breeding and nonbreeding seasons. Ten prey taxa were selected for reconstruction in this study; they had a frequency of occurrence ≥5.0 in the broader harbor seal diet study (Lance et al., 2012) or were species of conservation concern. Of these 10 taxa, 3 prey groups (unidentified gadid, skate species, and American Shad [*Alosa sapidissima*]) could not be used because we had insufficient methods (e.g., lack of correction factors) to reconstruct their presence in seal diet. Of the remaining prey, herring comprised the vast majority of reconstructed samples: ≥80% of wet weight in both breeding and nonbreeding seasons. Salmonids composed 15% and 9% in the breeding and nonbreeding seasons, respectively (Table 2). We were not able to identify rockfish otoliths to species in either season. In the breeding season, rockfish frequency of occurrence was 0.5% and therefore was assumed to contribute little in energetic terms to diet and was not further considered for calculation of consumption rates. Measurable otoliths were not found for rockfish species in the nonbreeding season; therefore, we were unable to determine species or size. During the nonbreeding season, rockfish frequency of occurrence was 1.4% (Lance et al., 2012); we set a hypothetical range for proportion of wet weight of rockfish in diet at 1.0–2.0%. Walleye Pollock and Shiner Perch constituted a relatively minor portion (averages 0.5–2.8%) of reconstructed diet (Table 2).

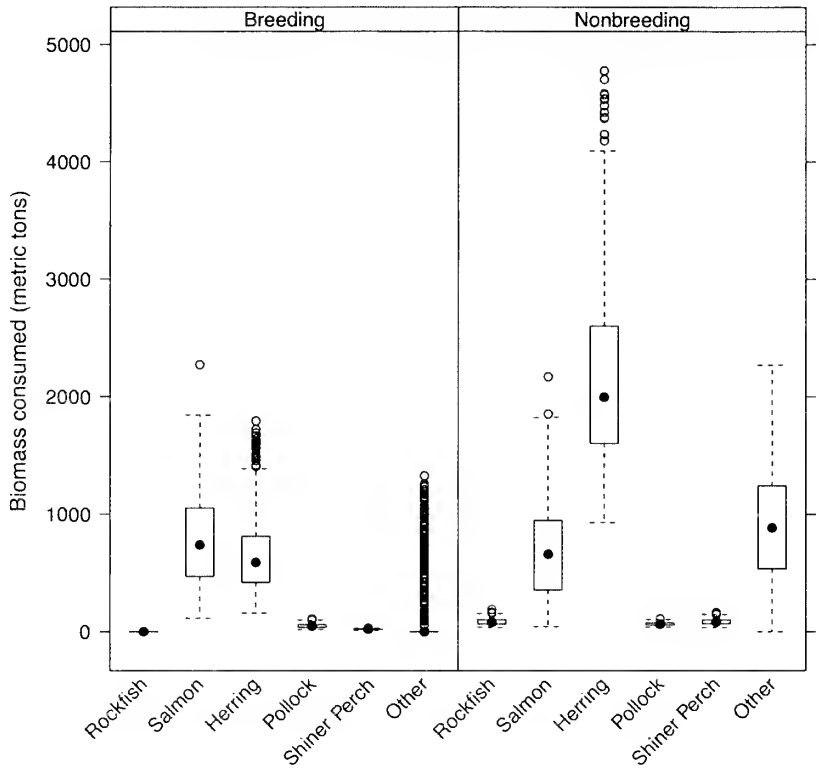

Figure 2

Estimates of consumption of prey species, relative to season (breeding or nonbreeding), for the harbor seal (*Phoca vitulina*) population in the San Juan Islands and eastern bays during 2007–08. "Other" category represents remaining prey in seal diet. Solid circles indicate medians, boxes enclose the interquartiles, vertical dashed lines represent 1.5× the interquartile range, and open circles indicate outliers.

During the seal breeding season, the average consumption for prey species calculated over 1000 simulations was 783 (±380) metric tons (t) of salmonids, 646 (±303) t of herring, 50 (±17) t of Walleye Pollock, and 22 (±4) t of Shiner Perch (Fig. 2). Subadult seals of both sexes consumed the greatest proportion of the total biomass (approximately 30–40% each), followed by adult females (27%). Adult males consumed a relatively small proportion of total biomass compared with adult females and subadults, and their consumption was only slightly higher than the biomass consumed by pups of both sexes (each <10%).

During the nonbreeding season, consumption of herring and salmonids had the widest range of values; rockfish, Shiner Perch, and Walleye Pollock were less variable. The average consumption for prey species calculated over 1000 simulations was 84 (±26) t of rockfish, 675 (±388) t of salmonids, 2151 (±706) t of herring, 66 (±13) t of Walleye Pollock, and 86 (±22) t of Shiner Perch (Fig. 2).

The per capita fish consumption rate predicted by the model was 2.1 kg day^{-1} seal^{-1} (annual average 2.9, 2.8, 2.0, 2.2, and 1.0 kg for adult females, adult males, subadult females, subadult males, and pups, respectively). As was evident during the breeding season, subadults (which included pups from the previous breeding season) of both sexes consumed the greatest proportion of the total biomass (approximately 30–45% each), followed by adult females (19%). Adult female consumption dropped slightly in the nonbreeding season. Adult males consumed the smallest proportion in the population (5%).

Sensitivity analyses and assessment of model uncertainty

Variation in seal body mass had the largest effect on energy use of the population and accounted for >80% of model variance in both seasons. Taken together, all bioenergetics variables (mass, growth rates, and activity) accounted for the majority of the variance in the simulation model. Fertility rates accounted for the next-greatest variance (7.3%) after body mass during the breeding season while population size contributed least (1.3%) to overall model variability (Fig. 3).

Consumption estimates of salmonids and herring were most sensitive to estimates of proportion of prey in the diet and energy density of prey. Variation in consumption estimates was low when the heat increment of feeding and assimilation efficiency parameters were varied within their estimated ranges. The variance in the nonbreeding season seen in the overall simulation model for both salmonids and herring was not well explained by any single prey variable (Fig. 4).

We estimated that adult seals used approximately 1,100,000 MJ of fat stores during the breeding season. Assuming an average prey energy density of 4000 J g^{-1}, this use of energy was equivalent to consumption of 300 t or approximately 6% and 21% of annual and breeding-season energy use, respectively. Increasing the number of adult seals in the population led to a positive increase in population energy use, although at a relatively slow rate of increase: even when we doubled the number of adults in the population, energy use increased only by 7% (Fig. 5).

Discussion

The prey consumption model was quite sensitive to body mass: when body mass was varied ±10% around the average, there was a corresponding ±10% change in the energy use outcome. Body mass controls many physiological functions in organisms, and because mass-based predictive relationships were used for metabolic rate, the sensitivity of the model to body mass was not entirely unexpected. By simply accounting for body size and number of harbor seals, the model captured the bulk of energy use in the population. In fact, omission of reproduction costs (lactation and gestation costs) did not affect estimates of nonbreeding season energy use and lowered breeding season estimates by approximately 10%.

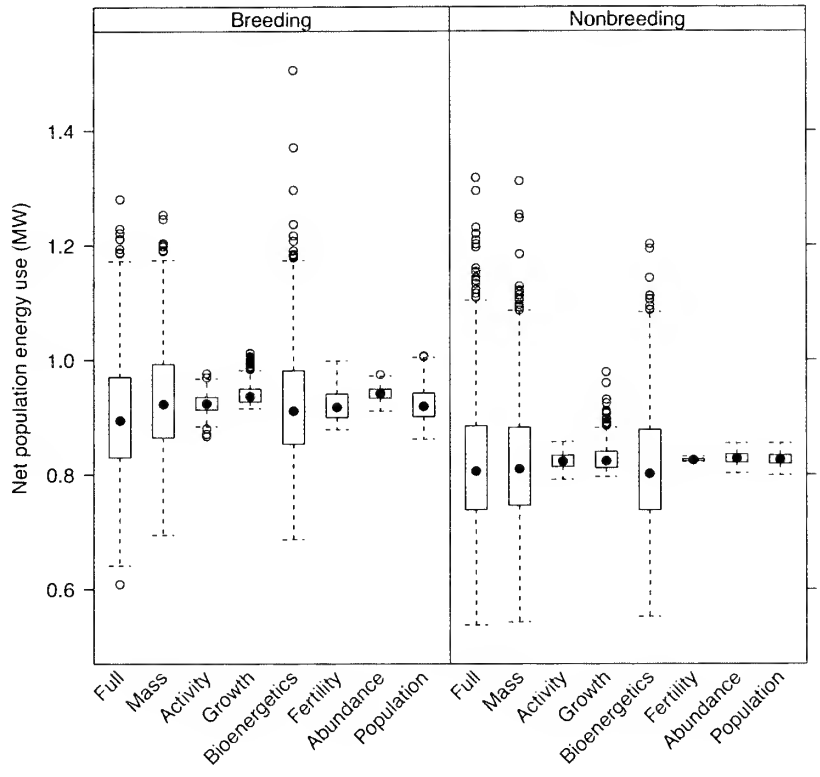

Figure 3

Effect of bioenergetics and population variables, relative to season (breeding or nonbreeding), on net population energy use (in megawatts) of harbor seals (*Phoca vitulina*) in the San Juan Islands and eastern bays during 2007–08. Distribution of model outputs after running 1000 simulations with all variables ("Full"), single (individual variables), or "groups of variables" ("Bioenergetics" [mass, activity, and growth rates]" or "Population" [fertility and abundance]) selected randomly. Solid circles indicate medians, boxes enclose the interquartiles, vertical dashed lines represent 1.5× the interquartile range, and open circles indicate outliers.

Predicted per capita fish consumption of 2.1 kg day^{-1} $seal^{-1}$ fell within the range estimated for the harbor seal populations in British Columbia, Canada, and Norway: 1.9 kg and 4 kg, respectively (Härkönen and Heide-Jørgensen, 1991; Olesiuk, 1993; Bjørge et al., 2002). Despite their large body size, adult males were the least numerous sex-and-age class in the population—information that explained their low proportion of total population consumption when the population was considered as a unit. Consumption was for the most part proportional to the biomass of the total seal population; therefore, any change in total population size would correspond to a roughly equal percent change in estimated consumption. With this prediction, all other model variables were assumed to be similar among years, and this assumption seems reasonable given that the total population size has stabilized during the last decade[2] (Jeffries et al., 2003). Nevertheless, at dramatically different population sizes, there may be different behavioral or population changes that would need to be taken into account (e.g., individual prey preferences, intraspecific competition, fertility rates, and mortality rates) to predict population consumption.

In contrast to the other population variables, only point estimates were used for mortality rates. The age structure of the harbor seal population used in the basic consumption model was heavily dominated by subadults, and the population structure was based on data from a time period when the harbor seal population was depressed. However, changing the age structure in our alternative model (see Appendix) caused relatively minor changes in the energy budget, especially compared with the sensitivity of the model to body mass. If the increase in population size since the 1970s has led to decreased juvenile survival rates, as is predicted to be the case for marine mammals (Fowler, 1981; Hiby and Harwood, 1985), and adult seals are now more dominant in the population, overall consumption rates still should be similar to those that we predicted, at least at the adult to subadult ratios that were tested in alternate model versions.

For species, such as harbor seals, that use fat stores during fasting periods, inferring consumption directly from energetic requirements may be somewhat misleading. Harbor seals fast or reduce feeding rates for 2–6 weeks and can lose up to 33% of body mass during the breeding season (Bowen et al., 1992; Coltman et

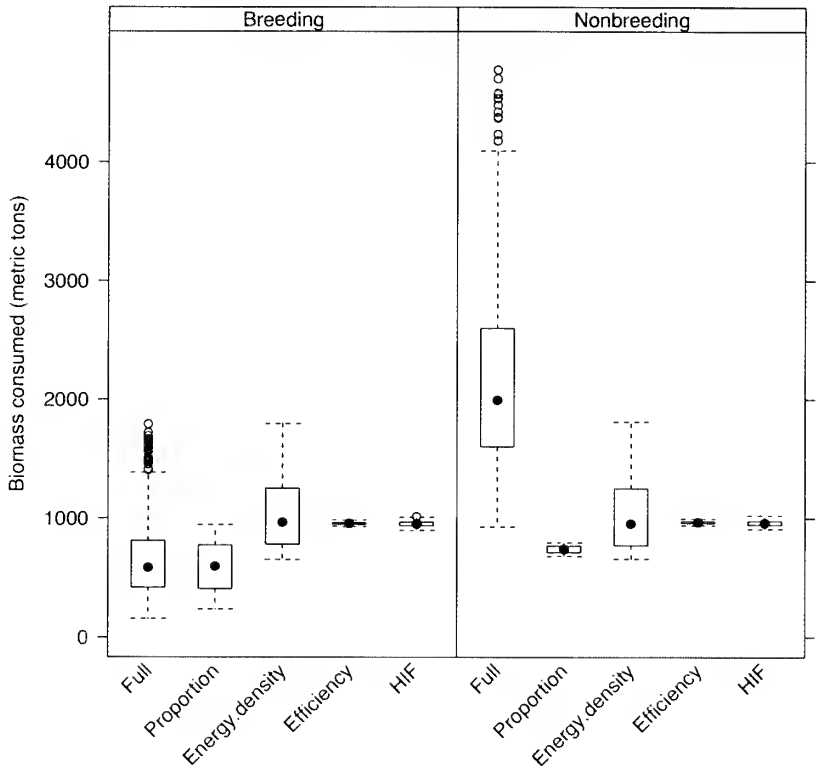

Figure 4

Effect of prey variables on herring consumption of harbor seals (*Phoca vitulina*) relative to season (breeding or nonbreeding), in the San Juan Islands and eastern bays during 2007–08. Distribution of model outputs after running 1000 simulations with all ("Full") or single variables selected randomly. Proportion=percent of total biomass in seal diet composed of herring (%). Energy density=energy contained in prey items (J g^{-1}). Efficiency=percent of gross energy available in prey item that is metabolizable (%). HIF=heat increment of feeding (%). Solid circles indicate medians, boxes enclose the interquartiles, vertical dashed lines represent 1.5× the interquartile range, and open circles indicate outliers. All simulations allowed variance in seal energetic requirements.

al., 1998). Pinnipeds increase feeding rates either immediately after the breeding season or before the next breeding season to regain fat stores (Beck et al., 2003). In addition, there are seasonal changes in energy intake that occur in harbor seals and other pinnipeds (Schusterman and Gentry, 1971; Rosen and Renouf, 1998). We addressed this discrepancy in timing of predicted energetic requirements and feeding through assessment of how much prey may be consumed by adult seals in the winter and spring and later used as fat stores. We found the amount to be a minor proportion of annual consumption but a more significant portion of the breeding season estimates. Therefore, the effect of consumption in the breeding season may be reduced, and consumption during the winter may be higher than we predicted.

Bioenergetic variables (especially body mass) contributed most to sensitivity in calculations of energy requirements in this study. Other pinniped consumption models similarly have identified body mass and body-mass predicted energetic requirements as a significant source of model variation (Mecenero et al., 2006; Chassot et al., 2009). When the full consumption model was examined, the assumed proportion of each prey species in the diet had the largest effect on consumption outputs—a result that was also similar to other pinniped consumption models (Mohn and Bowen, 1996; Shelton et al., 1997; Mecenero et al., 2006; Overholtz and Link, 2007), suggesting that future effort should be focused on refining the contribution of different prey to harbor seal diet. Genetic and molecular techniques increasingly are used to identify diet composition (Casper et al., 2007; Deagle and Tollit, 2007). It is likely necessary to evaluate the diet of generalist marine predators with a combination of techniques, given that these techniques often yield different results and can answer different questions (Tollit et al., 2006). The model described here can be used to test assumptions about the relative importance of salmonids and herring compared with other species in harbor seal diet as other data become available.

Estimates indicate that rockfish species constituted a relatively minor proportion of total consumption by harbor seals. There are more than 26 species of rockfish that occur in the inland waters of Washington State, and many species are listed as endangered by the state. Under the federal Endangered Species Act, 2 species are listed as threatened and 1 species is listed as endangered. The 2 most dominant species, Copper (*Sebastes caurinus*) and Quillback (*S. maliger*) Rockfish, for which abundance data are well documented, have both undergone serious declines and are considered vulnerable to extinction (Mills and Rawson, 2004). For depressed species such as these, even small amounts of predation may be significant. If we assume an average size of 1 kg for a rockfish in harbor seal diet (ignoring age- or species-size differences), harbor seals hypothetically consumed 84,000 rockfish individuals in 2007–08 in the San Juan Islands and eastern bays. However, to illustrate the importance of age or species preference by harbor seals, if we assume that harbor seals eat only

Puget Sound Rockfish (*S. emphaeus*; the smallest of the rockfish at ~40 g), they could have consumed more than 2 million individuals, a number that presumably can affect the rockfish population. It seems clear that prey that constitute even a minor proportion of harbor seal diet may be affected by predation, if such predation increases their natural mortality rates. Therefore, harbor seal interactions with prey species of management concern merit further attention, and modeling prey vulnerability to predation will require a multidisciplinary approach.

Consumption estimates calculated in this study illustrate the energetic importance of herring and salmonids to harbor seals in the San Juan Islands and the importance of considering predation effects on prey groups from multiple perspectives. In this study, we contrasted high consumption rates of prey species (salmonids and herring) with less commonly consumed prey groups, such as rockfish, to illustrate the capacity of models to test assumptions in situations with high uncertainty in input values, such as percentage by wet weight of rockfish in seal diet. We provided evidence that the apparently minor contribution of rockfish biomass to harbor seal diet may nevertheless indicate that large numbers of individuals are being consumed, but the number consumed is highly dependent on the species and age of prey. Harbor seals consumed large amounts of the more commonly consumed species, such as herring, even at the lower estimated limits of consumption rates calculated in this study. Many herring stocks have undergone critical declines, and there is concern that pinniped predation may have increased the natural mortality rate of herring in some areas (Musick et al., 2000), although it is acknowledged that there are likely many factors that contributed to the decline of herring (Stout et al., 2001). Spawner biomass of herring for the northern Puget Sound, an index of population abundance, remained low through the study period,[4] yet herring has been identified as one of the top prey species of harbor seals in a San Juan Islands diet study since 2005 (Lance et al., 2012).

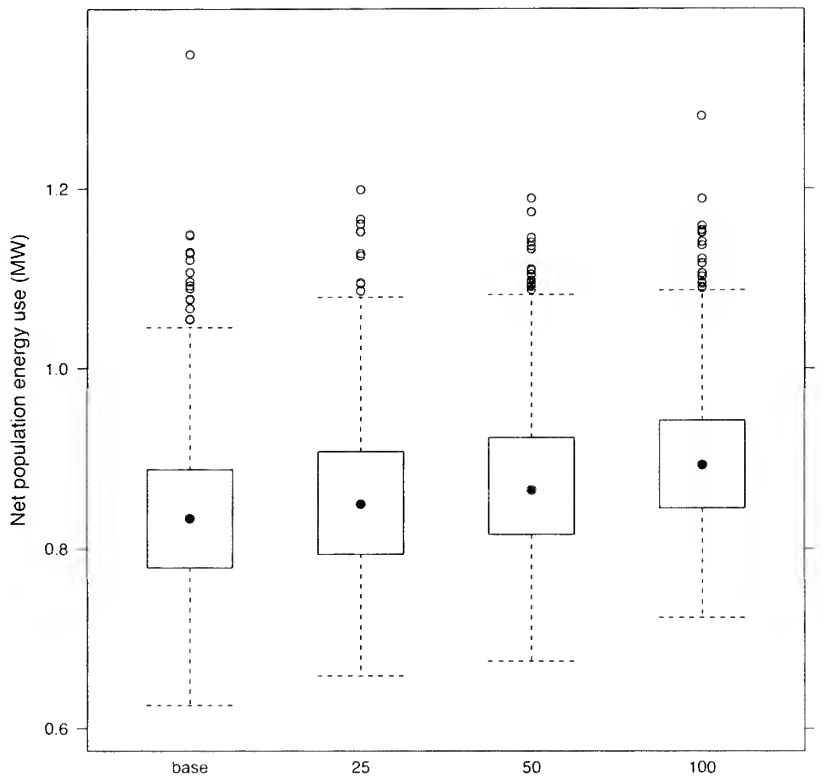

Figure 5

Effect of altering age structure on the net population energy use (in megawatts) of the harbor seal (*Phoca vitulina*) population in the San Juan Islands and eastern bays during 2007–08. Base=basic model with age structure from 1970s; for the other graph lines, 25, 50, and 100 correspond to percent increases in numbers of adults in population. Solid circles indicate medians, boxes enclose the interquartiles, vertical dashed lines represent 1.5× the interquartile range, and open circles indicate outliers.

Like herring populations, salmonid populations have undergone serious declines, and there is also concern that pinnipeds may affect salmonid recovery (NMFS, 1997; Wright et al., 2007). Five species of salmonid occur in the study area and all have been documented in harbor seal diet. Chinook Salmon (*Oncorhynchus tshawytscha*) was the only salmonid species confirmed by the scat samples of our study; however, Pink Salmon are the salmonid species most commonly consumed by harbor seals in the San Juan Islands (Lance et al., 2012). Pink Salmon runs in the northern Puget Sound were relatively abundant during the study period, but abundance indices indicate Chinook Salmon remained at critically depressed levels through 2008.[5] Salmonid

[4] Stick, K. C., and A. Lundquist. 2009. 2008 Washington State herring stock status report. Stock Status Report FPA 09-05, 111 p. Washington Department of Fish & Wildlife, Fish Program, Fish Management Division. [Available from http://wdfw.wa.gov/publications.]

[5] Salmonid stock inventory (SaSi). Washington Department of Fish & Wildlife. [Available from http://wdfw.wa.gov/mapping/salmonscape/index.html.]

abundance along the west coast of North America is linked to cooler than average ocean water temperatures. The high salmonid consumption values in our study may reflect higher than average salmonid abundance driven by changes (warm phase through 2005, neutral-to-cold phase after 2005) caused by the Pacific Decadal Oscillation since approximately 2006 (Mantua et al., 1997). We suggest that the overall high consumption rates of herring and salmonids (along with great uncertainty in these consumption rates) by harbor seals found in this study indicate that harbor seal consumption should be examined on broader spatial and historical scales to further explore the potential effect of harbor seal consumption on prey groups.

Conclusions

Harbor seals are a large-bodied and abundant predator whose consumption of depressed fish populations may conflict with regional fish recovery goals. This study established baseline consumption estimates for major prey groups and highlighted the potential range of consumption for the most common minor prey groups in the San Juan Islands region. Although there was great uncertainty in quantitative diet composition of harbor seals, salmonids and herring clearly constituted the majority of biomass consumed during the study period. Rockfish, one of the fish groups for which marine reserves are being planned, were among the minor prey groups consumed. The relative importance of prey items in harbor seal diet can be tested with future diet data in a model framework that incorporates estimates of uncertainty, similar to the one used in this study. Relation of consumption rates to mortality rates for any of the depressed fish species will require a multidisciplinary approach because of the complexity of harbor seal diet.

In this study, we explored how changes in the age structure of the harbor seal population influenced consumption values and found age structure to have relatively little influence. However, more work is needed to establish the current age structure of the harbor seal population because it may have significant implications for prediction of harbor seal body size, which strongly controlled model predictions. In further modeling exercises, the variables that most heavily influenced consumption values (body size of seals and quantitative diet composition) should be considered as some of the most important factors for prediction of consumption and food requirements of harbor seals in the study area.

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Appendix

Modified age structures (minimum–maximum number of seals) used in alternative model scenarios with increased numbers of adults in the harbor seal population. Pup numbers did not change from the basic age structure. +25%, 50%, and 100% correspond to percent increases in numbers of adults in the population.

Seal age class	Basic +25%	Basic +50% structure	Basic +100% structure
Adult female	1485–1673	1782–2007	2376–2676
Adult male	339–393	407–471	542–628
Subadult female	1997–2572	1688–2251	1068–1610
Subadult male	2388–3273	2316–3200	2170–3054

Quantification and reduction of unobserved mortality rates for snow, southern Tanner, and red king crabs (*Chionoecetes opilio, C. bairdi,* and *Paralithodes camtschaticus*) after encounters with trawls on the seafloor

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Abstract—Unobserved mortalities of nontarget species are among the most troubling and difficult issues associated with fishing, especially when those species are targeted by other fisheries. Of such concern are mortalities of crab species of the Bering Sea, which are exposed to bottom trawling from groundfish fisheries. Uncertainty in the management of these fisheries has been exacerbated by unknown mortality rates for crabs struck by trawls. In this study, the mortality rates for 3 species of commercially important crabs—red king crab, (*Paralithodes camtschaticus*), snow crab (*Chionoecetes opilio*) and southern Tanner crab (*C. bairdi*)—that encounter different components of bottom trawls were estimated through capture of crabs behind the bottom trawl and by evaluation of immediate and delayed mortalities. We used a reflex action mortality predictor to predict delayed mortalities. Estimated mortality rates varied by species and by the part of the trawl gear encountered. Red king crab were more vulnerable than snow or southern Tanner crabs. Crabs were more likely to die after encountering the footrope than the sweeps of the trawl, and higher death rates were noted for the side sections of the footrope than for the center footrope section. Mortality rates were ≤16%, except for red king crab that passed under the trawl wings (32%). Herding devices (sweeps) can expand greatly the area of seafloor from which flatfishes are captured, and they subject crabs in that additional area to lower (4–9%) mortality rates. Raising sweep cables off of the seafloor reduced red king crab mortality rates from 10% to 4%.

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The potential for unobserved mortality of crabs that encounter bottom trawls but are not captured has long been a concern for the management of groundfish fisheries in the Bering Sea (Witherell and Pautzke, 1997; Witherell and Woodby, 2005). Fisheries on the crab and groundfish stocks of the wide continental shelf of the eastern Bering Sea have made Dutch Harbor, the principal port for that area, the highest port by tonnage in the United States and 1 of the 2 highest ports by dollar value for more than 20 years.[1] Three major crab species—red king crab (*Paralithodes camtschaticus*), snow crab (*Chionoecetes opilio*), and southern Tanner crab (*C. bairdi*)—are targets of large commercial fisheries (Otto, 1990). The 2 *Chionoecetes* species have similar low, flat body shapes and inhabit deeper water with muddier substrates than that of the red king crab, which has a thicker body and inhabits shallower, sandier areas (Jadamec et al., 1999; Donaldson and Byersdorfer, 2005). Groundfish species, particularly gadids and flatfishes are targeted with trawls. Overlaps between crab habitat and areas trawled by groundfish fisheries can result in some mortality for crabs that encounter groundfish trawls, either through capture and discard (bycatch) or as unobserved mortality of crabs that remain on the seafloor (Witherell and Pautzke, 1997).

The current management measures to control and reduce bycatch of the major Bering Sea crab species in Alaska groundfish fisheries include extensive year-round trawl closure areas (Fig. 1) and bycatch limits outside these areas. The year-round closure areas were established to protect areas of known concentrations of female and juvenile crabs. Armstrong et al. (1993) and Witherell and Pautzke (1997) cited unobserved trawl–induced mortality, along with

[1] U.S. Department of Commerce. 1995–2011. Fisheries of the United States 1995 (1996,...,2011). Current Fishery Statistics 1995 (1996,...,2011). U.S. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Fisheries Statistics Division, Silver Spring, MD. [Available from http://www.st.nmfs.noaa.gov/commercial-fisheries/fus/index.]

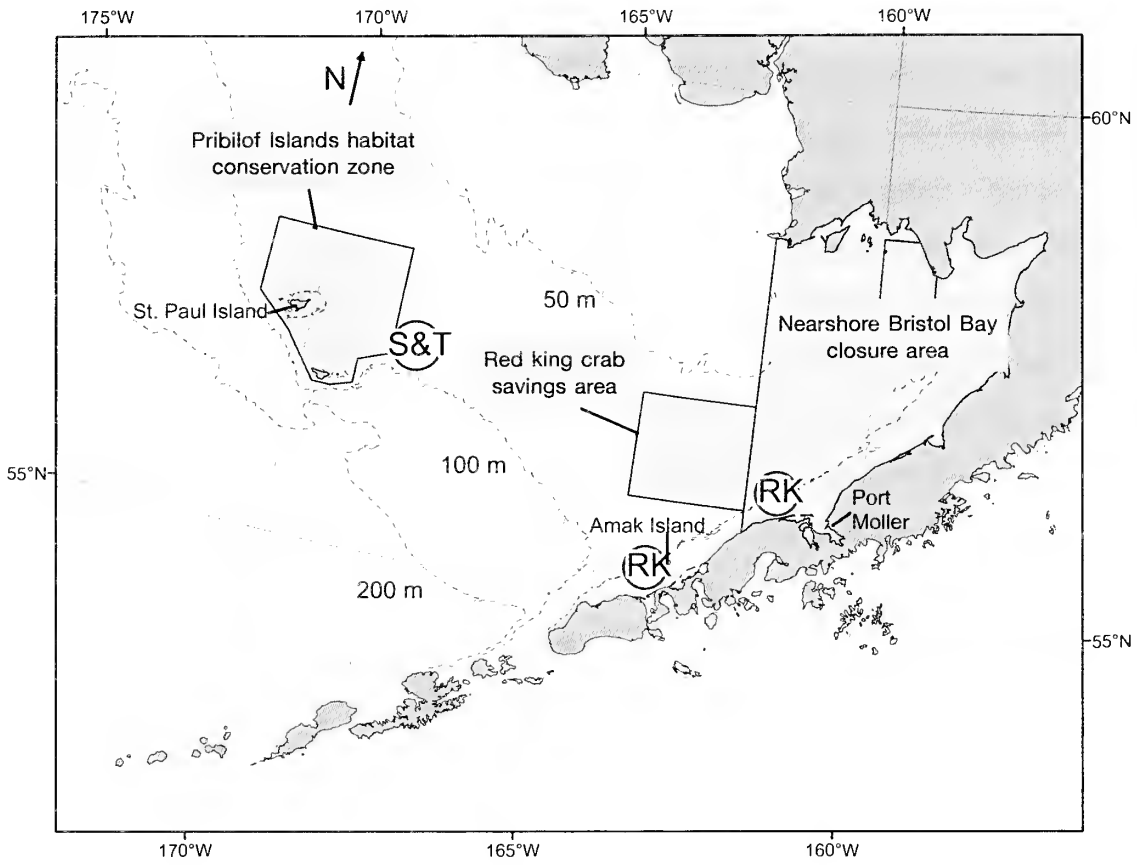

Figure 1

Sampling locations for snow (*Chionoecetes opilio*) (S) and southern Tanner (*C. bairdi*) (T) crabs in 2008 and red king crab (*Paralithodes camtschaticus*) in 2009 (RK), during our study of unobserved mortality rates from bottom trawling. Bottom trawl area closures (shaded) and depth contours are included for reference.

possible habitat degradation, as principal reasons for the establishment of these closures. Crab bycatch limits (on the basis of numbers caught) have also triggered additional closures if seasonal, species-specific (and sometimes area-specific) limits are reached. These bycatch numbers are obtained from onboard fishery observers on an in-season basis (Witherell et al., 2000). The species-specific crab bycatch limits (in estimated numbers of crabs brought aboard) are thought to have a biologically insignificant effect on the different crab populations because these limits have represented as little as 0.113% of the abundance index for snow crab and 0.5–1.0% of abundance for southern Tanner crab and red king crab (Witherell and Pautzke, 1997).

Critics of the existing framework of measures for crab bycatch management have from time to time asserted that, although bycatch limits appear to be sufficiently conservative, bycatch represents only a fraction of the actual mortality of different crab species caused by groundfish fisheries. Citing an unpublished technical paper, Thompson (1990) estimated actual trawl gear mortality for king crabs to be "10 to 15 times the number of crabs that are caught in the net (and estimated by [National Marine Fisheries Service] observers)." These concerns cannot be adequately evaluated without addition of valid estimates of the unobserved mortality rates for these crab species to the assessments of bycatch and discard. Some crab researchers in Alaska (Murphy et al., 1994) also have underscored the need for additional research on injury rates and unobserved or unaccounted for mortality from both directed crab fisheries and groundfish trawl fisheries. Dew and McConnaughey (2005) concluded that excessively high mortality rates on male Bristol Bay red king crab from the directed fishery and unaccounted for mortality of females from the groundfish fisheries explain the downward population trajectory of this crab species through the late 1970s and early 1980s better than does the more accepted scientific hypothesis that the low population levels of red king crab were explained by unfavorable climate conditions.

Worldwide, the recognition of unobserved mortalities as a potentially significant element by the fishing industry and by fishery managers has increased the number of studies that have addressed such mortalities and the range of methods used in their estimation.

Broadhurst et al. (2006) provided a thorough review of such studies. Although a great number of studies estimated mortalities of discarded catch, others dealt with mortalities of escaping animals not brought aboard the fishing vessel. Broadhurst et al. (2006) noted that studies of escaping animals, almost exclusively fishes, lately have emphasized methods where escaping animals are recaptured in cages that are then detached from the fishing gear while still at fishing depths. Those cages then are moved slowly to shallower depths, where they are maintained by divers long enough to assess delayed mortalities. Earlier methods involved capture of escaping animals in auxiliary nets before they were brought aboard and held long enough to evaluate mortality rates. However, stress and injury from recapture and extended towing and holding times could have easily masked or exacerbated the effects of the escape process, particularly for animals vulnerable to skin abrasion damage. More recent methods retain the experimental subjects in an environment closer to what they would experience after actual escape. The cost of these gains is that each collection of affected animals requires an extended series of activities that are time consuming and labor and resource intensive. These time and resource demands greatly restrict the number of experimental samples that can be collected and held and, hence, the number of experimental factors that can be addressed.

As an experimental subject, crab are significantly different from fish for which the in situ capture, transfer, and holding methods were developed. Exoskeletons protect crabs from the type of abrasion to which fish are particularly susceptible during net capture and crowded holding. As a trawl net approaches, fish continue swimming, often to exhaustion, to avoid contact with the net and other animals, but crab, being much slower, can flee only briefly before being overrun (Rose, 1995).

Another difference is how crabs interact with fishing gear. Broadhurst et al. (2006), describing research on fishes, noted, "Because most experiments have quantified escape mortality at the codend, the potential for mortalities as a result of collisions and escape through other parts of the gear have largely been ignored." Because of the sizes and behavior of Bering Sea crabs and the configurations of Bering Sea bottom trawls, most crabs escape under the forward parts of trawl systems, and interactions typically last only a few seconds as the crab passes the components of the net that directly contact the seafloor. Rose (1999) studied crab mortalities after such escapes under the forward sections of bottom trawls through assessment of visible injuries to red king crab that resulted from passes of crabs under different trawl footrope designs. The crabs were recaptured in an auxiliary net fished behind the main footropes. A control footrope, suspended with floats to allow crabs to pass beneath with minimal damage, also was used. A low rate of injuries for control crabs indicated that recapture of crabs to bring them aboard could be done without greatly increasing injury to crabs. The principal limitations of that study were the following: 1) crabs were not held beyond the initial assessment of injuries to observe delayed mortality; and 2) observations were limited to crabs that passed under the center section of the footrope, a small portion of the area swept during trawling.

Studying mortality of crabs discarded from trawl catches, Stevens (1990) effectively applied a strategy in which all subject crabs were assessed for selected condition attributes and a sample was held long enough to relate those attributes to delayed mortality. Since that study, such methods have been expanded and improved. Davis and Ottmar (2006) used assessment of a range of reflexes of Pacific Halibut (*Hippoglossus stenolepis*) to build a predictor of delayed mortality, the Reflex Action Mortality Predictor (RAMP). In a pilot study for this project, Stoner et al. (2008) found the RAMP technique effective for estimation of delayed mortalities for snow and southern Tanner crabs.

Our research addressed unobserved mortality rates for 3 principal commercial crab species of the Bering Sea: red king crab, southern Tanner crab, and snow crab. We improved methods for collection of crabs immediately after trawl encounters as used by Rose (1999) and applied the RAMP technique as described by Stoner et al. (2008) to assess the mortality probabilities for crabs that passed under the sweeps, wings, and central footrope of a commercial groundfish trawl. Raised sweeps, which reduce seafloor contact yet maintain herding of flatfishes (Rose et al., 2010), also were used at the red king crab sites to evaluate whether they would reduce crab mortality rates. Observations of control animals collected with identical recapture nets but no trawl encounter were used to adjust observed mortality rates for effects of capture and handling.

Materials and methods

A pilot study conducted in 2007 evaluated the RAMP and developed and tested techniques for 1) recapturing crabs after encounters with trawl components, 2) handling and assessing those crabs on deck, 3) holding selected crabs to determine their survival over several days, and 4) using the RAMP to estimate the mortality probability of each crab (Stoner et al., 2008). Our study followed those methods closely, and the following description summarizes them and highlights all modifications made to the methods of the pilot study for our later study.

Experimental tows for southern Tanner and snow crabs were made in August of 2008 ~111 km (~60 nmi) east of Saint Paul Island (Fig. 1). All tows included a mix of both species. Red king crab tows were made in August of 2009 at 2 sites in Bristol Bay, about 22 km (12 nmi) west of Amak Island and ~65 km (~35 nmi) northwest of Port Moller. Operations were conducted aboard the FV *Pacific Explorer*, a 47-m, 1800-hp com-

mercial trawler equipped with a trawl configured similarly to the one used by many of the bottom trawlers that are used in Bering Sea groundfish fisheries. The 2-seam trawl net had a 36.0-m headrope and a 54.6-m footrope, which was made of 19-mm-long link +steel chain and equipped with bobbins 46 cm in diameter. The ~70-cm sections between bobbins were covered with 2 steel-chain toggles, weighing 6.4 kg each, rubber disks of 4–20 cm, and one 5-kg circular weight. Wing extensions, installed ahead of the forward ends of the footrope, were made of 20-cm disks strung over 19-mm-long link chain. The cables (sweeps) that ran forward from the trawl to the doors were made of 48-mm combination rope, a product made of both steel and synthetic materials and used by most Bering Sea flatfish trawlers. The red king crab study included tests of sweeps equipped with disk clusters spaced at 14-m intervals and raised the combination rope 7.5 cm above the seafloor. Rose et al. (2010) found that such raised sweeps reduced seafloor contact while still herding groundfish effectively.

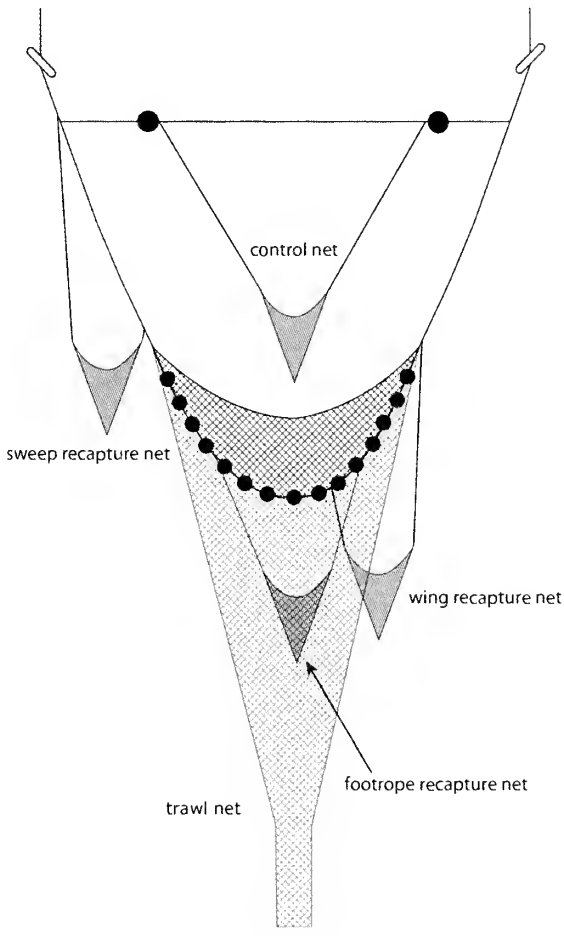

Figure 2

Diagram of the trawl net (not to scale) used in our study of unobserved mortality rates for snow crab *(Chionoecetes opilio)*, southern Tanner crab (*C. bairdi*), and red king crab *(Paralithodes camtschaticus)*, showing positions of recapture nets designed to retain crabs after contact with various trawl components. No more than 2 of these nets were fished during the same tow, and the control net always was fished separately. Illustration by Karna McKinney.

Crabs were captured immediately after contact with the components of the main trawl by small recapture nets fished behind these 3 gear regions: 1) at center of the footrope, 2) at the footrope wings (including their extensions), and 3) behind the sweeps (Fig. 2). These recapture nets were small trawls designed to minimize fish capture and maximize crab capture. The recapture nets used behind the wings and sweeps had unequal bridle lengths, which were adjusted until water passed perpendicular to the center of the headrope of each net, as observed with an underwater camera. An identical recapture net was fished ahead of the trawl as a control to assess damage and mortality due to handling. A rope between the sweeps ahead of the control net was necessary to avoid overspreading. That rope was raised 23 cm off the bottom of the seafloor to avoid affecting crabs. Only 1 recapture net was used at a time during every tow in 2008 to ensure that nets did not tangle when launched. Experience allowed us to expand to 2 nets (1 sweep and 1 footrope) at a time during some tows in 2009; however the control net was always fished alone because it would potentially have damaged crabs before they reached the footrope. The time required to change positions of the recapture nets on the trawls precluded alternating them between trawl components on a tow-by-tow basis; therefore, all tows that addressed each gear component were done in 1 or 2 blocks of sequential tows. To maximize holding times for crabs affected by the trawl, the control tows were done last. The codend of the main trawl was not closed because the tows were too short to represent typical mortality due to capture by the trawl, and catch volume was considered unlikely to significantly affect sweep and footrope mortality.

Towing speeds were 3–3.5 kn. Tow lengths were kept short to minimize damage to crabs from the recapture process but varied from 7 to 25 min to capture sufficient numbers of crabs. These speeds reflect industry practice, and, although commercial tows last much longer, the shorter lengths of the tows in our study did not change the relatively brief interactions between individual crabs and the ground contact components of the trawl. The main trawl was monitored with trawl sonar, which would detect any significant net asymmetry, and video observations of ground-gear components were used to check for atypical contact with the seafloor.

Tow sites (Fig. 1) were selected to provide adequate numbers of the targeted crab species during relatively short tows. Both snow and southern Tanner crabs were sufficiently abundant to be studied at a single site in 2008, but red king crab research in 2009 required an

additional site. Although one of the red king crab sites was in a closed area, both such sites were similar in depth and substrate to areas where Bering Sea groundfish fisheries encounter that species. If <7 individuals of a species were captured in one of the nets, crab assessments for that species were not used in the analysis. Tow tracks were arranged to minimize crossing the trawl tracks of previous tows and to keep such crossings close to perpendicular, to limit areas of overlap. Track crossings made up <1% of study tows. We also maximized the time elapsed between such crossings (always more than 1 day) so that immediate mortalities from earlier exposure would be easily recognizable.

Upon recovery, the recapture codend was opened and all *Chionoecetes* crabs in 2008, or red king crab in 2009, were removed and sorted by species and sex (Jadamec et al., 1999). To use our project's resources most efficiently, we used a 2-stage sampling procedure in which all of the subject animals were assessed immediately for selected condition attributes and a small sample of those subjects was held long enough to relate those attributes to eventual mortality rates.

All *Chionoecetes* crabs were assessed for the presence of the 6 reflex responses described in Stoner et al. (2008): leg flare, leg retractions, chela closure, eye retraction, mouth closure, and kick. For red king crab, the leg retraction reflex test was replaced with a test of antennae movement response. Antennae, minuscule in snow or southern Tanner crabs, were quite active and responsive for red king crab, providing a more sensitive reflex response. As in the Stoner et al. (2008) eye and mouth tests, the antennae were manipulated and responsive movements were recorded as a positive response.

Assessments were limited to presence or absence of reflexes, there was no evaluation of reflex strength. This simplification allowed for rapid assessments and reduced any ambiguity or observer variation (Stoner et al., 2008). Initial scans separated unimpaired crabs from those crabs with an injury or at least one reflex missing. Missing reflexes and any injuries were recorded. Reflex scores indicated the number of impairments; a score of 0 indicated an unimpaired crab, and a score of 6 indicated a moribund crab with no reflexes present. Sex and shell condition for all crabs and carapace width for the 2 *Chionoecetes* spp. and carapace length for red king crab were recorded. Shell conditions (Jadamec et al., 1999) included soft shell (shell soft and pliable), new hard shell (firm to hard shell that lacked wear or encrustment), old shell (wear and encrustment present) and very old shell (extensive signs of shell wear and encrustment). Catch processing generally took less than 15 min, and crabs were held in seawater when they were not being processed.

For each crab species, specimens representing each reflex score were tagged and held to estimate the relationship between reflex score and delayed mortality. Collections of snow and Tanner crabs in 2008 supplemented the RAMP results of the 2007 pilot study. Selection of crabs for holding emphasized those with reflex scores from 1 to 5, categories that had lower observed numbers in the earlier study. Holding procedures were identical to those of Stoner et al. (2008), with ~900 L on-deck tanks, supplied with seawater flow >20 L/min. Crabs were assessed daily, and those crabs that died were recorded and removed.

Early in the 2009 work, it became apparent that many of the red king crabs with no reflex impairments but apparent injuries were dying. This outcome indicated that fatally injured red king crab were not as likely to lose reflexes as were the *Chionoecetes* crabs and led us to adapt the full RAMP approach so that all red king crab that had either a missing reflex or an apparent injury were held. To ascertain how commonly fatal damage was completely hidden, 367 uninjured crabs displaying all reflexes were held.

Our estimator of the probability of mortality for crabs with each reflex score was the proportion of held crabs with that score that died for each species. To estimate overall mortalities, the proportions of crabs in each reflex class were multiplied by the probability mortality of that reflex class and summed (Eq. 1):

$$m_c = \Sigma_{r=0 \text{ to } 6}(m_r * p_{rc}), \quad (1)$$

where m_c = the mortality estimate for a species in catch c;
m_r = the mortality probability from the RAMP for that species for reflex score r; and
p_{rc} = the proportion of that species from catch c with reflex score r.

For red king crab, this formula was modified to use the actual mortality outcomes of the injured and reflex-impaired crabs, all of which were held for observation (Eq. 2):

$$m_c = (m_u * p_{uc}) + ((D_{ic}/N_{ic})*p_{ic}), \quad (2)$$

where D_{ic} = the number of impaired or injured crabs that died from catch c;
N_{ic} = the number of injured or impaired crabs in catch c; and
m and p have the same meaning as in Equation 1, except that i refers to injured or impaired crabs and u refers to those crabs that were uninjured with all reflexes present.

To estimate mortality for crabs that encountered a portion of the trawl, mortalities (m_c) for all catches from recapture nets installed in that area were averaged and weighted for the number of crabs in each catch.

To correct mortality estimates for handling damage, we assumed that gear and handling mortalities were independent and sequential. That is, where both processes occurred together in the recapture catches (m_{g+h}), the gear mortality (m_g) occurred first and only those crabs not killed by the gear ($1 - m_g$) were vulnerable to handling mortality (m_h, estimated as the mor-

tality rate from the control net), resulting in Equation 3:

$$m_{g+h} = m_g + ((1 - m_g) * m_h). \quad (3)$$

This equation was solved for m_g, resulting in Equation 4:

$$m_g = (m_{g+h} - m_h) / (1 - m_h). \quad (4)$$

If the cumulative effects of gear impact and handling caused additional mortalities, this estimator would attribute those mortalities to gear effects, resulting in overestimated gear-caused mortalities.

To account for variability due to the combination of reflex score assessments, RAMP prediction of mortality, and corrections for handling mortality, a randomization approach was used for hypothesis testing and estimation of confidence intervals. A model of the experiment was implemented with the Resampling Stats add-in for Microsoft Excel (Resampling Statistics, Inc., Arlington, VA., http://www.resample.com).[2] RAMP estimators were regenerated for each trial by making random binary draws for each reflex score category (Urn procedure) and by using the sample size and mortality probability for that score from the experiment. New probabilities, calculated from that draw, were then used in the mortality estimation procedure for that trial.

In resampling from the reflex assessments, we used each catch as our sample unit, choosing not to assume that individual crabs within a catch have independent mortality probabilities. To test null hypotheses that 2 groups of catches (e.g., catches from recapture nets at different trawl locations) actually came from the same population, the groups were combined and random draws were made from that combination, without replacement (Shuffle procedure), filling 2 new samples corresponding in number to the samples from the original experiment. A mortality estimate was generated for each trial by using the RAMP and assessment draws. For each test, 5000 trials were generated, and the proportion of those trials with differences greater than the observed estimate indicated the probability that our result occurred from a random process in which the mortality rates for both groups were equal.

Comparisons were made between catches from each of the 3 gear areas (center footrope, footrope wings and extension, and sweeps) and the control catches to determine whether those trawl encounters caused significant mortality. Subsequent tests were made for differences between the 2 footrope areas and between the sweeps and the combined footrope areas.

Confidence intervals were generated by a similar process, except that samples of the assessment catches for each group, including control catches, were randomly selected with replacement from the actual catches for that group. Handling corrections were applied to mortality estimates generated for each gear component, on the basis of the control estimate from each trial. Confidence intervals (95%) were generated by identification of the highest 2.5% and the lowest 2.5% of the estimates from 5000 trials.

Effects of sex, size, species, and shell condition on mortality rates were examined with logistic regression after the effects of each gear component were accounted for. Mortality was initially regressed against gear components, and the effects of these other factors were then tested against the residual variation. Because logistic regression requires binomial outcomes, specific RAMP probabilities of death could not be directly applied. Where direct observations from holding were not available, crab mortality outcomes were scored on the basis of whether RAMP probabilities for their mortality were less or greater than 50%. Significant effects also were tested for interactions of each significant factor with gear area.

Results

The 159 total tows included 17–21 tows for each species at each recapture position. Between 154 and 991 crabs from each of the 6 combinations of species and sex were assessed after their capture behind each gear component and in the control position, and a substantial range of crab sizes were recorded within each combination (Table 1).

Augmentation of the Stoner et al. (2008) RAMP relationships for the 2 *Chionoecetes* species by holding additional crabs in 2008 had only minor effects on mortality rate estimates (Table 2) other than to reduce uncertainty due to larger sample sizes (Hammond, 2009).

For all 3 species, injuries varied widely in affected body part, type of damage, and severity, and were correlated with both reflex score and mortality rate. Of the red king crab with at least one missing reflex (reflex scores of 1 to 6), 96% also had observable injuries, as opposed to only 5% of those crab with no missing reflexes (reflex score of 0). Crabs of all 3 species never survived removal of their abdomen or carapace, although autotomized legs (dropped off after injury) rarely caused fatalities. Crabs with either leg damage or carapace cracks normally survived, depending on extent, severity, and combination with other injuries.

Of the 485 surviving red king crab released at the end of this study, 482 had all reflexes present upon release, including all 14 that initially were missing at least one reflex. The 3 crab that were missing a reflex upon release were all missing the eye reflex, had been held for 9 or 10 days, and had significant injuries, including carapace cracks. Although 25% (122) of the surviving crab had detectable injuries, their survival through the holding period and vigorous state condition upon release indicated a low likelihood of significant later mortalities.

[2] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

Table 1

Number of crabs assessed and size ranges for each species and sex combination after they were captured behind 3 sections of bottom trawl gear, or with a control net. Size ranges, carapace width for snow (*Chionoecetes opilio*) and southern Tanner crabs (*C. bairdi*) and carapace length for red king crab (*Paralithodes camtschaticus*) are given in millimeters. The three gear components were the footrope wings or extensions, the center of the footrope, and the sweep. For red king crab only, a fourth component was added, a sweep raised off of the seafloor (Rose et al., 2010).

	Snow crab				Southern Tanner crab				Red king crab			
	Male		Female		Male		Female		Male		Female	
	No.	Size range	No.	Size range	No.	Size range	No.	Size range	No.	Size range	No.	Size range
Control	467	50–130	154	54–92	567	62–148	157	56–100	448	53–183	433	82–145
Sweep	407	47–126	218	52–93	281	60–147	518	59–98	370	64–188	226	63–150
Raised sweep									321	63–179	278	68–148
Footrope center	991	46–140	353	50–85	677	50–145	756	49–102	753	69–189	393	68–164
Footrope wing	696	48–130	540	50–110	288	51–143	494	52–97	203	61–167	263	70–156

Most southern Tanner and snow crabs captured behind the main trawl components had all reflexes present (76–93% reflex score of 0, Fig. 3), and the next most frequent category was dead crabs (reflex score of 6, no reflexes present) upon capture (2–17%). Similarly, a substantial majority (66–83%) of red king crab captured behind the trawl gear was uninjured and had all reflexes present. Very few of these animals died during holding. Of the red king crab, 6% were dead upon capture, making up 71% of mortalities. Therefore, nearly all of the observed crabs were either extremely likely to survive or moribund; relatively few crabs displayed an intermediate condition where the holding and RAMP results were critical to estimation of their probability of mortality.

For both red king and southern Tanner crabs, the control net yielded 97% uninjured crab with all reflexes present and no crabs were dead upon capture. Snow crab had more immediate mortalities in the control net (2%) and only 88% had all reflexes present. Mortality estimates for crabs from the control nets (snow crab 7.1%, southern Tanner crab 8.5%, and red king crab 2.9%) were significantly lower than the estimates for crabs captured behind trawl components.

Estimates of the rates of mortality due to contact with the trawl gear, adjusted for capture and handling, were below 16% (Fig. 4), with the exception of red king crab that encountered the wing section of the footrope, for which mortality was estimated at 31%. Overall, estimated mortality rates for all 3 species were sig-

Table 2

Number of crabs held to observe delayed mortality and resulting mortality rates by reflex score (number of reflexes missing; 6 reflexes were assessed) and species for snow crab (*Chionoecetes opilio*), southern Tanner crab (*C. bairdi*), and red king crab (*Paralithodes camtschaticus*). Crabs from Stoner et al. (2008) were included for both *Chionoecetes* species.

	Number of reflexes missing							
	None missing	None missing + injury *	1	2	3	4	5	All 6 missing
Snow crab	500	—	78	70	57	79	67	61
Southern Tanner Crab	375	—	53	35	37	47	38	18
Red king crab	367	145	49	55	60	38	21	1
	Mortality rate (%)							
Snow crab	1.4%	—	20.5%	30.0%	43.9%	75.9%	88.1%	100.0%
Southern Tanner Crab	7.2%	—	32.1%	51.4%	86.5%	91.5%	92.1%	100.0%
Red king crab	1.9%	23.4%	81.6%	94.5%	98.3%	100.0%	100.0%	100.0%

* This category was used only for red king crab.

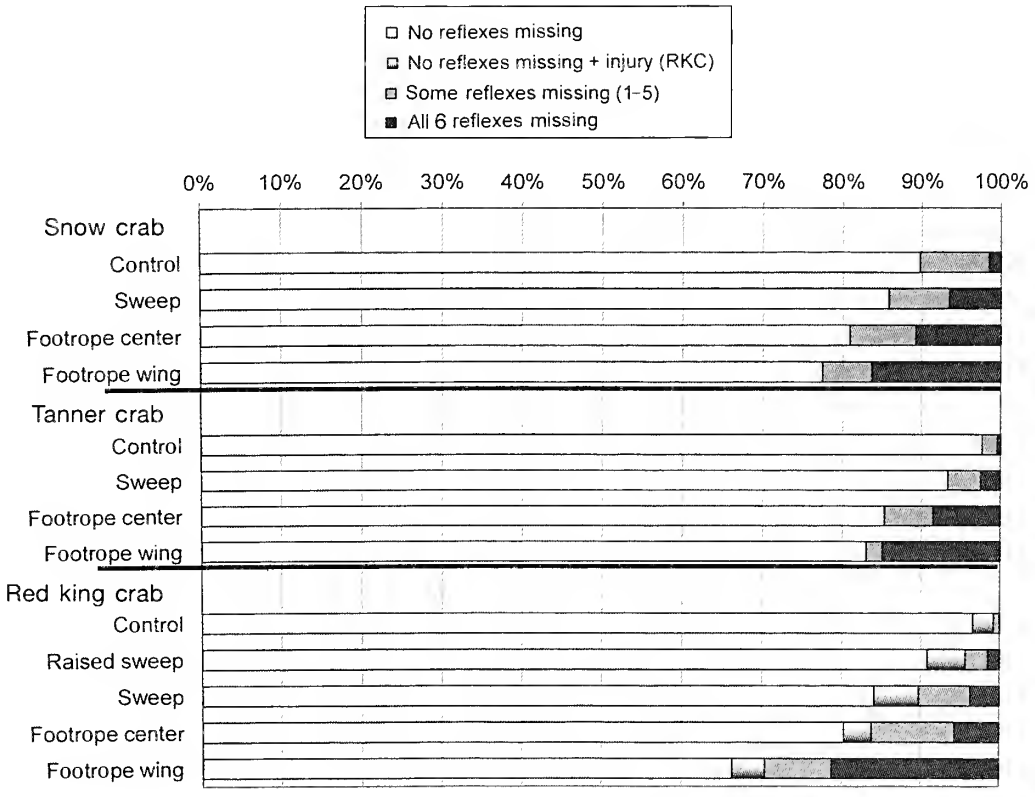

Figure 3

Percentage of crabs that displayed a range of reflex states in our study of unobserved mortality rates for snow crab *(Chionoecetes opilio)*, southern Tanner crab (*C. bairdi)*, and red king crab *(Paralithodes camtschaticus)*. Reflex states were assigned on the basis of the number of reflexes that were missing; 6 reflexes were assessed. Crabs were captured after they contacted 1 of the 3 components of a bottom trawl representative of the gear used in Bering Sea bottom trawl fisheries—the center of the footrope, the footrope wings or extensions, or the sweep—or, for red king crab only, a sweep raised off of the seafloor (Rose et al., 2010). Crabs were captured with no gear contact during control tows. Injured red king crab with no missing reflexes were categorized separately. RKC = red king crab.

nificantly lower for crabs that encountered the sweeps than for those crabs that encountered the footrope and were higher for those crabs that encountered the wing portion of the footrope as opposed to the center footrope. Although the mortality rates for the southern Tanner and snow crabs were similar, both had lower mortality rates than did the red king crab for all trawl components. Raising the sweeps with widely spaced disk clusters reduced red king crab mortality from 10% to 4%.

Holding only samples of the large numbers of crabs with no missing reflexes (no missing reflexes and uninjured for red king crab) greatly reduced the number of held crabs and produced minimal effects on precision of the mortality estimates. For example, the confidence interval estimation process was run with a sample size of 2581, representing all such red king crab observed, instead of the 367 crabs actually held. The resulting confidence range (high limit to low limit) for footrope wing mortality was reduced only from 14.25% to 13.99% by holding 7 times as many crabs. Confidence ranges for footrope center and sweep mortalities were reduced even less (3.53% to 3.50% and 5.98% to 5.95%, respectively).

Logistic regression was used to examine whether mortality rates varied by species, sex, size, and shell condition, after the effects of gear were removed. Nearly all crabs had either a new hard shell or old shell. For southern Tanner and snow crabs, marginally significant effects were detected between species, sexes, and sizes. When the mean effects across the combinations of those factors were examined, it was apparent that most of those effects were the result of higher mortalities for snow crab with carapace widths >95 mm; those large snow crab were nearly all males. Large snow crab were approximately twice as likely to die as smaller

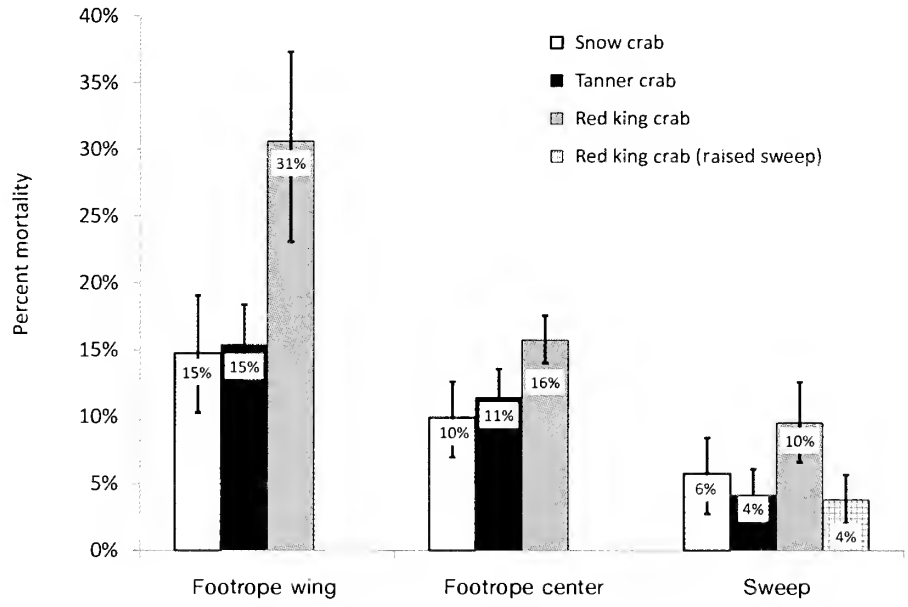

Figure 4

Estimates and 95% confidence intervals of rates of mortality for snow crab (*Chionoecetes opilio*), southern Tanner crab (*C. bairdi*), and red king crab (*Paralithodes camtschaticus* that resulted from contact with 1 of 3 different components of a bottom trawl representative of the gear used bottom trawl fisheries in the Bering Sea—the footrope wings or extensions, the center of the footrope, or the sweep—and, for red king crab only, a sweep raised off of the seafloor (Rose et al., 2010).

snow crab or as any size of southern Tanner crab, and this difference persisted across all gear components and control catches.

Large red king crab had higher mortality than smaller king crabs ($P<0.001$), although this effect explained <1% of the variability in mortality, compared with 12% for the difference between gear components. The interaction between crab size and gear component was not statistically significant; therefore, there was no indication that this difference in vulnerability between sizes varied between gear components. Mortality of red king crab did not vary significantly between sexes or between new-hard-shell and old-shell crab. Although the percentage of mortalities was high for soft-shell crab (4 of 5 died) and crab with very old shells (3 of 5 died), those shell types were too rare for a statistical validation of difference.

Discussion

Our study provides the first reliable estimates of mortality rates following noncapture (not bycatch or discard) bottom trawl encounters for 3 commercially important crab species. Mortality rates varied by species but depended mainly on that part of the trawl system they encountered.

Crabs that passed under the trawl footrope, particularly in the wing sections, died at higher rates than those crab struck by the sweeps. Effective herding by sweeps greatly expands the area of seafloor from which flatfishes are captured. Mortality rates were substantially lower for crabs that encountered these herding devices in that expanded area than for crabs that encountered the trawl net itself, specifically the footrope. Therefore, enhancement of fish capture rates through effective herding can also reduce overall crab mortalities (i.e., capture of equivalent quantities of fishes without herding would expose more crabs to footrope components). The effective reduction of crab mortality through use of sweeps was further augmented for red king crab with modifications to raise sweeps a few centimeters above the seafloor (Rose et al., 2010).

The lower rates of unobserved crab mortalities from herding devices (i.e., sweeps), compared with mortality rates from trawl footropes, only partially indicate the potential of herding to reduce crab mortalities. Mortalities of crabs that encounter the footrope also would include those crabs retained in the net (bycatch). Stevens (1990) found that mortality rates were much higher for both captured red king crab (79%) and captured southern Tanner crab (78%) than for escaping crabs. Some herding of crabs is conceivable, but their much slower

locomotion, compared with that of commercial fish species, led us to assume that the number of crabs that encountered each part of the trawl system is roughly proportional to the area swept by each part.

Red king crab had higher mortalities (6–32%) than 2 species of *Chionoecetes*, snow and southern Tanner crabs (4–15%)—a result that was expected given the generally smaller size and flatter body shape of *Chionoecetes* crabs. Overall mortality rates, weighted for the approximate relative areas swept by each trawl component for modern Bering Sea flatfish trawls (90% sweeps, 6% footrope wings, 4% footrope center) were 6% for snow crab, 5% for southern Tanner crab, and 11% for red king crab. The raised sweeps reduce mortality rate for red king crab to 6%. Such sweep modifications were required by the North Pacific Fishery Management Council for Bering Sea flatfish trawlers beginning in January 2011.

The trawl gear and methods selected for our experiment represented those gear and methods used in the Bering Sea flatfish fisheries. The gear is characterized by long, combination rope sweeps and footropes built with large-diameter, rubber bobbins or disks to keep the net mouth more than 20 cm above the seafloor. This footrope selection by the fleet has been driven partially by pressure to reduce crab bycatch. Decreasing bycatch through changes to gear means that more crabs pass under the trawl net. Although other Alaska bottom trawl fisheries (e.g., for Pacific Cod [*Gadus macrocephalus*]) use similar footropes, they use much shorter sweeps. Therefore, although cod trawls cover less seafloor (and hence contact fewer crabs) per kilometer towed than flatfish trawls, a higher proportion of the crabs might die because more of them would encounter the footrope components. The other major trawl fishery that can affect Bering Sea crabs is the fishery for Walleye Pollock (*Theragra chalcogramma*). Pollock trawls must meet a number of requirements that allow them to be considered "pelagic" trawls, but this fishery commonly has been fished with substantial seafloor contact.

Because regulations disallow any protective bobbins, none of the crab mortality estimates for gear components examined in our study can be used to estimate mortalities used for the pollock fishery, where chain footropes are used. The differences we found in mortality rates between different gear components indicate that changes in the specific gear configurations could improve or worsen crab mortality rates. The rates found here should not be applied to trawls with substantially different ground gear (e.g., chain footropes used in the Bering Sea pollock fishery). Component-specific mortality differences also present an opportunity to reduce crab mortality through identification of less damaging footrope configurations that sustain effective capture of target species. A companion study where an alternative footrope was tested has been completed (Hammond, 2009).

Because crabs were held for periods <14 days, our results did not include mortalities delayed over longer periods. The rapid drop of new mortalities after the first few days and the presence of all reflexes at the end of the study suggest that little additional mortality would be expected unless some other mechanism, such as infection or problems with molting, created a pulse of mortalities outside of the time period observed (see also Stoner et al., 2008). Likewise, holding crabs in on-deck tanks protected them from predation that would have increased delayed mortality if vulnerability to predation was enhanced by injury or stress after trawl exposure. Predators and scavenger species have been observed to move into areas recently swept by bottom trawls (Prena et al., 1999). Although this potential for additional mortality was not addressed directly in this study, the vast majority of the surviving crabs retained their full suite of assessed reflexes, including mobility of walking legs and defensive reactions. If predators initially focused on the more severely impaired and injured crabs that ended up as mortalities in our study, less impaired crabs might have some respite, allowing some time for recovery and reducing any difference between our results and the actual unobserved mortality due to predation.

All retained red king crab were held until the end of the study, 4 days after the control tows were completed. Because control crabs were held for only 4–6 days, we examined the proportions of delayed mortalities of crabs held for longer periods. For crabs held more than 10 days, 93% of the mortalities occurred in the first 4 days and 95% in the first 6 days. Because only 9 of the 881 red king crab caught in the control net died, the possibility of missing one additional mortality because of a shortened holding time was not considered to introduce a significant potential bias. Short holding time was even less of a concern for southern Tanner and snow crab because all of those crabs were held 7 days or longer and the low proportion of deaths after the first days noted during the pilot project (Stoner et al., 2008) continued during our 2008 observations.

In this study, the RAMP procedure (Stoner et al., 2008) was successful in prediction of mortality rates for many more crabs than we could have held to observe delayed mortality. Of all crabs assessed, 85% had either all reflexes present (*Chionoecetes* spp.) or were uninjured with all reflexes present (red king crab). Holding only one-eighth of these crabs provided generous samples (>350 crabs per species) for estimation of their low mortality probabilities. If we had followed a conventional study method and held all crabs regardless of reflex state, more than 4 times as many crabs would have been held, with minimal reductions in uncertainty.

Although only representing a small proportion of the observed crabs, the RAMP procedure also allowed us to efficiently account for crabs with intermediate reflex assessments (reflex scores of 1 to 5). Because significant mortalities occurred to injured red king crab with all

reflexes present, we held all those crabs, as well as all crabs of any of the 3 species with any missing reflexes. This procedure maintained the primary advantage of our RAMP assessments, accounting for a large group with high survival, and avoided the need to rely on injury assessments to estimate mortality. Both Stevens (1990) and Stoner et al. (2008) applied scoring systems for injuries, but the variety of injury types makes injury assessment more subjective and less likely to be repeatable than the reflex assessments.

We provided specific estimates of the unobserved mortality rates of crabs swept over by trawl gear common to bottom trawl fisheries in the Bering Sea. However, assessment of the effects of such mortalities on the populations of those crabs will require estimation of the portion of those populations exposed to trawling each year. Although the distribution of trawling effort is well documented by automated position monitoring of vessels and onboard observers, the spatial distribution of crabs throughout the year is not well known. A reliable estimate of the distribution of crabs, including seasonal variability, would be needed to estimate their exposure to trawling and allow for use of our mortality rate estimates in order to estimate resulting mortalities to the population. This approach would be subject to error from interannual and seasonal variations in crab distribution—variations that are not well understood and would be difficult to monitor.

The number of crabs captured in bottom trawls is monitored through catch sampling by onboard observers. Another way to estimate the number of crabs encountering trawls would be to learn the proportion of crabs that are caught in the path of a trawl. Crab bycatch data could then be expanded to estimate the number encountered, a value to which our mortality rates could be applied to estimate overall, unobserved mortality. One significant source of error for this approach is variability or changes in the specific footropes used across the fishery—differences that could substantially alter the proportion of crabs retained by the trawl. Also, should the trawl fishery approach its goal of eliminating crab bycatch, the base bycatch data could become sparse and even more variable.

Conclusions

Unobserved mortality is an important component of bycatch that is both easily overlooked and difficult to assess. Mortality rates for commercial crab species overrun by bottom trawls used in the Bering Sea varied substantially between the different components of trawls, with lower mortality for crabs that encountered sweeps than for crabs that encountered footropes. Reduction of mortality rates of red king crab from 10% to 4% by raising the sweeps off the seafloor showed that gear modifications can mitigate unobserved mortality.

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Abstract—We examined the reactions of fishes to a manned submersible and a remotely operated vehicle (ROV) during surveys conducted in habitats of rock and mud at depths of 30–408 m off central California in 2007. We observed 26 taxa for 10,550 fishes observed from the submersible and for 16,158 fishes observed from the ROV. A reaction was defined as a distinct movement of a fish that, for a benthic or hovering individual, was greater than one body length away from its initial position or, for a swimming individual, was a change of course or speed. Of the observed fishes, 57% reacted to the ROV and 11% reacted to the submersible. Aggregating species and those species initially observed off the seafloor reacted most often to both vehicles. Fishes reacted more often to each vehicle when they were >1 m above the seafloor (22% of all fishes >1 m above the seafloor reacted to the submersible and 73% to the ROV) than when they were in contact with the seafloor (2% of all reactions to the submersible and 18% to the ROV). Fishes reacted by swimming away from both vehicles rather than toward them. Consideration of these reactions can inform survey designs and selection of survey tools and can, thereby, increase the reliability of fish assemblage metrics (e.g., abundance, density, and biomass) and assessments of fish and habitat associations.

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Reactions of fishes to two underwater survey tools, a manned submersible and a remotely operated vehicle

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Visual surveys of fishes in deep water and untrawlable areas have been conducted more frequently in recent years than in the past largely because of increased availability of underwater vehicles and the need for nonextractive assessments, particularly in no-take areas. These vehicles have provided researchers with the opportunity to gather valuable information on species composition, habitat associations, population density, and various behavioral traits that was previously unattainable in these deep (>30 m), structurally complex areas (Carlson and Straty, 1981; Pearcy et al., 1989; Yoklavich et al., 2007; Laidig et al., 2009; Love et al., 2009). Visual surveys present advantages over traditional sampling methods (e.g., trawling, hook and line, traps) through the use of nondestructive, in situ observations of fishes in their natural habitats.

One concern in counting fishes is their reaction to an observer (e.g., in scuba or snorkel surveys) or underwater vehicle (e.g., submersibles, remotely operated vehicles [ROVs], and camera sleds; Stoner et al., 2008). The vehicles, in particular, can produce a number of electronic and mechanical stimuli (e.g., lights, motion, and noise) that could alter behavior (Krieger, 1993; Uiblein et al., 2003; Ryer et al., 2009). Accounting for these reactions is an important aspect of accurate population assessments. To this end, Yoklavich et al. (2007) quantified the reactions of fishes to a manned submersible during a survey of Cowcod (*Sebastes levis*). Cowcod were found to react very little to the submersible, and that low level of reaction strengthened the accuracy of the survey results and associated stock assessment. Other studies have reported fish reactions to both ROVs (Johnson et al., 2003; Trenkel et al., 2004a; Lorance and Trenkel, 2006) and manned submersibles (Murie et al., 1994; Krieger and Sigler, 1996; Gibbons et al., 2002). However, most of these studies were qualitative, simply noting that fishes moved out of the way of the vehicles. More quantitative studies are needed to improve our understanding of the nature and magnitude of reactions of various fish species to a variety of underwater survey vehicles.

The goal of our study was to characterize the reactions of a wide range of marine fish species to 2 commonly used underwater vehicles (a manned submersible and an ROV) during surveys conducted along the seafloor. We quantified the degree of species- and size-specific reactions of fishes living both on and above the seafloor.

Materials and methods

Fish surveys were conducted off central California (Fig. 1) inside and outside of 3 recently created marine protected areas (MPAs)—Point Lobos, Portuguese Ledge, and Soquel Canyon—with the 2-person *Delta* submersible (Delta Oceanographics, Torrance, CA) and a Phantom DS4[1] ROV (Deep Ocean Engineering, San Jose, CA). The manned submersible surveys occurred during the period of 20 September–5 November, 2007, at depths of 30–365 m, and the ROV surveys were conducted during the period of 18–23 November, 2007, at depths of 71–408 m. All surveys were conducted during daylight hours from 0800 to 1700. Each submersible dive comprised 2–6 transects, each of a 10-min duration. The ROV dives were 1–3 h in duration. The ROV surveys were conducted along the same path of only a subset of the submersible transects; in other words, not all submersible transects were paired with an ROV dive (Fig. 1).

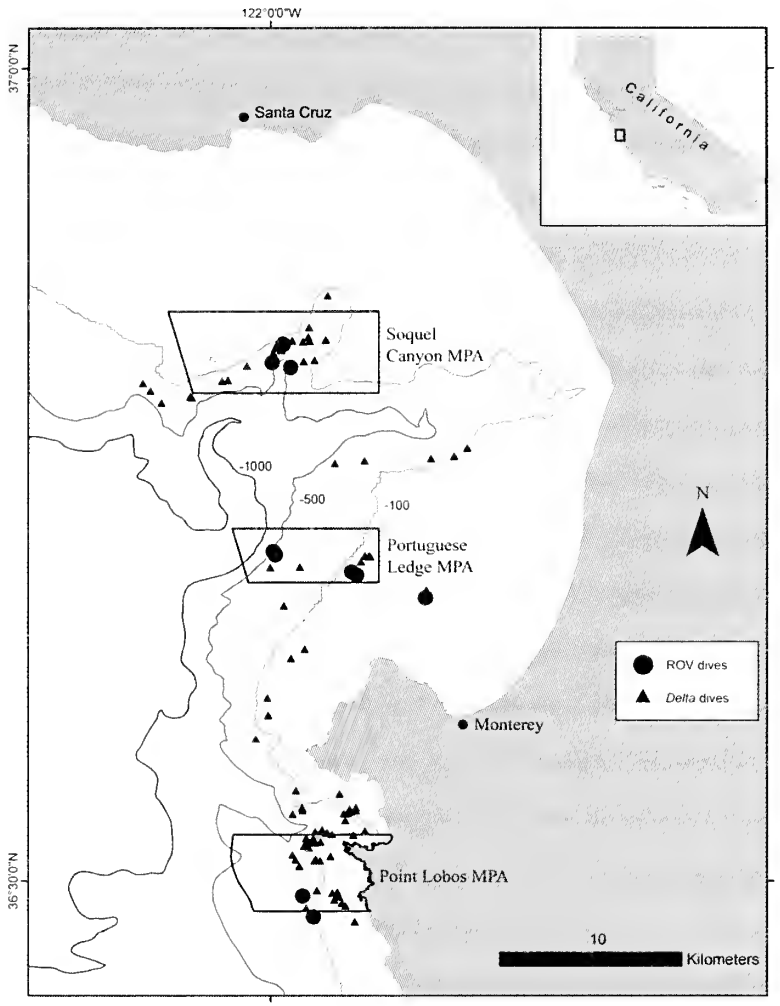

Figure 1

Map of the area inside and outside of 3 marine protected areas (MPAs) off central California that was surveyed in 2007 for our study of the reactions of fishes to the manned *Delta* submersible and a remotely operated vehicle (ROV). Polygon shapes outline the MPAs, and triangles and circles indicate dive locations for the submersible and ROV. Bathymetry is in given meters.

The *Delta* submersible (Fig. 2A) was launched from the FV *Velero IV* and operated by experienced pilots from Delta Oceanographics. An experienced scientific observer accompanied the pilot inside the untethered submersible. This yellow-orange submersible was 1.8 m tall, 4.6 m long, and from 0.4 m wide at its forward-most part to 1.1 m wide at mid-vehicle. The submersible was equipped with 2 video cameras: 1) a forward-facing, low-light, wide-angle, monochrome camera (Super SeaCam 5000, DeepSea Power and Light, San Diego, CA), and 2) a starboard-mounted, custom-built, color zoom camera with 400 lines of resolution and an illumination range of 2–100,000 lux (Yoklavich and O'Connell, 2008). The position of the *Delta* submersible was tracked from the support vessel with WinFrog integrated navigation software (Fugro Pelagos, San Diego, CA) and an ORE Trackpoint-II ultra-short baseline (USBL) acoustic system (EdgeTech, West Wareham, MA). The distance traveled was estimated with a ring laser gyro and Doppler velocity log attached to the outside of the submersible. A single 24-volt propeller provided thrust. During surveys, the *Delta* traveled at an average speed of 0.5 m/s, ~1 m above the seafloor, following a directional heading given to the pilot by scientists aboard the support vessel. The submersible was equipped with ten 150-watt halogen bulbs; only 3 of the starboard lights and 1 of the front-mounted forward-facing lights were used to illuminate the transect area.

We also used an unmanned Phantom DS4 ROV launched from and tethered to the NOAA Ship *David Starr Jordan* and operated by experienced pilots from the National Marine Fisheries Service, Southwest Fisheries Science Center, in La Jolla, California (Fig. 2B). The ROV had a yellow body and black frame and was 1 m tall, 2 m long, and 1.4 m wide. The ROV was equipped with a forward-facing, color video camera (Sony FCB-IX47C, Sony Corp., Tokyo, Japan) with 470 lines of horizontal resolution and an 18× optical zoom. Like the position of the submersible, the position of the ROV was tracked with WinFrog software and

[1] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

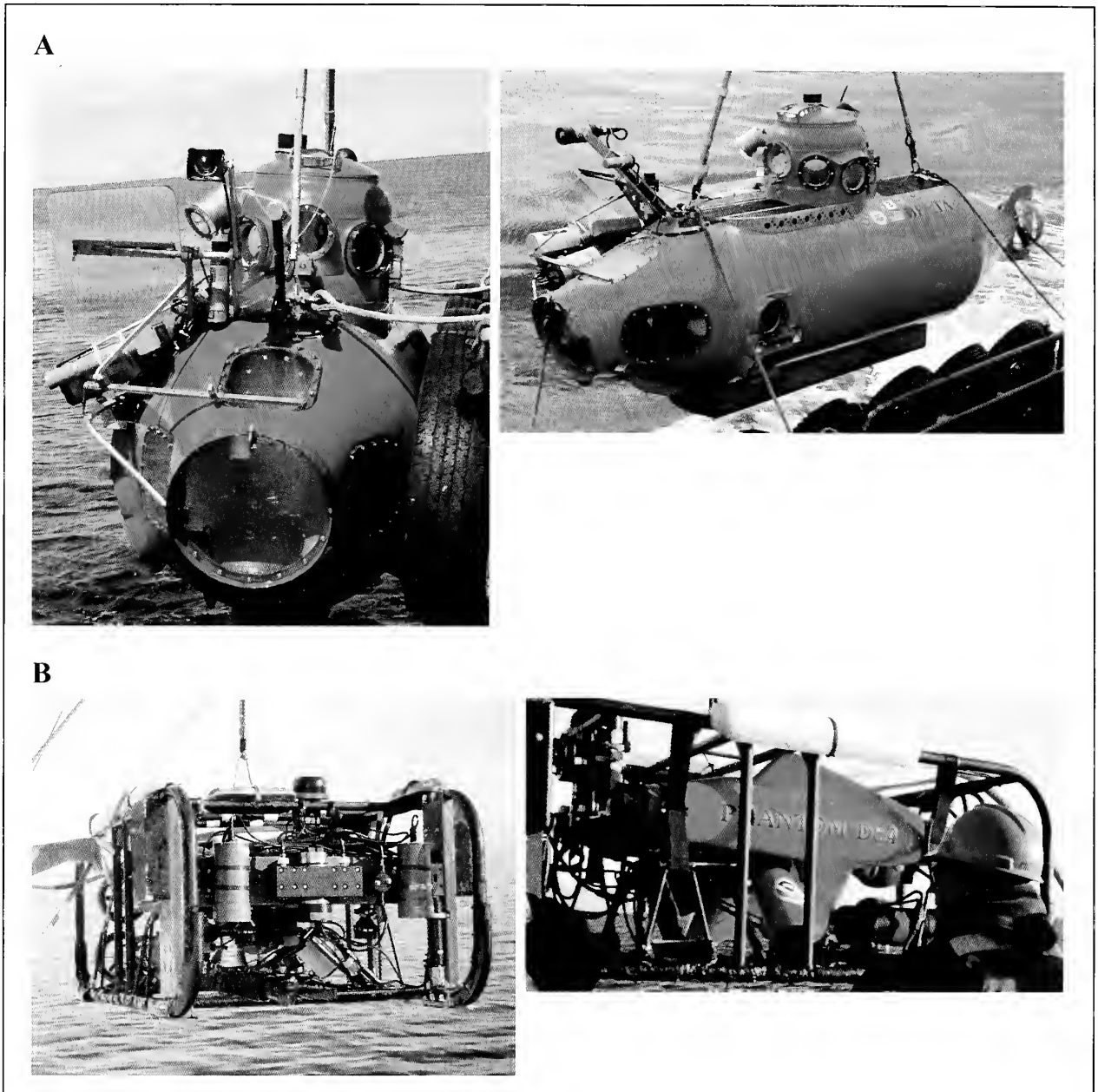

Figure 2

(A) Photos of the front and port side of the *Delta* submersible and **(B)** views of the front and port side of the Phantom DS4 remotely operated vehicle (ROV), the 2 survey tools that were used in 2007 off central California in our study of the reactions of fishes to underwater vehicles. The *Delta* measures 1.8 m tall, 1.1 m wide (tapering to 0.4 m at front port), and 4.6 m long. The ROV is 1.0 m tall, 1.4 m wide, and 2 m long.

an ORE Trackpoint-II system. The ROV was propelled by 6 electric thrusters (2 angled and 4 perpendicular to the seafloor). Surveys were conducted at a target speed of 0.5 m/s and a target height of 1 m above the seafloor. Illumination was provided by two 250-watt Multi-SeaLite halogen lights from DeepSea Power and Light.

The forward-facing video cameras on each vehicle were used to document fish reactions because these cameras had similar orientations and captured fish reactions in front of both vehicles. Both vehicles also were equipped with lasers to help the observers estimate size of fishes and their distance from the vehicle. The *Delta* submersible had a pair of parallel lasers

mounted 20 cm apart on either side of the color video camera and were visible to the observer inside the submersible. For both vehicles, fishes were measured to the nearest 5 cm. Five lasers were mounted on the front of the ROV; these lasers included 2 pairs of parallel lasers (20 and 60 cm apart) and a single crossing laser used to determine depth of field. The laser spots on the video footage were used in postsurvey analysis to estimate both the size (total length) of fishes and the distance ahead of the ROV at which a fish reaction occurred. An effort was made to measure all fishes; however, some fishes were either too far away or partially obscured, and, therefore, they could not be measured.

In an important distinction in survey methodology between the 2 vehicles, the scientific observer inside the submersible identified, counted, and estimated length of fishes (as annotated on the audio channel of the video camera), but these tasks were performed only with video footage from the ROV surveys. Video footage from both vehicles was reviewed after completion of the surveys. Fishes in both surveys were identified to the lowest possible taxon with taxonomic keys (Love et al. [2002] for rockfishes, and Miller and Lea [1972] and Eschmeyer et al. [1983] for the remaining fishes).

All fish reactions were determined solely from video footage of the forward-facing cameras on both vehicles in order for the methods to be similar between survey vehicles. A reaction was defined as a distinct movement of a fish if that movement was greater than one body length away from the initial position of the fish. Some fishes that were hovering off the seafloor would turn and face the vehicle as it passed by, but this movement was not considered a reaction unless a fish actively swam at least one body length in any direction. If a fish was swimming in a particular direction when first observed and continued swimming in the same direction at the same speed during the entire time on video, that fish was considered to have no reaction. However, a reaction was noted if a fish changed course or swimming speed.

The initial position of a fish was recorded as 1 of 3 categories: resting on the seafloor, ≤1 m above the seafloor (but not touching the seafloor), or >1 m above the seafloor. Direction of fish reaction was recorded as swimming 1) toward the vehicle, 2) parallel, forward, and away from the vehicle, 3) perpendicular to the left, 4) perpendicular to the right, or 5) down toward the seafloor. No fishes were ever recorded moving upward. We used time and an average vehicle speed of 0.5 m/s to estimate the distance between a reacting fish and the front of the *Delta* submersible. The distance between a reacting fish at first sighting and the front of the ROV was estimated with the laser array. This distance was binned to <3 m or 3–6 m. A 20-cm fish could be seen at a maximum distance of about 6 m in front of the ROV and about 9 m in front of the submersible (because images could be distinguished farther with the low-light, monochrome camera on the submersible compared with the color camera on the ROV). To ensure that results from the ROV and submersible were comparable, we used data only from fishes that occurred at a distance of at most 6 m from the submersible.

Hagfishes (*Eptatretus* spp.), thornyheads (*Sebastolobus* spp.), and young-of-the-year (YOY) rockfishes (*Sebastes* spp.) were included as taxonomic groups in our analyses. Hagfishes often were seen hiding in holes or under structure and could not be identified to species. However, all the hagfishes that could be identified were Pacific Hagfish (*Eptatretus stoutii*). The thornyhead group comprised Shortspine Thornyhead (*Sebastolobus alascanus*), a few Longspine Thornyhead (*S. altivelis*; 1 observed from the submersible and 4 from the ROV), and mostly unidentified thornyheads. YOY rockfishes were a mix of many species, and each was recorded as 5 cm in total length.

We determined fish reactions only while the vehicles traveled forward in survey mode. No fish reactions were counted if the seafloor, which we used as a stationary reference for fish movement, was not observed in the video footage for ≥5 s (as when either vehicle transited over narrow ravines or when the ROV was pulled off transect by the ship). A number of species were not considered in our analyses. For instance, pelagic schooling fishes, such as Northern Anchovy (*Engraulis mordax*), Jack Mackerel (*Trachurus symmetricus*), and Pacific Chub Mackerel (*Scomber japonicus*), swam around the vehicles for extended periods of time (possibly because they were attracted to the vehicles, but this idea was not verified), and these long periods of time increased the possibility that fishes would be double counted. These species also darted in and out of the view of the cameras, making it difficult to assess individual reactions to the vehicles. Only species that accounted for ≥1% of the total number of fish observed from either vehicle were included in the analyses of reactions to the vehicles. A chi-square test was used to evaluate reactions relative to initial fish position.

Results

A total of 223 transects (56 h) were surveyed with the *Delta* submersible in hard (70% rock, boulder, and cobble) and soft (30% mud and sand) seafloor habitat, and 10,550 fishes were observed (Table 1). A total of 10 ROV dives (21 h) were conducted, and 16,158 fishes were observed. Although the ROV covered only a subset of all submersible dives, the type of habitats surveyed with the ROV (60% hard and 40% soft) were similar to those habitats surveyed with the submersible. Water visibility during submersible dives ranged from 4 to 13 m (as estimated by the submersible pilot during each dive), averaged 8 m, and was greatest at depths >100 m. Observations made from the submersible were limited more by light penetration from the submersible (9 m) than by water visibility. Observations made from the ROV video footage were confined to ~6 m, because

Table 1

The number and percentage of reactions of various fish taxa observed during visual surveys conducted from a manned submersible and remotely operated vehicle (ROV) in 2007 off central California for our study of the reactions of fishes to underwater vehicles. YOY=young-of-the-year.

		Submersible					ROV				
Common name	Scientific name	No. of fish	% of total no. of fish	No. of fish that reacted	% of fish that reacted	Size range of fish (cm)	No. of fish	% of total no. of fish	No. of fish that reacted	% of fish that reacted	Size range of fish (cm)
Bank Rockfish	*Sebastes rufus*	381	4	46	12	10–55	58	0	17	29	20–45
Blackeye Goby	*Rhinogobiops nicholsii*						337	2	91	27	5–15
Blue Rockfish	*Sebastes mystinus*	2504	24	181	7	10–40	6	0	2	33	30
Bocaccio	*Sebastes paucispinis*	342	3	65	19	25–70	98	1	35	36	30–60
Canary Rockfish	*Sebastes pinniger*	139	1	20	14	15–60	63	0	35	56	30–50
Cowcod	*Sebastes levis*	60	1	5	8	25–100	15	0	3	20	45–70
Dover Sole	*Microstomus pacificus*	27	0	0	0	15–40	147	1	21	14	15–50
Greenblotched Rockfish	*Sebastes rosenblatti*	59	1	3	5	20–50	40	0	5	13	15–50
Greenspotted Rockfish	*Sebastes chlorostictus*	154	1	16	10	10–50	190	1	87	46	10–50
Greenstriped Rockfish	*Sebastes elongatus*	126	1	5	4	10–40	164	1	76	46	15–45
Hagfishes	*Eptatretus* spp.	22	0	1	5	20–30	91	1	6	7	20–40
Halfbanded Rockfish	*Sebastes semicinctus*	2646	25	218	8	5–20	9086	56	6431	71	5–25
Pacific Hake	*Merluccius productus*	88	1	17	19	15–40	19	0	15	79	35–45
Pink Seaperch	*Zalembius rosaceus*	7	0	0	0	15–20	261	2	219	84	10–20
Pygmy Rockfish	*Sebastes wilsoni*	1259	12	0	0	5–15	3616	22	1782	49	5–20
Rosethorn Rockfish	*Sebastes helvomaculatus*	190	2	4	2	10–35	29	0	3	10	15–30
Rosy Rockfish	*Sebastes rosaceus*	145	1	9	6	10–30	62	0	11	18	15–35
Shortspine Combfish	*Zaniolepis frenata*	16	0	1	6	15–25	84	1	27	32	15–25
Splitnose Rockfish	*Sebastes diploproa*	458	4	54	12	10–45	441	3	39	9	15–40
Spotted Ratfish	*Hydrolagus colliei*	83	1	16	19	15–50	9	0	6	67	25–60
Squarespot Rockfish	*Sebastes hopkinsi*	769	7	412	54	10–25	283	2	112	40	15–25
Stripetail Rockfish	*Sebastes saxicola*	201	2	5	2	10–35	26	0	4	15	15–40
Thornyheads	*Sebastolobus* spp.	70	1	0	0	10–40	246	2	10	4	10–35
Widow Rockfish	*Sebastes entomelas*	338	3	67	20	15–45	133	1	50	38	20–40
Yellowtail Rockfish	*Sebastes flavidus*	296	3	16	5	25–55	234	1	119	51	20–55
YOY rockfishes	*Sebastes* spp. (YOY)	170	2	0	0	5	420	3	0	0	5
Total		10,550	100	1161	11		16,158	100	9206	57	

of the type of lighting and camera (see *Materials and methods* section).

We used 26 taxa of fishes in the analyses of fish reactions to the submersible and ROV (Table 1). Halfbanded (*Sebastes semicinctus*; 25%), Blue (*S. mystinus*; 24%), and Pygmy (*S. wilsoni*; 12%) Rockfishes were the most abundant species observed from the *Delta* submersible, and Halfbanded (56%) and Pygmy (22%) Rockfishes were most abundant in the ROV survey. In total, observations of 1161 fishes for the *Delta* submersible and 9206 fishes for the ROV were used in the analyses of directional movements and distance of reaction from each vehicle.

Fewer fishes reacted to the manned submersible (11% of all fishes; Table 1) than to the ROV (57% of all fishes). The minimum distance of a fish reaction was 0.5 m from the submersible (96% of reactions were at a distance ≥1 m) and 1 m from the ROV. The percent reaction varied from 0% for several species to 54% for the Squarespot Rockfish (*S. hopkinsi*) observed from the submersible and from 0% for some species to 84% for Pink Seaperch, (*Zalembius rosaceus*) for fishes observed from the ROV. Of those taxa observed from the submersible, only Squarespot Rockfish had a reaction rate of at least 50%. Six taxa observed with the ROV had a reaction rate of at least 50%: Pink Seaperch, Pacific Hake (*Merluccius productus*), Spotted Ratfish (*Hydrolagus colliei*), and Yellowtail (*S. flavidus*), Canary (*S. pinniger*), and Halfbanded Rockfishes. Cowcod, Bocaccio (*S. paucispinis*), and Canary Rockfish are of particular concern to fishery managers and in need of improved assessments (Hilborn et al., 2011; PFMC, 2011). The reaction rate of these 3 species to the submersible ranged from 8% to 19%; their reactions to the ROV varied from 20% to 56%. Thornyheads, YOY rockfishes, and hagfishes had reaction rates <10% to either vehicle. Fishes of 5 taxa did not react at all to the submersible, and 1 group of taxa (YOY rockfishes) that did not react to the ROV.

Fish reactions to both vehicles increased significantly as fish distance above the seafloor increased, and this trend in reaction was greater for the ROV than for the submersible (all fishes combined, $P<0.001$; Table 2; Fig. 3). Only 2% of the fishes observed on the seafloor during submersible surveys (i.e., 27 of 1261 fishes) and 7% observed near the seafloor (i.e., 410 of 6009) reacted to this vehicle. However, 18% of fishes on the seafloor (i.e., 512 of 2895) reacted to the ROV, with Halfbanded Rockfish and Blackeye Goby (*Rhinogobiops nicholsii*) accounting for 71% of these reactions (361 out of 512 fishes that reacted; Table 2). During the ROV surveys, fishes near the seafloor reacted more than fishes in contact with the seafloor (59% versus 18%, respectively), with Halfbanded and Pygmy Rockfishes representing 93% of these reactions (3800 out of 4083 fishes that reacted). Fishes in the midwater, a region defined as >1 m above the seafloor, reacted the most to either vehicle (22% to the submersible and 73% to the ROV). Squarespot and Blue Rockfishes represented 80% of the midwater reactions to the submersible, and Halfbanded and Pygmy Rockfishes accounted for 90% of the reactions of midwater fishes to the ROV. This pattern of greater percentage of reactions with increased height off the seafloor was observed for most individual taxa. Even those species that are primarily demersal, like Cowcod and Greenstriped (*S. elongatus*) and Greenspotted (*S. chlorostictus*) Rockfishes, exhibited this pattern in observations from both the submersible and ROV.

The fishes that demonstrated any type of reaction to each vehicle primarily swam away rather than toward the vehicles (Fig. 4; Table 3, A and B). Only a small percentage (0–8%) of fishes swam toward either vehicle; most of these fishes were Bocaccio near the seafloor, and 19 of 50 of those Bocaccio reacted by swimming toward the submersible. Most fishes either moved away (forward, ahead of the vehicle) or sideways (to the left or right). However, 37% of all fishes in the midwater reacted by swimming downward when initially encountered by the submersible (Table 3A). This group was dominated by Blue, Widow (*S. entomelas*), and Splitnose (*S. diploproa*) Rockfishes (representing 96% of those midwater fishes that reacted by swimming down). Only 13% of all fishes near the seafloor moved downward as the submersible approached; Bocaccio and Widow Rockfish reacted the most in this category (20% and 25% of all fish that reacted, respectively). Only 1% of fishes in the midwater or near the seafloor reacted to the ROV by swimming downward (Table 3B).

The distance at which a fish reaction occurred varied between vehicles (Table 4; Fig. 5). Blue, Halfbanded, Widow, Bank (*S. rufus*), and Splitnose Rockfishes moved at distances >3 m in front of the submersible. These species often were located in the midwater or near the seafloor. However, some species located most often near the seafloor (e.g., Bocaccio and Canary and Squarespot Rockfishes) reacted more often when the vehicle came closer to them (<3 m). Seafloor-dwelling species did not react often to the submersible, and, when they did, there was no clear pattern in reactions related to distance in front of the vehicle. The species that reacted farthest in front of the ROV were Halfbanded, Widow, and Yellowtail Rockfishes, all of which were found near the seafloor or in the midwater. Species that reacted closer (<3 m) to the ROV included fishes living almost entirely on the seafloor (e.g., Blackeye Goby, Shortspine Combfish [*Zaniolepis frenata*], and Greenstriped Rockfish), as well as some near the seafloor and in the midwater (e.g., Bocaccio and Canary, Greenspotted, Rosy [*S. rosaceus*], Splitnose, and Squarespot Rockfishes).

Body length was determined for all fishes that were observed during the submersible surveys (n=10,550), but only 9177 fishes (57%) of all fishes observed in video footage from the ROV surveys were measured. Total length ranged from 5 to 100 cm for fishes observed from the submersible and from 5 to 70 cm for fishes seen during the ROV surveys. Most fishes were

Table 2

Number and percentage of fishes that reacted to the manned submersible and the remotely operated vehicle (ROV) that were used in our surveys in 2007 off central California, relative to the initial position of those fishes: on the seafloor, near (≤1 m above) the seafloor, or in the midwater (>1 m above the seafloor). YOY=young-of-the-year, s=submersible, r=ROV. Significance levels: *=0.05, **=0.01, ***=0.0001.

	Seafloor						Near seafloor						Midwater					
	Submersible			ROV			Submersible			ROV			Submersible			ROV		
	No. of fish	No. of fish that reacted	% of fish that reacted	No. of fish	No. of fish that reacted	% of fish that reacted	No. of fish	No. of fish that reacted	% of fish that reacted	No. of fish	No. of fish that reacted	% of fish that reacted	No. of fish	No. of fish that reacted	% of fish that reacted	No. of fish	No. of fish that reacted	% of fish that reacted
Bank Rockfish (s ***)(r ***)	68	5	7	17	2	12	278	22	8	31	5	16	35	19	54	10	10	100
Blackeye Goby	0	0	0	326	86	26	0	0	0	8	3	38	0	0	0	3	2	67
Blue Rockfish (s ***)	10	0	0	0	0	0	270	1	0	4	1	25	2224	180	8	2	1	50
Bocaccio (s ***) (r *)	40	0	0	21	4	19	264	50	19	34	9	26	38	15	39	43	22	51
Canary Rockfish (r ***)	4	0	0	0	0	0	132	20	15	35	12	34	3	0	0	28	23	82
Cowcod (s **)	49	1	2	5	0	0	11	4	36	10	3	30	0	0	0	0	0	0
Dover Sole (r **)	27	0	0	143	18	13				3	2	67				1	1	3
Greenblotched Rockfish (s **)	54	1	2	24	3	13	5	2	40	16	2	13	0	0	0	0	0	0
Greenspotted Rockfish (s **) (r *)	114	7	6	45	12	27	40	9	23	133	68	51	0	0	0	12	7	58
Greenstriped Rockfish (s **) (r ***)	123	4	3	53	13	25	3	1	33	78	37	47	0	0	0	33	26	79
Hagfishes	21	1	5	88	4	5	1	0	0	3	2	67						
Halfbanded Rockfish (r ***)	44	0	0	921	275	30	2596	218	8	3682	2793	76	6	0	0	4483	3363	75
Pacific Hake	10	0	0	3	1	33	16	2	13	3	2	67	62	15	24	13	12	92
Pink Rockfish (r ***)	1	0	0	1	0	0	6	0	0	55	27	49				205	192	94
Pygmy Rockfish***	5	0	0	61	11	18	1253	0	0	2484	1007	41	1	0	0	1071	764	71
Rosethorn Rockfish (s *) (r **)	163	2	1	19	1	5	27	2	7	9	1	11	0	0	0	1	1	100
Rosy Rockfish	10	1	10	21	3	14	134	8	6	39	8	21	1	0	0	2	0	0
Shortspine Combfish	15	1	7	80	26	33	1	0	0	2	0	0				2	1	50
Splitnose Rockfish (s ***) (r ***)	237	2	1	312	16	5	22	2	9	104	11	11	199	50	25	25	12	48
Spotted Ratfish	7	0	0	0	0	0	75	16	21	5	3	60	1	0	0	4	3	75
Squarespot Rockfish (s ***)	0	0	0	33	15	45	274	12	4	121	53	44	495	400	81	129	44	34
Stripetail Rockfish (s ***)	189	2	1	19	2	11	12	3	25	6	1	17	0	0	0	1	1	100
Thornyheads	70	0	0	246	10	4	0	0	0	0	0	0	0	0	0	0	0	0
Widow Rockfish (s ***)	0	0	0	5	0	0	192	27	14	37	4	11	146	40	27	91	46	51
Yellowtail Rockfish	0	0	0	32	10	31	227	11	5	74	29	39	69	5	7	128	80	63
YOY rockfishes	0	0	0	420	0	0	170	0	0	0	0	0	0	0	0	0	0	0
Total	1261	27	2	2895	512	18	6009	410	7	6976	4083	59	3280	724	22	6287	4611	73

20 cm or less in length (68% of all fishes in the submersible surveys and 89% in the ROV surveys). The fishes that were ≤20 cm in total length accounted for 75% of all reactions observed from the submersible and 94% of all reactions observed in video footage from the ROV surveys.

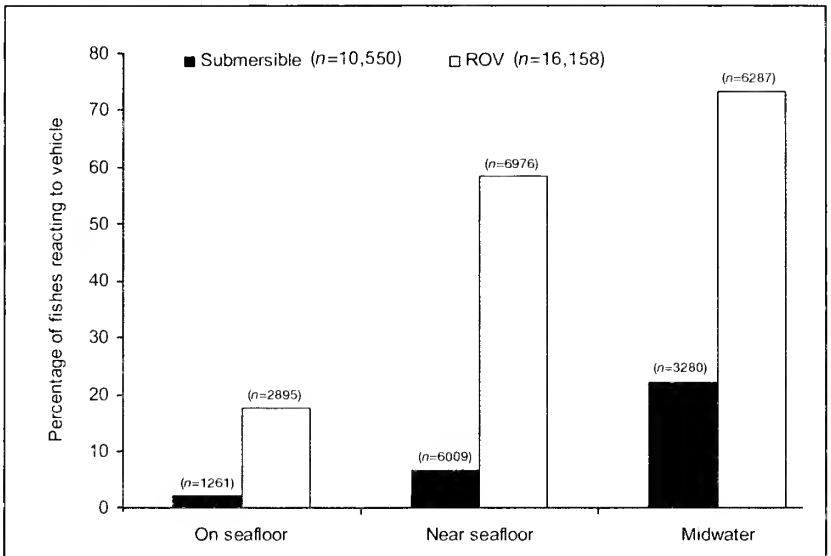

Figure 3

Percentages of fishes that reacted to the manned submersible or the remotely operated vehicle (ROV) that were used in 2007 off central California in our study of the reactions of fishes to underwater vehicles relative to the initial position of those fishes: on the seafloor, near (≤1 m above) the seafloor, or in the midwater (>1 m above the seafloor). The total number of reactions is indicated for each vehicle. Numbers above bars indicate the total number of fishes (i.e., sample size) in each category for each vehicle.

Discussion

Although fishes reacted to both survey vehicles, there were proportionally greater numbers of reactions to the ROV than to the submersible. The ROV and submersible traveled at similar speeds and maintained similar heights off the seafloor, yet substantial differences were observed in fish reactions to the 2 vehicles. Possible reasons for these differences in reactions include the presence of a tether that attaches the ROV to the support ship (the manned submersible is autonomous and untethered), forward lighting on the ROV compared with lighting largely on the starboard side of the submersible, differences in vehicle noise, and disparity in vehicle dimensions.

Both vehicles were much larger than common predators (e.g., large fishes and pinnipeds) of most of these species, and we, therefore, surmise that size alone was not the factor that caused fishes to react. It is possible that the smaller ROV, which was about one-half the height and length of the submersible, appeared to be more like a large predator to the fishes than did the submersible, but this idea is difficult to establish.

The magnitude of pressure waves generated in front of each vehicle could have differed because the submersible was of solid construction and the ROV comprised a frame with attached instruments and a trailing tether. Indeed, pressure waves generated from a deepwater drop-camera system that operated about 130 m above a midwater aggregation of Orange Roughy (*Hoplostethus atlanticus*) off Tasmania caused those fishes to disperse rapidly up to 40 m (Koslow et al. 1995).

Fish reactions to vehicles can also depend on environmental conditions (e.g., type of seafloor sediments, relief, ambient light levels, and water currents) and some attributes of the survey itself (e.g., vehicle speed and height off the seafloor). To reduce the effects of some of these conditions, we surveyed only during daylight hours, in similar habitats, during the same time of the same year, at similar speeds, and at similar heights off the seafloor.

Whatever the reasons that fishes react to survey vehicles, the reaction of the target species must be considered in selection of underwater vehicles to conduct surveys on fish abundance. Population abundance can be either over-or under-estimated if fish reactions to the survey vehicles are not quantified. Once the reaction rates are determined, correction factors can be developed to account for species-specific differences in reaction to the survey vehicles and to adjust resultant abundance estimates. Knowledge of fish reactions associated with each survey tool can help ascertain the most appropriate survey method for target species.

Clear description and quantification of fish reactions to underwater survey vehicles are not common in the literature. From a review of the literature, fish reactions were defined in only 2 of 37 published papers that reported on the reactions of fishes to underwater vehicles (see review in Stoner et al. 2008; Davis et al., 1997; Krieger and Ito, 1999; Else et al., 2002; Moore et al., 2002; Uiblein et al., 2003; Costello et al., 2005; Gartner et al., 2008; Luck and Pietsch, 2008; Benefield et al., 2009; Trenkel and Lorance, 2011; Baker et al., 2012; O'Connell et al.[2]). A fish reaction was defined in one of these 2 articles as a "disturbed" behavior or "differenc-

[2] O'Connell, V., D. Carlile, and C. Brylinsky. 2001. Demersal shelf rockfish stock assessment and fishery evaluation report for 2002. Regional Information Report 1J01-35, 42 p. Alaska Dept. Fish Game, Division of Commercial Fisheries, Juneau, AK.

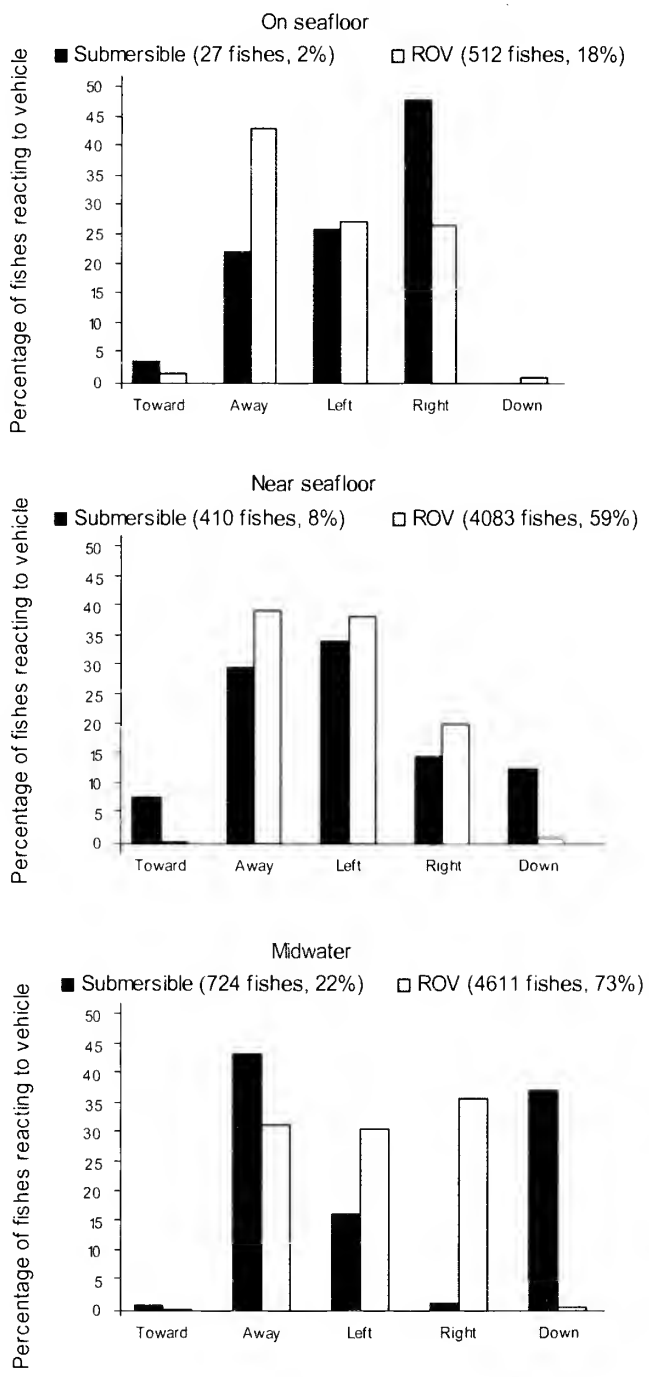

Figure 4

Percentage of fishes that reacted in a particular direction to the manned submersible or the remotely operated vehicle (ROV) that were used in 2007 off central California in our study of the reactions of fishes to underwater vehicles relative to the initial position of those fishes: on the seafloor, near (≤1 m above) the seafloor, or in the midwater (>1 m above the seafloor). Total number of fishes that reacted to each vehicle, and the percentage of the total number of fishes in the survey that reacted, are shown in parentheses for each initial position.

es in natural behavior" (Lorance and Trenkel, 2006) and as "a marked change in activity level and/or locomotion behavior" in the other article (Uiblein et al., 2003). General categories of reactions (such as a fish avoided or was attracted to a vehicle, or a fish had no reaction) were used in 6 studies (Adams et al., 1995; Trenkel et al., 2004a; Trenkel et al., 2004b; Costello et al., 2005; Trenkel and Lorance, 2011, Baker et al., 2012), but specific definitions of the reactions (in contrast to natural movements) were not reported for these studies.

In our surveys, reaction of a nonmoving fish was defined as a distinct movement greater than one body length. We used this proportional measure instead of a specific distance because the total length of observed fishes varied from 5 to 100 cm. The use of our definition of a reaction as at least one body length could be problematic, especially for quantification of relatively small movements. However, in our study, the minimum distance that any fish traveled was 0.5 m in reaction to the submersible (with 96% of these fishes moving 1 m or greater) and 1.0 m in reaction to the ROV. Therefore, the reactions of even the smallest fishes could be readily discerned.

It can be argued that a fish in motion when first seen in a video footage was already moving in reaction to the survey vehicles (Uiblein et al., 2003; Lorance and Trenkel, 2006). In our study, we surveyed numerous benthopelagic species that were slowly moving when first observed in the video footage. Such movement was not considered a reaction unless a fish obviously changed course or speed. Because a fish could not be seen before it came into view on a video footage, it could not be determined if that fish was initially motionless and then reacted as the vehicle approached. This type of behavior could be indicated by signs like a dust cloud where a fish had contact with the seafloor, a fish quickly darting into the video footage, or loose aggregations of fishes moving in many different directions. In our study, these types of behavior were rarely, if ever, observed.

Few quantitative studies have been conducted on fish reactions to a submersible or an ROV, and no direct comparisons between the reactions of specific fish species to a submersible and ROV have been found in the literature. General reactions to an ROV (fishes moving into and out of a video frame) were quantified during surveys on mud habitats off central California (Adams et al., 1995). In that study, most fishes that occurred on the seafloor did not react to a relatively large working-class ROV, although 2 species typically observed off the seafloor exhibited avoidance behavior: 44% of all

Table 3A

Number of fishes that reacted in a particular direction (toward, away, etc.) to a manned submersible in our surveys in 2007 off central California, relative to the initial position of those fishes: on the seafloor, near (≤1 m above) the seafloor, or in the midwater (>1 m above the seafloor). YOY=young-of-the-year.

	Seafloor							Near seafloor							Midwater						
			No. of fish that reacted							No. of fish that reacted							No. of fish that reacted				
Fish name	No. of fish	No. that reacted	Toward	Away	Left	Right	Down	No. of Fish	No. that reacted	Toward	Away	Left	Right	Down	No. of Fish	No. that reacted	Toward	Away	Left	Right	Down
Bank rockfish	68	5	1	2		2		278	22	1	2	7	10	2	35	19			15	1	3
Blackeye goby	0	0																			
Blue rockfish	10	0						270	1				1		2224	180					180
Bocaccio	40	0						264	50	19		3	8	20	38	15		15			
Canary rockfish	4	0						132	20		11	1	7	1	3	0					
Cowcod	49	1				1		11	4	3				1							
Dover sole	27	0						0	0												
Greenblotched rockfish	54	1			1			5	2				2								
Greenspotted rockfish	114	7		2	1	4		40	9		2	2	4	1							
Greenstriped rockfish	123	4			2	2		3	1		1										
Hagfish	21	1			1			1	0												
Halfbanded rockfish	44	0						2596	218	1	86	115	16		6	0					
Pacific hake	10	0						16	2			2			62	15	1		2	3	9
Pink surfperch	1							6	0												
Pygmy rockfish	5	0						1253	0						1	0					
Rosethorn rockfish	163	2				2		27	2	1				1							
Rosy rockfish	10	1			1			134	8	3	1	3	1		1	0					
Shortspine combfish	15	1			1			1	0												
Splitnose rockfish	237	2		2				22	2					2	199	50	6	1	3	1	39
Spotted ratfish	7	0						75	16	2	1	6	7		1	0					
Squarespot rockfish								274	12		11		1		495	400		300	100		
Stripetail rockfish	189	2				2		12	3		1		2								
Thornyhead	70	0																			
Widow rockfish								192	27		1		1	25	146	40					40
Yellowtail rockfish								227	11	2	6	2	1		69	5		1		4	
YOY								170	0												
Total	1261	27	1	6	7	13	0	6009	410	32	123	141	61	53	3280	724	7	317	120	9	271
Percent		2.1	4	22	26	48	0		6.8	8	30	34	15	13		22.1	1	44	17	1	37

Table 3B

Number of fishes that reacted in a particular direction to a remotely operated vehicle (ROV) in our surveys in 2007 off central California, relative to the initial position of those fishes: on the seafloor, near (≤1 m above) the seafloor, or in the midwater (>1 m above the seafloor). YOY=young-of-the-year.

	Seafloor							Near seafloor							Midwater						
			No. of fish that reacted							No. of fish that reacted							No. of fish that reacted				
Fish name	No. of fish	No. that reacted	Toward	Away	Left	Right	Down	No. of fish	No. that reacted	Toward	Away	Left	Right	Down	No. of fish	No. that reacted	Toward	Away	Left	Right	Down
Bank rockfish	17	2		2				31	5			2	3		10	10			1	8	1
Blackeye goby	326	86	2	24	31	28	1	8	3			2	1		3	2			2		
Blue rockfish								4	1				1		2	1	1				
Bocaccio	21	4		3	1			34	9		2	3	4		43	22		3	7	12	
Canary rockfish								35	12		6	1	5		28	23		1	9	13	
Cowcod	5							10	3		2		1								
Dover sole	143	18		7	4	7		3	2		1		1		1	1		1			
Greenblotched rockfish	24	3	1	2				16	2		1		1								
Greenspotted rockfish	45	12		8	1	2	1	133	68	5	42	10	8	3	12	7	2	2	2	1	
Greenstriped rockfish	53	13		5	2	6		78	37		19	13	5		33	26		3	14	9	
Hagfish	88	4			4			3	2			1	1								
Halfbanded rockfish	921	275	1	137	72	64	1	3682	2793	9	1114	1062	590	18	4483	3363	14	929	1081	1332	7
Pacific hake	3	1		1				3	2		1		1		13	12		1	2	7	2
Pink surfperch	1	0						55	27	1	9	7	10		205	192	1	5	35	151	
Pygmy rockfish	61	11			2	9		2484	1007	1	381	442	179	4	1071	764	3	465	211	77	8
Rosethorn rockfish	19	1		1				9	1			1			1	1		1			
Rosy rockfish	21	3	1	2				39	8		7	1			2						
Shortspine combfish	80	26	1	8	12	5		2	0						2	1		1			
Splitnose rockfish	312	16		9	3	4		104	11		10		1		25	12		3	5	3	1
Spotted ratfish								5	3		1	1	1		4	3		1		2	
Squarespot rockfish	33	15		3	6	3	3	121	53	2	8	18	15	10	129	44		19	17	2	6
Stripetail rockfish	19	2		2				6	1				1		1	1			1		
Thornyhead	246	10	1	5		4															
Widow rockfish	5							37	4	1		2	1		91	46	1	24	13	8	
Yellowtail rockfish	32	10	1	2	3	4		74	29	1	12	9	6	1	128	80		9	29	41	1
YOY	420	0																			
Total	2895	512	8	221	141	136	6	6976	4083	20	1616	1575	836	36	6287	4611	22	1468	1429	1666	26
Percent		17.7	2	43	28	27	1		58.5	0	40	39	20	1		73.3	0	32	31	36	1

Table 4

Number of fishes reacting within 3 m or >3 m from the front of the manned submersible and the remotely operated vehicle (ROV). "Position" refers to the location where each fish taxon was most frequently observed. S=on the seafloor, N=near the seafloor, M=in midwater (>1 m above seafloor). The total number of fishes (*n*) observed is indicated for each vehicle.

		Submersible (*n*=10,550)			ROV (*n*=16,158)		
		Number of reacting fishes			Number of reacting fishes		
	Position	<3 m	>3 m	Total	<3 m	>3 m	Total
Bank Rockfish	N	8	38	46	14	3	17
Blackeye Goby	S			0	74	17	91
Blue Rockfish	M	2	179	181	0	2	2
Bocaccio	N	57	8	65	30	5	35
Canary Rockfish	N	18	2	20	32	3	35
Cowcod	S	3	2	5	2	1	3
Dover Sole	S			0	10	11	21
Greenblotched Rockfish	S	2	1	3	5	0	5
Greenspotted Rockfish	S, N	10	6	16	55	32	87
Greenstriped Rockfish	S	2	3	5	66	10	76
Hagfishes	S	1	0	1	4	2	6
Halfbanded Rockfish	N, M	84	134	218	1258	5173	6431
Pacific Hake	M	11	6	17	13	2	15
Pink Seaperch	N, M			0	211	8	219
Pygmy Rockfish	N			0	1201	581	1782
Rosethorn Rockfish	M	0	4	4	2	1	3
Rosy Rockfish	N	7	2	9	11	0	11
Shortspine Combfish	S	1	0	1	23	4	27
Splitnose Rockfish	S, N, M	14	40	54	29	10	39
Spotted Ratfish	N	10	6	16	4	2	6
Squarespot Rockfish	N, M	301	111	412	68	44	112
Stripetail Rockfish	S	2	3	5	2	2	4
Thornyheads	S			0	4	6	10
Widow Rockfish	N, M	26	41	67	4	46	50
Yellowtail Rockfish	N, M	12	4	16	37	82	119
YOY Rockfishes	S, N			0			0
Total		571	590	1161	3159	6047	9206
Percentage of fish reactions		49	51		34	66	
Percentage of all fishes		5	6		20	37	

Sablefish (*Anoplopoma fimbria*) and 39% of all Pacific Hake. Lorance and Trenkel (2006) observed that all 8 taxa seen in the Bay of Biscay, in habitat types ranging from flat to gentle slopes and from fine sediments to boulders, reacted to a large working-class ROV with rates from 10% to 90%. Uiblein et al. (2003), also in the Bay of Biscay, worked with a 3-person submersible to study fish behavior and found that most of the 7 more abundant taxa reacted to the vehicle by markedly changing their activity level. Two species observed in both of these studies (Roundnose Grenadier [*Coryphaenoides rupestris*] and Orange Roughy) reacted more often to the ROV than to the submersible.

In our study, fishes that lived in the midwater above the seafloor reacted to both the *Delta* submersible and the Phantom ROV at a higher rate than did fishes on the seafloor. Similar results have been reported in other studies. Krieger and Ito (1999) observed that all Shortraker (*Sebastes borealis*) and Rougheye (*S. aleutianus*) Rockfishes that occurred above the seafloor reacted by swimming toward the seafloor as the *Delta* submersible approached, but only 5 out of the 531 recorded fishes of these 2 species moved when initially seen on the seafloor. Lorance and Trenkel (2006) examined the reactions of 8 fish taxa in the Bay of Biscay and observed that most species reacted to the working-class ROV; only the seafloor-dwelling, deep-sea Atlantic Thornyhead (*Trachyscorpia cristulata echinata*) had little reaction to the vehicle. In that study, 2 of the 3 taxa that had the greatest reactions (shark species of the order Squaliformes and the family Scyliorhinidae) were commonly encountered as they swam high in the water column. Adams et al. (1995)

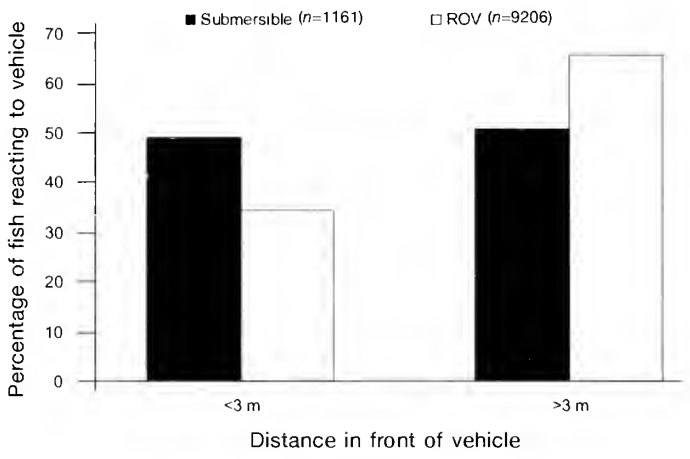

Figure 5

Percentage of fishes that reacted at a specific distance in front of the manned submersible and the remotely operated vehicle (ROV). These percentages were used in 2007 off central California in our study of the reactions of fishes to underwater vehicles. The total number of reactions (n) is indicated for each vehicle.

used a working-class ROV and Starr et al. (1996) used the *Delta* submersible to estimate fish abundance; both studies determined that these vehicles were not reliable in assessment of the abundance of fishes well above the seafloor.

Conclusions

What are the implications of the reaction of a fish to a survey vehicle? If the reaction occurs over a small distance and the fish remains inside the survey transect, then the fish would be counted and its reaction would not affect the outcome of the survey. However, some reactions (both large and small in magnitude) could cause a fish to move out of the survey transect or out of view (e.g., into a hole or behind a rock)—behavior that would, thereby, bias the resultant abundance estimate. Similarly, overestimates of abundance could be made if a fish moves into a transect because of its reaction to a survey vehicle.

Reactions of the target species need to be considered in selection of a survey vehicle, and the limitations of vehicles need to be evaluated relevant to the goals of a study. For instance, a comparative study can be undertaken to estimate abundance and reaction rates of fish species with various underwater vehicles (e.g., a submersible, ROV, camera sled, an autonomous underwater vehicle, or drop camera) within a specific survey area or over particular transects. From this type of study, the reaction of fishes and abundance estimates can be ascertained for each vehicle, thereby aiding in the selection of an appropriate survey vehicle for target species and environmental conditions. Through such efforts, researchers will gain a better understanding of the effectiveness and limitations of potential survey vehicles.

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Seabed classification for trawlability determined with a multibeam echo sounder on Snakehead Bank in the Gulf of Alaska

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Abstract—Rockfishes (*Sebastes* spp.) tend to aggregate near rocky, cobble, or generally rugged areas that are difficult to survey with bottom trawls, and evidence indicates that assemblages of rockfish species may differ between areas accessible to trawling and those areas that are not. Consequently, it is important to determine grounds that are trawlable or untrawlable so that the areas where trawl survey results should be applied are accurately identified. To this end, we used multibeam echosounder data to generate metrics that describe the seafloor: backscatter strength at normal and oblique incidence angles, the variation of the angle-dependent backscatter strength within 10° of normal incidence, the scintillation of the acoustic intensity scattered from the seafloor, and the seafloor rugosity. We used these metrics to develop a binary classification scheme to estimate where the seafloor is expected to be trawlable. The multibeam echosounder data were verified through analyses of video and still images collected with a stereo drop camera and a remotely operated vehicle in a study at Snakehead Bank, ~100 km south of Kodiak Island in the Gulf of Alaska. Comparisons of different combinations of metrics derived from the multibeam data indicated that the oblique-incidence backscatter strength was the most accurate estimator of trawlability at Snakehead Bank and that the addition of other metrics provided only marginal improvements. If successful on a wider scale in the Gulf of Alaska, this acoustic remote-sensing technique, or a similar one, could help improve the accuracy of rockfish stock assessments.

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Rockfish (*Sebastes* spp.) stocks are difficult to assess because of their propensity to aggregate near the seafloor in areas that are difficult to trawl, such as rocky, cobble, or generally rugged areas. Consequently, data from bottom-trawl surveys conducted in trawlable areas typically are extrapolated to all areas within the boundaries of a survey, regardless of whether the seafloor is trawlable or not (Wakabayashi et al., 1985). Such extrapolation may result in biased biomass indices if, for example, there is a shift in biomass between strata with variable but unknown amounts of untrawlable seafloor (Cordue, 2006). Evidence also indicates that species assemblages differ between trawlable and untrawlable areas (Matthews and Richards, 1991; Jagielo et al., 2003; Rooper et al., 2010), and remote-sensing techniques with acoustic or optical sensors may be able to help identify these differences. Equally important is the need to have a quantitative assessment of those grounds that are trawlable or untrawlable to more accurately estimate the areas where the results of different stock assessment methods are valid.

In many bottom-trawl surveys, trawlability has been assessed through the subjective interpretation of normal-incidence backscatter (echoes) from downward-looking single-beam echo sounders. These backscatter echoes are examined by vessel captains with different levels of experience, with different echo sounders, and with different echosounder settings. Multibeam echo sounders (MBES), which have been successful previously for characterizion of the seafloor for the purposes of mapping habitat and surficial geology (e.g., Kostylev et al., 2001; Goff et al., 2004; Brown and Blondel, 2009), may offer an alternative solution for assessment of trawlability. In addition to the wider, high-precision coverage of the seafloor that results from the use of multiple beams, MBES offer the potential for more accurate discrimination between different types of seafloor substrate (e.g., silt, sand, cobble, and rock) than does the use of downward-looking single beams because of the angle-dependent na-

ture of the seafloor backscatter strength, S_b. For example, the normal-incidence (i.e., 0° incidence angle) S_b that would typically be expected for both cobble and fine sand are predicted to be very similar but are appreciably different at increased incidence angles (Fig. 1). Angle-dependent metrics that describe the backscatter from the seafloor have been extracted from MBES data in previous studies to determine the nature of seafloor sediments (e.g., Fonseca and Mayer, 2007).

Seafloor backscatter collected with an MBES, as are the predictions shown in Figure 1, are often treated as the ensemble average of a large number of random realizations of scattered acoustic intensity. Higher order statistics that describe the scattered intensity may also provide information that can be used to characterize the seafloor. Often, the amplitude of the backscatter echoes is expected to follow a Rayleigh distribution, with the underlying assumption that there are a large number of contributors to the backscatter from the seafloor at any instant in time (Jackson and Richardson, 2007). Abraham and Lyons (2002) have linked heavy-tailed, non-Rayleigh distributions of backscatter to a model with a relatively small number of objects on the seafloor that have high levels of backscatter strength. In other words, the details of the probability density function that describe the amplitude of the acoustic echoes are likely to be related to the size and density of the scattering objects and their relative role in the overall scattering response. Measures that indicate non-Rayleigh backscatter may give an indication of distributed cobble or rock that would render a seafloor untrawlable.

In this study, we examined the angle-dependent nature of S_b, as well as measures of non-Rayleigh distribution of the backscatter and the seafloor rugosity (roughness) derived from bathymetric soundings, in an attempt to discriminate between trawlable and untrawlable seafloors. The data were collected with a Simrad[1] ME70 MBES (Kongsberg AS, Horten, Norway) at a study area on Snakehead Bank in the Gulf of Alaska, ~100 km south of Kodiak Island (Fig. 2). To test the efficacy of the acoustic measures as classifiers of the seafloor as either trawlable or untrawlable, we compared metrics derived from a MBES with observations collected with a stereo drop camera (SDC) system (Williams et al., 2010) along with cameras mounted on a remotely operated vehicle (ROV) (Rooper et al., 2012). The results of this comparison were then extracted to the entire multibeam data set that was collected with the Simrad ME70 during our Snakehead Bank surveys.

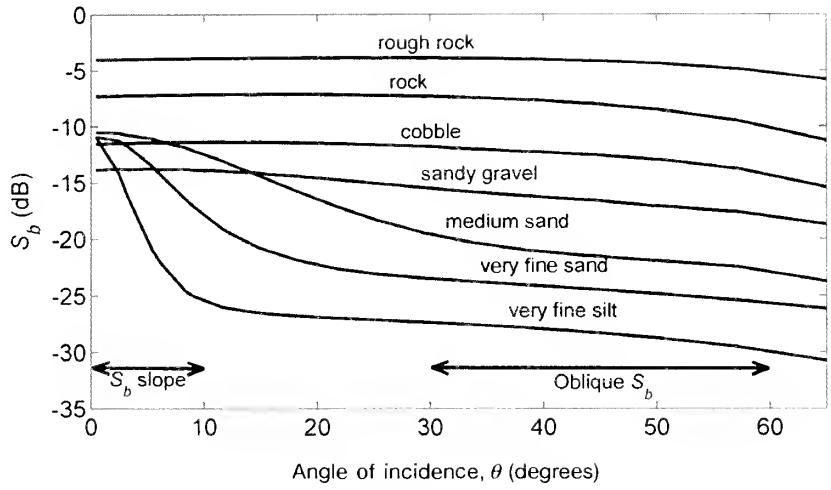

Figure 1

A prediction of the angle-dependent seafloor backscatter strength, S_b (dB), according to APL [1994], for the beam configuration used for the Simrad ME70 multibeam echo sounder at Snakehead Bank in the Gulf of Alaska during a cruise of the NOAA Ship *Oscar Dyson* in October 2009. The areas over which the oblique-incidence S_b and the slope of the angle-dependent backscatter within 10° of normal incidence (S_b-slope) were calculated are shown. Normal-incidence S_b was calculated at 0° incidence angle.

Methods

MBES data were collected with a Simrad ME70 MBES mounted on the hull of the NOAA ship *Oscar Dyson*. The Simrad ME70 was developed specifically for fisheries applications (Trenkel et al., 2008), although it also has been used for bathymetric mapping (e.g., Cutter et al., 2010). The Simrad ME70 is configurable in terms of 1) the number of beams generated, 2) acoustic frequency for each beam, and 3) direction and opening angle of the beams. For our surveys at Snakehead Bank, the Simrad ME70 was configured to generate 31 beams at frequencies ranging from 73 to 117 kHz and at beam opening angles that ranged from 2.8° to 11.0°. The 31 beams were steered to 0° in the alongship direction and from −66° to +66° in the athwartship direction, with the lowest frequencies steered to the highest beam steering angles to mimimize the possibility of ambiguities associated with grating lobes (angular regions within a beam pattern of a transducer array that have equal sensitivity to the main angular region, or lobe, and cause ambiguities in the determination of

[1] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

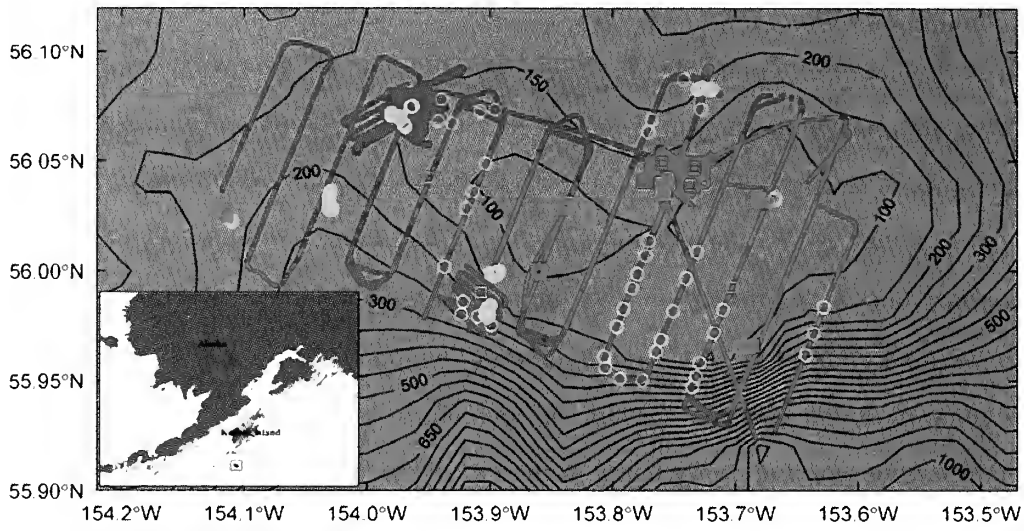

Figure 2

The study area at Snakehead Bank in the Gulf of Alaska, south of Kodiak Island. Bathymetric contours are drawn at 50-m intervals. The locations where data were collected in 2009 with a Simrad ME70 multibeam echo sounder from the large-scale trackline and during focused surveys are shown in red (classified as untrawlable) and blue (classified as trawlable). Camera data collected in 2009 and 2010 with a stereo drop camera and a remotely operated vehicle are shown as green squares (untrawlable) and cyan circles (trawlable).

target angle direction; the occurrence of grating lobes is specific to the design of the transducer array that generates beams). A pulse duration of 1.5 ms was used for each beam. During transmission and reception, the beam-pointing directions were compensated for pitch and roll of the ship with a GPS-aided inertial motion unit (IMU). The IMU was also used to georeference the data collected with the MBES. The standard target method was used to calibrate the combined transmit-receive sensitivity of each beam (Foote et al., 1987).

In comparison with the Simrad ME70, most hydrographic MBES are capable of generating an order of magnitude more beams with beam opening angles of a fraction of a degree and, therefore, produce a relatively high density of bathymetric soundings and measurements of seafloor backscatter. To achieve a similarly high density of data with fewer beams, we processed the Simrad ME70 data with a hybrid multibeam and phase-differencing technique (Lurton, 2010) that provided hundreds of independent seafloor soundings (each of which was associated with a measure of S_b) over a swath that nominally covered ±60°. At beam angles away from normal incidence, the insonified portion of the seafloor (the area on the seafloor defined by the intersection of the sonar pulse within the beam pattern of the transducer array) acts as a discrete target; therefore, each beam was processed as if it were a phase-measuring bathymetric sonar (Lurton, 2010, section 8.2.3). Because this approach is more accurate at higher incidence angles (Jin and Tang, 1996), a weighted mean amplitude detection (Lurton, 2010, section 8.3.3) was used for beams with incidence angles of only a few degrees. For our data, the transition between these 2 bottom detection approaches corresponded to an incidence angle of approximately 15°. The raw soundings were then merged with vessel position and attitude data and corrected for refraction through the water column. The georeferenced soundings were used to extract the rugosity in a grid of 25-m squares, or cells, by computing the ratio of the observed surface area within each grid cell to the area of a plane fitted to the same data.

A measure of the acoustic power was associated with each bottom detection and was converted to S_b by accounting for system gains and calibration offsets, spherical spreading and absorption in the water column, and area insonified. Area insonified was estimated with the assumption that the seafloor was flat and with the method described by Lurton (2010, section 3.4.3). Applications of these radiometric corrections provided a realization of the angle-dependent seafloor backscatter, which was used to help characterize the seafloor, on each ping. Figure 1 shows predictions of the angle-dependent S_b for different substrate types that range from very fine silt to rough rock, on the basis of a scattering model that includes estimates for acoustic impedance, seafloor roughness, and sediment volume scattering strength (APL, 1994). In general, it can be difficult to disambiguate between the different factors that underlie these scattering curves (Fonseca and Mayer, 2007), but they do offer some separation between different substrate types. On the basis of an

examination of the predictions of S_b shown in Figure 1, 3 different metrics that describe S_b were used, similar to those of Fonseca and Mayer (2007): the normal-incidence S_b, the slope of the angle-dependent backscatter within 10°of normal incidence (S_b-slope), and the average oblique-incidence S_b (30° <θ< 60°).

The acoustic power associated with each bottom detection also was converted to acoustic backscatter intensity and used to derive an estimate of the scintillation index, *SI*, which is defined here as

$$SI = \frac{\sigma_I^2}{\mu_I^2}, \quad (1)$$

where σ_I^2 and μ_I^2 = the variance and mean of the backscatter intensity, respectively.

The *SI* is a measure of how the backscatter intensity fluctuates: for Rayleigh-distributed backscatter, the *SI* is equal to 1; for heavier tailed distributions that are a potential indicator of a relatively few strong scatterers contributing to the backscattered echo, the *SI* would be >1. The *SI* was calculated independently for each beam with a minimum of 50 samples (pings) and then averaged across beams. One important caveat to such *SI* estimation is that it is dependent on the sonar footprint on the seafloor (Abraham and Lyons, 2004), which changes as a function of incident angle and seafloor depth for MBES. To reduce changes in *SI* that were associated with the sonar footprint rather than the substrate type, we used only the beam angles between 34° and 50° to generate this parameter. This restriction of angles essentially reduced the resolution to that of a single multibeam swath.

The MBES data were compared with image data (both video and still images) from an SDC and a ROV. The SDC contained identical Sony TRD-900 camcorder units (Sony Corp., Tokyo, Japan) capable of collecting progressive scan video images at a pixel resolution of 1280×720. Both SDC camcorder units were mounted on a sled in an aluminum frame and lowered to the seafloor with a dedicated winch, and illumination was provided by 2 lights mounted above the camera housings inside the aluminum frame (Williams et al., 2010). MBES data also were compared with data collected with a Phantom DS4 ROV (Deep Ocean Engineering, Inc., San Jose, CA). Video footage was recorded from the ROV with a forward-looking color camera (Sony FCB-IX47C module with 470 lines of horizontal resolution and 18× optical zoom). Two pairs of parallel lasers on the ROV were used to estimate substrate size and horizontal field of view.

Data were collected during 3 cruises conducted at Snakehead Bank, south of Kodiak Island in the Gulf of Alaska (Fig. 2). During the first cruise, the *Oscar Dyson* and the FV *Epic Explorer*, a commercial fishing vessel, visited the study site on 4–12 October 2009. Data were collected aboard the *Oscar Dyson* with the Simrad ME70 and ROV, and data were collected with the stereo drop camera aboard the *Epic Explorer*. Several repeat large-scale surveys were conducted with The *Oscar Dyson* along a series of parallel transect lines spaced 2.2 km (1.2 nmi) apart and 9.3–14.8 km (5–8 nmi) long. Three of these surveys were used for this analysis. In addition to the large-scale surveys, 4 small-scale, focused surveys were conducted in the same area during the first of the 3 cruises. The focused surveys were designed to achieve "full coverage" (i.e., no unsampled regions of the seafloor) of the seafloor with the Simrad ME70 in areas where a relatively strong indication of fish had been observed in the acoustic data. For the small-scale surveys, transects were 1.9–3.7 km (1–2 nmi) long and spaced 0.2–0.4 km (0.1–0.2 nmi) apart (depending on the water depth).

The drop camera was deployed 9 times during the October 2009 cruise, and locations were chosen where the acoustic data indicated that rockfishes were most abundant. During each of the drop-camera deployments, the camera sled moved over the bottom at speeds of <1.5 kn as the *Epic Explorer* drifted along transects that lasted up to 1 h and, as a result, collected relatively dense data in 9 small regions. The horizontal field of view of the drop camera averaged 2.43 m (standard error of the mean [SE]=0.14).

The ROV was deployed in 5 different areas where the acoustic data indicated that rockfishes were most abundant. Each deployment lasted for a few hours. The horizontal field of view for the ROV averaged 2.61 m (SE=0.20).

During the other 2 cruises in March and June of 2010, the study site was revisited and the SDC deployed 51 times aboard the *Oscar Dyson*. During these additional deployments, the seafloor was recorded in only 1 of the 2 available stereo cameras, preventing collection of stereographic images. Each of these deployments was short: the drop camera was deployed to the bottom for a couple of minutes before it was retrieved to the surface. The resulting images were all from single, small patches (<25 m radius) of seafloor, rather than from the drift transects described for the first cruise.

The seafloor substrate observed during the underwater video transects was classified with a commonly used scheme (Stein et al., 1992; Yoklavich et al., 2000). The classification consisted of 2-letter codes for substrate types that denoted a primary substrate with >50% coverage of the seafloor bottom and a secondary substrate with 20–49% coverage of the seafloor. There were 7 identified substrate types: mud (M), sand (S), pebble (P, diameter <6.5 cm), cobble (C, diameter 6.5–25.5 cm), boulder (B, diameter >25.5 cm), exposed low-relief bedrock (R), and exposed high-relief bedrock and rock ridges (K). The size of substrate particles was measured or estimated from a known horizontal field of view (~2.4 m) for the SDC and estimated with a paired laser system for the ROV. With this classification scheme, a section of seafloor covered primarily in cobble but with boulders over more than 20% of the surface would receive the substrate-type code cobble-

boulder (Cb), with the secondary substrate indicated by the lower-case letter. Because the video collected with the SDC and ROV provided a continuous display of substrata, the substrate-type code was changed only if a substrate type encompassed more than 10 consecutive seconds of video.

For this study, the substrate observed in the underwater video transects was further classified as either untrawlable or trawlable with reference to the standard Poly-Nor'eastern 4-seam bottom trawl used in biennial bottom-trawl surveys of the Gulf of Alaska and Aleutian Islands by the Alaska Fisheries Science Center (Stauffer, 2004). The Poly-Nor'eastern bottom-trawl footrope comprised 10-cm disks interspersed with bobbins 36 cm in diameter. The untrawlable areas were defined as any substrate containing boulders that reached >20 cm off the bottom of the seafloor or any substrate with exposed bedrock that was so rough that the standard bottom-trawl footrope would not easily pass over it. Therefore, the trawlable grounds were those areas mostly composed of small cobble, gravel, sand, and mud without interspersed boulders or jagged rocks. The untrawlable grounds were those areas that contained any boulder or high-relief rock substrates. The same experienced observer classified the substrate for both the ROV and SDC video transects.

The video data thus classified were partitioned in a grid of 25-m squares, or cells—a length scale that is a rough estimate for the accuracy of the positioning systems associated with both video systems. The primary and secondary substrate types were given a numeric value based on a nominal substrate size, and each grid cell was assigned substrate types associated with the median values for all data within the cell boundaries. Grid cells also were assigned as trawlable or untrawlable if all data within a cell supported such a classification; otherwise, the grid cell was assigned a "mixed" classification. The gridded video classifications were then compared with the seafloor parameters (e.g., rugosity or normal-incidence S_b) derived from data collected with the Simrad ME70, where both types of data existed at the same position, to provide an indication of how each acoustically derived seafloor parameter was able to discriminate between trawlable and untrawlable areas. This comparison was done for each parameter separately and then done for various combinations of parameters to find a combination of parameters that best discriminated between trawlable and untrawlable substrate. For each parameter, a t-test was used to determine whether it was able to distinguish between trawlable and untrawlable seafloor at the significance level of α=0.05 (i.e., where erroneous rejection of the null hypothesis is expected 5% of the time), and values of standard difference (the difference between the sample means divided by the pooled standard deviation) were computed. When combinations of parameters were tested, a best-fit separation (for the goal of minimizing the classification error rate) within the multidimensional parameter space was found through examination of the entire parameter space. To maintain a clear link back to the underlying data distribution, the separation between trawlable and untrawlable was assumed to be a line, plane, or hyperplane (a generalization of a plane into more than 2 dimensions), depending on the dimension of the parameter space.

Results

The data showed a wide range of values and, presumably, associated substrate types. The shallowest (<100-m) portion of Snakehead Bank contained the highest oblique-incidence S_b (approximately –12 dB). This region contained similar values for the normal-incidence S_b, and small S_b-slope (<0.75 dB/°). Taken together, these data indicate a cobble seafloor on the top of the bank. On the northeastern side of the bank at depths ~200 m, the oblique-incidence S_b reached its lowest value of approximately –30 dB with a normal-incidence S_b of –15 dB and S_b-slope of ~1.1 dB/°—values consistent with a substrate composed of very fine silt.

The region with the highest normal-incidence S_b (–10 to –7 dB) occurred between 154°W and 153.9°W and near 56.07°N in the northwest region of the bank. The S_b-slope was also high in this region, reaching up to 1.5 dB/°, and the oblique-incidence S_b was between –18 dB and –15 dB. These results for the seafloor parameters are confounding, given that the S_b-slope was large enough to indicate a fine sand or silt, but the normal-incidence and oblique-incidence S_b both indicated a coarser sediment or a higher-than-anticipated volume scatter contribution due to heterogeneities or gas (Jones et al., 2012) within the sediment.

The *SI* shows a complicated pattern that did not appear to be well correlated with any certain substrate type, although there were large (hundreds of meters) contiguous regions that exhibited high *SI* values (i.e., the data did not appear to be simply random noise). The rugosity levels show the bank to be relatively smooth along the top, except at a sharp transition along its northeastern edge between the 100- and 150-m contours. The rugosity analysis also indicates the appearance of what may be large (wavelength ~150 m) sand waves in the extreme southeastern portion of the study area and smaller pockmarks in the southwestern portion of the study area.

The results of a comparison of the seafloor parameters derived from the backscatter data that was collected with the Simrad ME70 and the substrate types derived from the data collected with the SDC and ROV are shown in Figure 3. These data show that, although substrate types Bb, Cb, and Gb are difficult to distinguish with backscatter parameters, these 3 types are clearly separate from substrate type Ss. The oblique-incidence S_b values for substrate type Ss appeared to be bimodal, with the majority of the values residing between –17 and –15 dB and a substantial number of values between –29 and –26 dB. According to the notional

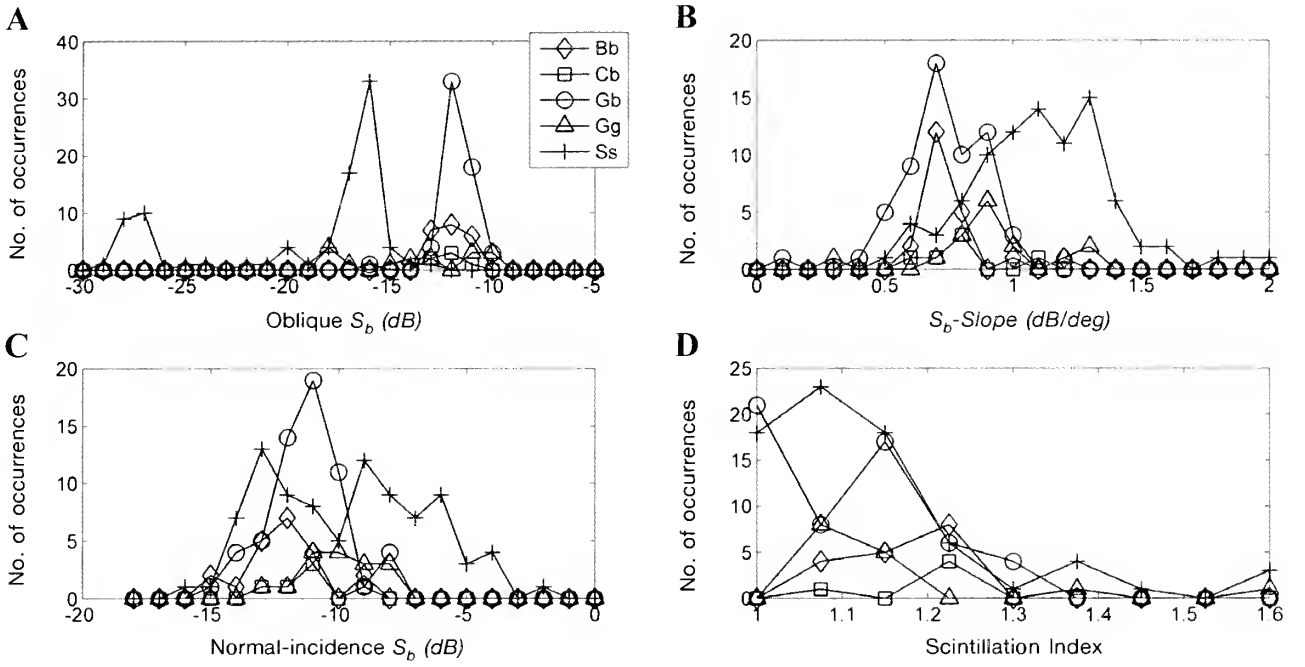

Figure 3

The frequencies of occurrence for major and minor substrate combinations, classified from the data collected in 2009 and 2010 with a stereo drop camera and a remotely operated vehicle as a function of different seafloor characteristics derived from the data collected with a Simrad ME70 multibeam echo sounder. Major (capital letter) and minor (lowercase letter) substrate types included Bb=boulder; C=cobble; Gg=gravel; and Ss=sand.

values shown in Figure 1, these 2 regions would correspond to sandy gravel and very fine silt, respectively. The lower set of oblique-incidence S_b values were found in the deepwater off the northern side of the bank at depths of 200–250 m and also on the south side of the bank at depths of 120–150 m. On average, the largest S_b-slope and the widest range of normal-incidence S_b were observed on sandy substrate. The normal-incidence S_b for areas classified as sandy substrate extended to ranges higher than would be expected, a finding that could be a result of unusually high volume-backscatter caused by gas or heterogeneities within the sediment volume. The harder substrates (Bb and Cb) all had small S_b-slope, as expected, and on average had higher *SI* than the sandy sediments.

To determine how each parameter discriminated between trawlable or untrawlable seafloor, using classified SDC and ROV video data as verification, the frequencies of occurrence for each parameter were extracted for each substrate type (Fig. 4). *T*-tests indicated that the distributions of trawlable and untrawlable areas of seafloor were distinguishable at the α=0.05 significance level (Table 1), although each parameter did not perform equally when discriminating between the 2 classifications. The 3 best individual discriminators were the normal-incidence S_b, S_b-slope, and the oblique-incidence S_b with standard differences of 0.74, 1.12, and 1.89, respectively. Of these 3 parameters, the oblique-incidence S_b demonstrated the clearest separation between trawlable and untrawlable seafloor, with a boundary at −13.4 dB. According to modeled data (Fig. 1), this S_b level discriminates cobble and rock from gravel, sand, and silt. The *SI* and rugosity were separated less well with standard differences of 0.25 for each.

With the oblique-incidence S_b considered alone, the combined error rate (erroneous classifications of both trawlable and untrawlable seafloor) reached a minimum of 5.6% (n=303) with a boundary set at S_b=−13.4 dB. To determine whether this error rate could be lowered, additional parameters derived from the data collected with the Simrad ME70 were linearly combined with the oblique-incidence S_b. Figure 5 shows the combination of the oblique- incidence S_b with each of these other parameters, along with a line that best discriminated between the trawlable and untrawlable classifications. The largest reduction in classification error rate was achieved when the oblique-incidence S_b was combined with either the normal-incidence S_b or the *SI*, both of which had a marginally improved error rate of 5.0%. When 3 parameters were combined to discriminate between trawlable and untrawlable sea-

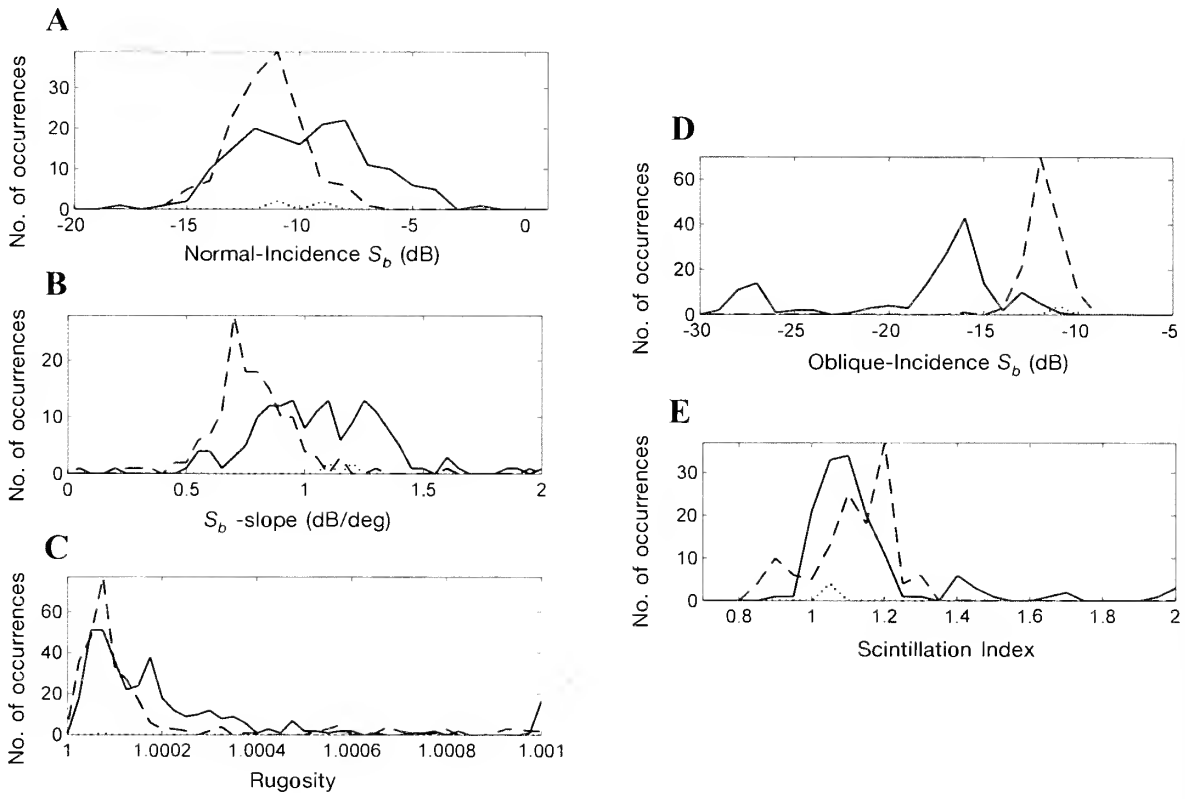

Figure 4

The frequencies of occurrence for trawlable (solid lines) and untrawlable (dashed lines) seafloor as a function of different seafloor parameters—(**A**) normal-incidence seafloor backscatter strength, S_b; (**B**) the slope of the angle-dependent backscatter within 10°of normal incidence (S_b-slope); (**C**) rugosity (roughness); (**D**) oblique-incidence S_b; and (**E**) scintillation index—derived from the data collected with a Simrad ME70 multibeam echo sounder in 2009. A classification of mixed (dotted lines) indicates a 25-m^2 area of the seafloor that included classifications of both trawlable and untrawlable data.

Table 1

Results of a 2-sample *t*-test and the standard difference in a comparison of trawlable and untrawlable populations for different parameters derived from the data collected with the Simrad ME70 multibeam echo sounder during a cruise in 2009 aboard the NOAA Ship *Oscar Dyson*. These parameters are normal-incidence seafloor backscatter strength (S_b), oblique-incidence S_b, the slope of the angle-dependent backscatter within 10° of normal incidence (S_b-slope), scintillation index (SI), and rugosity (roughness).

	t-statistic	Degrees of freedom	*P*-value	Standard difference
Normal-incidence S_b	6.6	260	2×10^{-10}	0.74
Oblique-incidence S_b	17.2	170	4×10^{-39}	1.89
S_b-slope (0–10°)	9.9	287	5×10^{-20}	1.12
SI	2.1	216	0.04	0.25
Rugosity	3.6	418	0.0004	0.25

floor, the error rate did not change appreciably except in the case of a combination of the oblique-incidence S_b, the normal-incidence S_b, and the *SI*, in which case the class error rate was reduced to 3.8%; similar error rates were found with 4 classes separated by a best-fit hyperplane.

Because only marginal improvements in class error rate were achieved when multiple parameters were combined and maintenance of simplicity in the interpretation of the results was desired, the oblique-incidence S_b was chosen as the sole discriminator between the trawlable and untrawlable seafloor at the study site. The classifications of trawlable and untrawlable seafloor classifications area shown in Figure 2 for both the from the Simrad ME70 and the data from the SDC and ROV. The classification based on the data from the Simrad ME70 is accurate throughout most

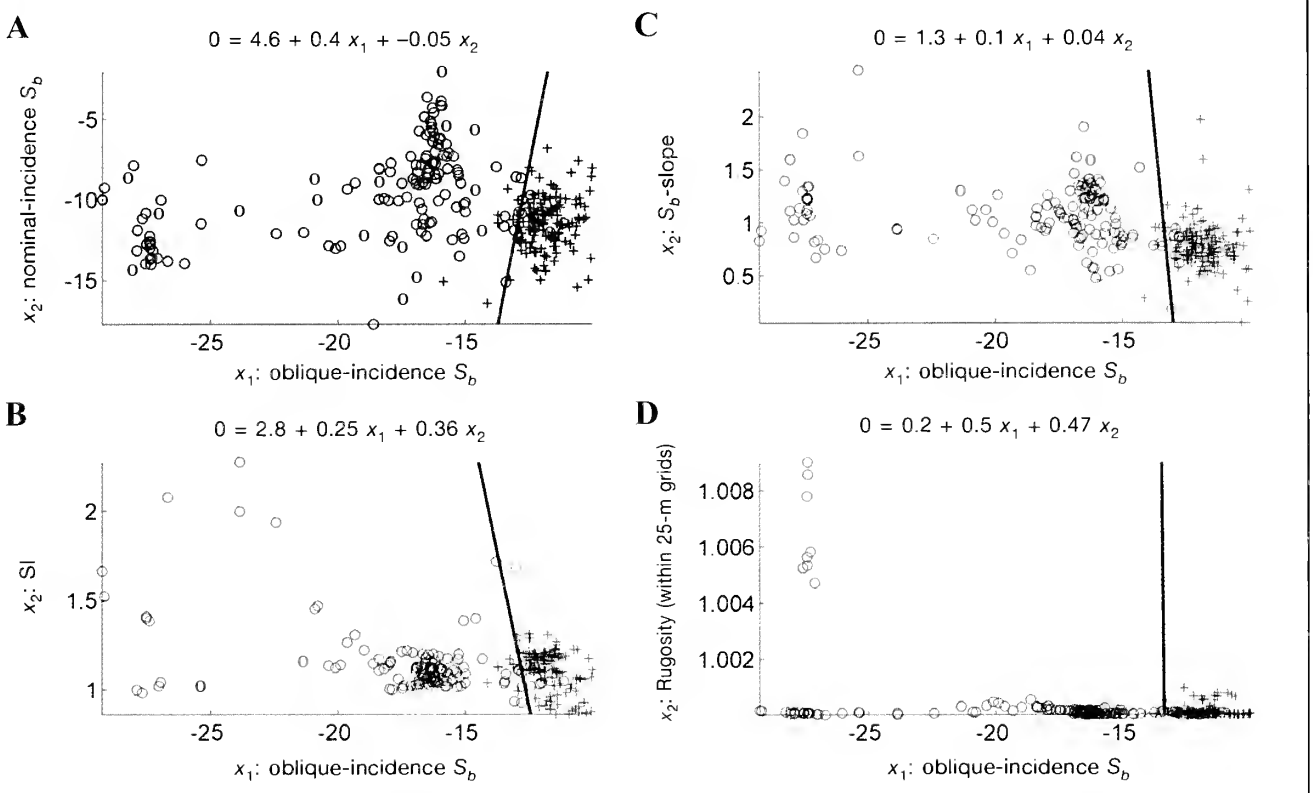

Figure 5

Scatter plots of the oblique-incidence seafloor backscatter strength (S_b) with each of the other seafloor parameters examined in our work and a best-fit line that discriminates between trawlable and untrawlable seafloor. The other seafloor parameters shown here are the (**A**) normal-incidence S_b, (**B**) scintillation index, (**C**) slope of the angle-dependent backscatter within 10°of normal incidence (S_b-slope); and (**D**) rugosity (roughness).

of the study site, and the most obvious error occurred on the north–south transect intersected 153.9°W in an area with high oblique-incidence S_b.

Discussion

The oblique-incidence S_b and the S_b-slope followed the expected trends when separated into trawlable and untrawlable classes and these trends were verified from video data collected with the SDC and ROV. Untrawlable areas were expected to have a larger oblique incidence S_b and S_b-slope than trawlable areas on the basis of backscatter models (e.g., Fig. 1). The normal-incidence S_b did not appear to discriminate very well between trawlable and untrawlable seafloor and tended to have a wider distribution of backscatter values than would have been expected on the basis of consideration of the oblique-incidence S_b and the modeled values shown in Figure 1. There are several possible reasons for the lack of discrimination with normal-incidence S_b, including higher-than-expected normal-incidence S_b in the sands and silts caused by gas or heterogeneities within the sediment volume in some trawlable areas and higher-than-expected roughness in the areas of cobble and rock that caused a larger-than-anticipated reduction in the normal-incidence S_b for some untrawlable areas.

Although quite variable throughout the study area, the mode of the *SI* was slightly higher for the untrawlable seafloor than it was for the trawlable seafloor. This difference seems plausible when we consider the SI to be a metric for how many scatterers are contributing to the sonar return within a beam footprint. A *SI* value near 1 suggests that there are a large number of scatterers (i.e., the central limit theorem applies, and the backscatter amplitude is Rayleigh distributed), as might be expected from a sand or silt seafloor. On the other hand, a larger SI indicates that there are only a few dominant scatterers within the beam footprint, as might be expected from a seafloor of cobbles or boulders. Although the data indicate a trend in the correct direction, SI alone has not provided a clear separation between trawlable and untrawlable seafloor (e.g., a

threshold of 1.2 would result in a high classification error rate).

Rugosity derived from the data collected with the Simrad ME70 was a poor discriminator of trawlable versus untrawlable seafloor, generally with lower values (e.g., smoother seafloor) in areas where the validation data from the SDC and ROV surveys indicate that the seafloor is untrawlable. The areas that contained high values of rugosity generally were dominated by larger scale features: the ridgeline on the northern edge of the bank, the sand waves in the southeast, or the pockmarks in the southwest. It is likely that the spatial resolution of the MBES was insufficient to provide a useful estimate of the rugosity level and that an MBES with higher frequencies and higher resolution might provide more useful results.

The oblique-incidence S_b alone provided a low error rate as a discriminator between trawlable and untrawlable seafloor. When combined with the other metrics, it was possible to slightly lower the error rate, but an examination of the scatter plots in Figure 5 indicates that the error rates were not been lowered in any meaningful way. For example, the best-fit line that discriminates between the combination of oblique-incidence S_b and normal-incidence S_b shows that a combination of high oblique-incidence S_b and low normal-incidence S_b gives a better indication of untrawlable seafloor than high oblique-incidence S_b on its own. This finding is contrary to what the modeled seafloor return (Fig. 2) would predict: high oblique-incidence S_b and high normal-incidence S_b are a better predictor of an untrawlable seafloor. Therefore, it is likely that the marginal improvement in classification error rate with these extra parameters combined is simply a result of variations in the tails of the underlying data distributions. With only marginal improvements (5.6–3.8%) in classification error rate when up to 4 parameters are combined, with a hyperplane separating the 2 classes, it is reasonable to choose the simpler approach of using only the oblique-incidence S_b as a predictor of trawlable or untrawlable seafloor.

Conclusions

The results described here indicate that acoustic remote sensing of substrate type with an MBES, and oblique-incidence acoustic S_b in particular, offer useful insight into whether the seafloor is untrawlable. This conclusion is in qualitative agreement with the work of Jagielo et al. (2003), who used seafloor backscatter collected with a sidescan sonar as part of an a priori assessment of trawlability (note that much of the sidescan record was collected at oblique incidence angles). Whether these types of acoustic metrics can provide a similar level of confidence regarding the distribution of untrawlable seafloor in areas throughout the entire Gulf of Alaska needs to be determined. If successful on a wider scale, this type of acoustic remote sensing can help refine the interpretation of bottom-trawl surveys. In particular, techniques such as those described here could increase the accuracy in identification of areas with seafloor characteristics similar to areas where bottom-trawl surveys of rockfish were conducted (i.e., areas where results from the trawl surveys can be applied). As a result, the precision and accuracy of biomass estimates from bottom-trawl surveys and their resultant stock assessments would be improved.

Acknowledgments

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Distribution of ommastrephid paralarvae in the eastern tropical Pacific

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Abstract—Jumbo squid (*Dosidicus gigas*) and purpleback squid (*Sthenoteuthis oualaniensis*) (Teuthida: Ommastrephidae) are thought to spawn in the eastern tropical Pacific. We used 10 years of plankton tow and oceanographic data collected in this region to examine the reproductive habits of these 2 ecologically important squid. Paralarvae of jumbo squid and purpleback squid were found in 781 of 1438 plankton samples from surface and oblique tows conducted by the Southwest Fisheries Science Center (NOAA) in the eastern tropical Pacific over the 8-year period of 1998–2006. Paralarvae were far more abundant in surface tows (maximum: 1588 individuals) than in oblique tows (maximum: 64 individuals). A generalized linear model analysis revealed sea-surface temperature as the strongest environmental predictor of paralarval presence in both surface and oblique tows; the likelihood of paralarval presence increases with increasing temperature. We used molecular techniques to identify paralarvae from 37 oblique tows to species level and found that the purpleback squid was more abundant than the jumbo squid (81 versus 16 individuals).

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Adult squid of the oceanic family Ommastrephidae are active generalist predators and key prey for a wide variety of marine fishes, birds, and mammals. They are also the primary targets of the world's larger squid fisheries (Nigmatullin et al., 2001; Markaida et al., 2005; FAO, 2011). Many questions remain unanswered about the reproduction and early life history of these oceanic squid (Young et al., 1985; Boletzky, 2003). Logistical challenges impede direct observation of reproduction and development in the wild, but the collection of paralarvae in net tows often can be used to elucidate ommastrephid spawning grounds and the habitat needs of early life stages (e.g., Okutani and McGowan, 1969; Zeidberg and Hamner, 2002).

Two ommastrephid species that reproduce in the eastern Pacific are *Dosidicus gigas*, the jumbo or Humboldt squid, and *Sthenoteuthis oualaniensis*, the purpleback squid (Vecchione, 1999). The jumbo squid is currently the target of the world's largest squid fishery (628,579 t in 2009 [FAO, 2011]), and commercial interest in purpleback squid is growing (Zuyev et al., 2002; Xinjun et al., 2007). The adult ranges of these 2 species overlap in the eastern tropical and subtropical Pacific (Roper et al., 1984), but the location and extent of spawning grounds of either species over this large region are not well established. Paralarvae of these species cannot be reliably distinguished morphologically; molecular techniques must be used (Gilly et al., 2006; Ramos-Castillejos et al., 2010). When molecular identification is not possible because of formalin preservation or other limitations, paralarvae in this broad geographic region are generally assigned to the "SD complex" (*S. oualaniensis* and *D. gigas* [Vecchione, 1999]).

Ommastrephid paralarvae are relatively rare off California (e.g., Okutani and McGowan, 1969; Watson and Manion, 2011), and none have been attributed to jumbo squid or purpleback squid. Both species, however, have been identified off the Pacific coast of the Baja California Peninsula (Hernández-Rivas et al.[1]; Ramos-Castillejos et al., 2010). With-

[1] Hernández-Rivas, M. E., R. De Silva-Dávila, S. Camarillo-Coop, J. Granadores-Amores, and R. Durazo. 2007. Ommastrephid paralarvae during 1997–1999 IMECOCAL cruises. Abstract in California Cooperative Oceanic Fisheries Investigations Annual Conference 2007, Program and Abstracts; San Diego, CA, 2–28 November, p. 41. Calif. Coop. Oceanic Fish. Invest., La Jolla, CA.

in the Gulf of California only the jumbo squid has been reported to spawn (Gilly et al., 2006; Staaf et al., 2008; Camarillo-Coop et al., 2011), and, to our knowledge, no other adult ommastrephid has been described from this region, although adults of purpleback squid have been reported from the area near the mouth of this gulf (Olson and Galván-Magaña, 2002). In the southern hemisphere, the Peru Current System has yielded only jumbo squid paralarvae (Sakai et al., 2008). In the large intervening equatorial region, paralarvae of both purpleback squid and jumbo squid are present (Okutani, 1974; Ueynagi and Nonaka, 1993).

The data that form the basis of this knowledge were collected through a variety of methods. Samples from both the Pacific and Gulf coasts of the Baja California Peninsula and from the Peru Current were collected primarily during subsurface oblique tows with bongo nets (Ramos-Castillejos et al., 2010; Camarillo-Coop et al., 2011; Sakai et al., 2008). By contrast, the central region of the eastern tropical Pacific (ETP) has been sampled extensively during surface tows with neuston nets, yielding higher densities of paralarvae (Ueynagi and Nonaka, 1993; Vecchione, 1999). In the ETP, densities can be extremely high, as in the case of more than 10,000 very small paralarvae of the SD complex from a single surface tow conducted during the 1986–87 El Niño (Vecchione, 1999). By contrast, the greatest number of SD-complex paralarvae reported from the Baja California Peninsula is 20, collected with a bongo net (Camarillo-Coop et al., 2011).

Surface tows effectively sample only the top 10–20 cm of the water column, but subsurface oblique tows typically sample from the surface to depths of about 200 m. Because oblique tows sample a broader, deeper range of habitats than surface tows, discrepancies in paralarval abundance and size between the 2 types of tows may reflect different vertical habitat preferences at different stages of development. For example, if recently hatched paralarvae exhibit a preference for surface waters, surface tows would be far more effective at capturing these animals because oblique tows spend very little time at the surface (10–20 cm). And if paralarvae begin to occupy greater depths as they grow, while their numbers decrease because of natural mortality, oblique tows would be likely to capture fewer, larger individuals than would surface tows, as has been seen for the ommastrephid *Todarodes pacificus* (Yamamoto et al., 2002; 2007).

Although high surface abundances can be representatively sampled by surface tows, any narrow subsurface band of high abundance, as might occur at a pycnocline, would be undersampled by oblique tows. However, a strong association of paralarvae with a subsurface feature in preference to the surface could still be detected by a greater likelihood of capture in oblique rather than in surface tows, as has been found for the northern shortfin squid (*Illex illecebrosus*), which shows a relationship with the subsurface interface between slope water and the Gulf Stream in the Atlantic (Vecchione, 1979; Vecchione et al., 2001).

Diel vertical migrations, typical of adult ommastrephids, also could drive different abundances in surface and oblique tows. This result was found in loliginid paralarvae (Zeidberg and Hamner, 2002), but the situation is less clear for ommastrephids (Piatkowski et al., 1993; Young and Hirota, 1990). The few surface tows during which paralarvae of northern shortfin squid were collected in the Middle Atlantic Bight were conducted at night (Vecchione, 1979)— a finding that could indicate a nighttime migration to the surface, but the numbers are too small to strongly support this idea. No significant differences in paralarval abundance of purpleback squid have been found between daytime and nighttime tows in Hawaii (oblique and horizontal tows from the surface to a depth of 200 m; [Harman and Young, 1985]) or Japan (horizontal tows from the surface to a depth of 200 m; [Saito and Kubodera, 1993]).

On cruises conducted by NOAA in the ETP, ecosystem data (including plankton samples) from a large geographic area have been collected regularly and archived for many years. In this study, we present the first analysis of planktonic squid from this data set, focusing on the ommastrephids jumbo squid and purpleback squid. Our aims are 1) to compare surface and oblique tows conducted at the same location and time to determine differences in paralarval distribution and abundance due to sampling method, 2) to address questions of species-specific depth preference and vertical migration, 3) to uncover relationships between paralarval abundance and oceanographic features, and 4) to use molecular techniques on a subset of samples to determine whether the 2 species have distinct spawning areas or habitat preferences within their range overlap. Paralarval distribution is also contrasted with adult distribution data, collected during the 2006 cruise, to confirm that the study region is within the adult range of both species and to enhance our understanding of the ETP as a feeding and spawning area.

Materials and methods

Study area and data collection

The ETP, where the ranges of jumbo squid and purpleback squid overlap, is defined by 3 large surface currents and 2 water masses (Fiedler and Talley, 2006; Fig. 1A). The westward-flowing North and South Equatorial Currents derive from the temperate California and Peru Currents, respectively. The Equatorial Countercurrent flows eastward from the western Pacific to the coast of Central America. These currents define 2 water masses: Tropical Surface Water and Equatorial Surface Water, the latter cooler and fresher than the former. Two smaller-scale oceanographic features are prominent: 1) a distinct thermocline ridge at the interface between the North Equatorial Current and

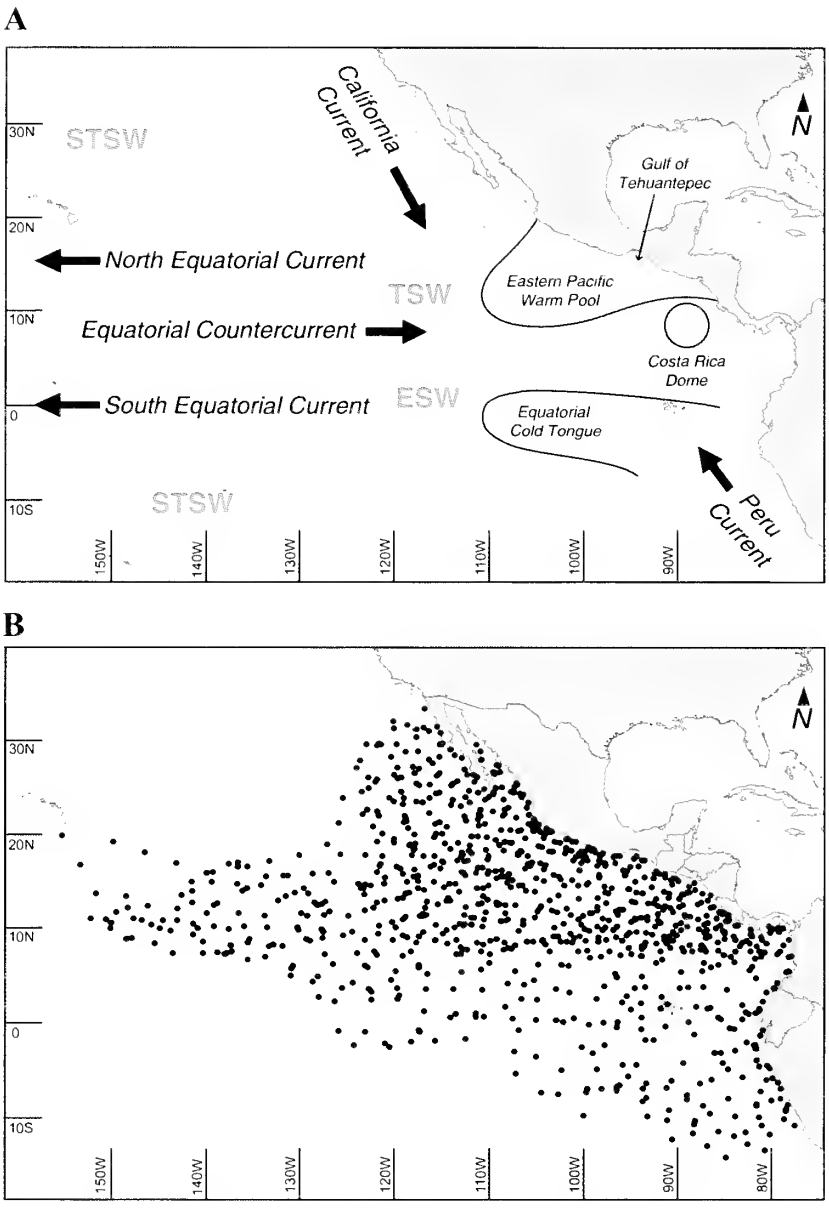

Figure 1

Map of study area in our examination of the distribution of ommastrephid paralarvae in the eastern tropical Pacific with (**A**) oceanography (after Fiedler and Talley, 2006) and (**B**) sampling stations from cetacean and ecosystem assessment surveys conducted by the Southwest Fisheries Science Center (NOAA) from 1998 to 2006. Two plankton tows, one each with a manta and a bongo net, were conducted each evening approximately 2 h after sunset. STSW=Subtropical Surface Water. TSW=Tropical Surface Water. ESW=Equatorial Surface Water.

the Equatorial Countercurrent, nominally along 10°N latitude (although the exact location varies seasonally) and 2) the Costa Rica Dome, an area of thermocline doming, nominally at 9°N latitude, 90°W longitude, although this feature too varies in location and degree of development through time, seasonally and interannually.

The study area for this research forms a polygon that circumscribes the oceanic waters from the U.S.–Mexico border west to Hawaii, and south to central Peru. Cetacean and ecosystem assessment cruises were conducted in this region by the Southwest Fisheries Science Center (NOAA Fisheries) from late July to early December of 1998, 1999, 2000, 2003, and 2006 (Fig. 1B), with the University-National Oceanographic Laboratory System (UNOLS) research vessel *Endeavor* (1998), and the NOAA Ships *David Starr Jordan* (all years), *McArthur* (1998, 1999, 2000), and *McArthur II* (2003, 2006). Plankton were sampled with 2 types of net tows, conducted ~2 h after sunset each day, for a total of 979 manta (surface) tows and 762 bongo (oblique) tows over the 8-year period. On the *McArthur II* in 2006, during one leg of the cruise, medium-size jigs and rods were used to fish for adult squid from 1 to 2 h after sunset.

Manta nets (Brown and Cheng, 1981) with 0.505-mm mesh were towed for 15 min at a ship speed of 1.0–2.0 kn, with all deck lights off. Bongo nets (McGowan and Brown[2]; Smith and Richardson, 1977), consisting of a pair of circular net frames with 0.505-mm or 0.333-mm mesh, were towed for a 15-min double oblique haul to a depth of ~200 m at a ship speed of 1.5–2.0 kn. The net was lowered continuously at about 35 m/min, held at ~200 m for 30 s, and then was retrieved at about 14 m/min, with the angle of stray always maintained at ~45°.

Volume of water filtered during manta and bongo tows was estimated with a flowmeter suspended across the center of the net. Contents of the codends were preserved in 5% formalin buffered with sodium borate. In 2003 and 2006, the contents of one codend of each bongo tow were frozen in seawater at –20° C, and the contents of the other were preserved in 5% formalin. Also in 2006, the contents of one codend of every fourth bongo tow (38 samples total) were preserved in 70% ethanol instead of formalin.

[2] McGowan, J. A., and D. M. Brown. 1966. A new opening-closed paired zooplankton net. Univ. Calif. Scripps Inst. Oceanogr. Ref. 66-23, 56 p. Scripps. Inst. Oceanogr., Univ. Calif., San Diego, CA.

Water column data were collected with conductivity-temperature-depth (CTD) profilers 1 h before sunrise and 1 h after sunset on each survey day and with expendable bathythermographs (XBTs) during daylight hours at intervals of ~55 km. Samples of surface water were collected in bottles during the CTD casts and in buckets concurrent with XBT casts at depths from 1 to 3 m. Precruise calibration factors (fluorometer calibration factor, F, and acid ratio of pure chlorophyll, τ) were used to calculate chlorophyll-*a* and phaeophytin values from digital fluorometer readings of these surface water samples. Sea-surface temperature (SST) and salinity (SSS) were measured continuously (around the clock) with a thermosalinograph while the ship was underway. Details of the complete data set are available in NOAA data reports (Philbrick et al., 2001, a–c; Ambrose et al., 2002, a and b; Watson et al., 2002; Jackson et al., 2004; 2008).

Sample processing

Cephalopods were removed manually from 654 bongo (1998, 2000, 2003, 2006) and 784 manta (1998, 1999, 2003, 2006) samples. Bongo samples with >25 mL of plankton were fractioned to ~50% of the original sample volume before they were sorted. The absolute count from each tow was divided by the volume of water filtered during that tow, as computed from flowmeter readings, to give paralarvae densities per cubic meter (following techniques described in Kramer et al., 1972).

Adult and paralarval specimens were identified by morphological characteristics (Wormuth et al., 1992). Adults were identified to species by the presence of a fused funnel-locking cartilage in purpleback squid and the absence of the fused structure in jumbo squid. Ommastrephid paralarvae are known as rhynchoteuthions; their distinctive form is recognized easily by the presence of a proboscis. For individuals missing the proboscis or in which the proboscis already had separated into tentacles, identification was based on the characteristic inverted-T funnel-locking cartilage of this family. When proboscis suckers were visible, they were checked to separate individuals of the genera *Hyaloteuthis*, *Eucleoteuthis*, and *Ommastrephes* (enlarged, lateral suckers on proboscis) from individuals of the genera *Dosidicus* and *Sthenoteuthis* (equal-size suckers on proboscis). *Hyaloteuthis*, *Eucleoteuthis* and *Ommastrephes* are relatively rare in the ETP (all the molecularly identified ommastrephids in this study were *Sthenoteuthis* or *Dosidicus*; see also Yatsu[3]), and only 9 specimens were tentatively identified as *Eucleoteuthis* and 2 specimens were tentatively identified as *Ommastrephes* by proboscis suckers and photophores (6 others were excluded from *Dosidicus* or *Sthenoteuthis* but were too small to be assignable to the other 3 genera). Therefore, any specimens damaged such that the terminal suckers were not preserved were assigned to the SD complex. The presence of paralarvae from other cephalopod families was recorded, but these specimens were not identified to genus or species, or counted.

Morphological techniques for reliable differentiation between paralarvae of jumbo squid and purpleback squid are not available. Wormuth et al. (1992) and Yatsu[3] used proboscis length and photophores as distinguishing characters, but the muscular proboscis can extend and retract (Staaf et al., 2008), and reactions to fixatives have not been quantified. Additionally, there may be variability in ontogenetic timing of photophore formation (Gilly et al., 2006). Ramos-Castillejos et al. (2010) suggested several distinguishing indices that used morphometric ratios; however, samples in this study were prepared in different fixatives (ethanol for jumbo squid and formalin for purpleback squid) that can distort or shrink specimen proportions. The efficacy of indices for diagnoses of individual specimens of unknown species also were not tested. Therefore, we attempted no species-level identification of SD-complex specimens that were preserved in formalin.

Molecular identification of SD-complex ommastrephids from ethanol-preserved samples followed protocols described in Gilly et al. (2006). Two frozen bongo samples were also sent to Hopkins Marine Station for sorting and molecular identification. The frozen samples were selected on the basis of a high abundance of ommastrephid paralarvae in the matching codend, and they were sorted primarily to test whether it is possible to reliably identify paralarvae from a frozen plankton sample.

Mantle lengths (ML) of ommastrephid paralarvae from 1998 manta and bongo tows were measured with an ocular micrometer. For tows with 10 or fewer ommastrephids, all individuals were measured. For tows with more than 10 ommastrephids, 10 individuals were selected for measurement. Selection was arbitrary and aimed to be representative; e.g., the largest (or smallest) specimens were not always included.

Data analysis and modeling

We constructed a data set of ommastrephid paralarval abundance and 5 in situ oceanographic variables: SST, SSS, mixed-layer depth (MLD), temperature at thermocline (TT), and surface concentration of chlorophyll-*a* (CHL). MLD is defined as the depth at which temperature is 0.5°C less than SST (Fiedler, 2010). TT is temperature at the depth of the thermocline as determined by the "maximum slope by difference" method (Fiedler, 2010). MLD, TT, and CHL values were collected from the station nearest the net tow; these data were used

[3] Yatsu, A. 1999. Morphological and distribution of rhynchoteuthion paralarvae of two ommastrephid squids, *Dosidicus gigas* and *Sthenoteuthis oualaniensis*, collected from eastern tropical Pacific Ocean during 1997–preliminary report. *In* Report of the *Kaiyo Maru* cruise for study on the resources of two ommastrephid squids, *Dosidicus gigas* and *Ommastrephes bartramii*, in the Pacific Ocean, during September 11–December 24, 1997 (A. Yatsu, and C. Yamashiro, eds.), p. 193–206. Fisheries Agency of Japan, Tokyo.

only if the station was located within 18.5 km (10 nautical miles) and was sampled within 12 h of the net tow. SST and SSS were averaged over a 2-h window centered on the time of the net tow. In total, 137 bongo and 164 manta samples were discarded according to these criteria, leaving 517 bongo and 620 manta samples. Many of the discards (56 bongo and 57 manta) were collected aboard the *McArthur* in 2003, when the thermosalinograph malfunctioned. Three outlier points were also removed: an abnormally low value for each of CHL and SST, and an abnormally high value for MLD.

Relationships between ommastrephid abundance and oceanographic variables were explored with generalized linear models in the R statistics package, vers. 2.1.1 (R Development Core Team, 2005). We used generalized linear models because of their utility in modeling relationships between cetaceans and oceanographic habitat (Redfern et al., 2006) and between cephalopod paralarvae and oceanographic habitat off western Iberia (Moreno et al., 2009). Typical of marine survey counts, our paralarval abundance data were overdispersed, with a high proportion of zeros and a few very large samples. Therefore, we followed Aitchison (1955) and Pennington (1983) in performance of a 2-step analysis, in which we separated the data into a binomial presence and absence data set (hereafter referred to as *paralarval presence*) and an abundance data set that included only stations at which paralarvae were present (hereafter referred to as *paralarval abundance*). To analyze paralarval presence, we used a binomial distribution with a logit link; for paralarval abundance we used a lognormal distribution. We used an automated forward/backward stepwise approach based on Akaike's information criterion (AIC) to select the variables for inclusion in the model.

Results

Abundance of paralarvae

Paralarvae of the SD complex were found in 781 of the 1438 formalin-preserved plankton samples. By type of tow, 355 of 656 oblique bongo tows (54.28%) and 426 of 784 surface manta tows (54.34%) contained SD-complex paralarvae. The greatest abundance in a single manta tow was 1588 paralarvae versus 64 paralarvae in a single bongo tow. SD-complex paralarvae taken in bongo tows were distributed over a somewhat broader geographical area than were those paralarvae captured in manta tows (Fig. 2), but density of captured paralarvae was typically at least an order of magnitude greater in manta tows.

Size of paralarvae

Average mantle length in manta tows was 1.94 ±1.29 mm (n=779; range 0.7–15 mm ML) versus 1.86 ±1.0 mm (n=148; range 0.6–7 mm ML) in bongo tows. No significant difference was found between these distributions (1-way analysis of variance [ANOVA], P= 0.44).

Relationship of presence and abundance of paralarvae to environmental variables and modeling

The stepwise approach for the presence models selected SST, SSS, and TT as predictor variables for manta data, and SST and MLD for bongo data (Table 1). The decrease in the AIC values for these models and the increase in the percentage of explained deviance came primarily from SST for both bongo and manta tows, with minimal contribution from MLD, SSS, and TT. Therefore, SST emerged as the strongest predictor for presence of SD-complex paralarvae, and the probability of capture increased monotonically as SST increased from 15°C to 32°C (Fig. 3).

Analysis of paralarval abundance, rather than presence, revealed no strong predictors (Table 2). For bongo tows, the stepwise approach selected CHL, TT, and SST in the final model (7.5% explained deviance). For manta tows, CHL, SST, MLD, and TT were all selected (12.1% explained deviance). There appears to be little relationship between these variables and nonzero paralarval abundance, which varied over a wide range of each environmental variable for both manta and bongo tows.

Species identification

In total, 97 SD-complex paralarvae were found in 12 of the 38 ethanol-preserved samples. Of these paralarvae, 81 were identified genetically as *Sthenoteuthis oualaniensis* and 16 as *Dosidicus gigas*. Paralarvae of purpleback squid were found over a much greater area than paralarvae of jumbo squid (Fig. 4A). Eight ommastrephid paralarvae were removed from the 2 frozen samples and identified genetically as purpleback squid.

Non-ommastrephid cephalopods were identified in many of the tows, most commonly as taxa in the teuthid families Enoploteuthidae, Onychoteuthidae, Gonatidae, Chtenopterygidae, Cranchiidae, and Brachioteuthidae and in the octopod genera *Argonauta* and *Tremoctopus*; all have previously been reported from the ETP (Ueyanagi and Nonaka, 1993; Vecchione, 1999).

Of the 129 adult squid captured in jigging sessions, 118 were jumbo squid and 11 were purpleback squid. Jumbo squid adults were found primarily in the southernmost sampling sites off Peru, but the few purpleback squid adults were more evenly distributed (Fig. 4B).

Discussion

This study represents the most extensive sampling to date in the ETP of paralarvae of jumbo squid and purpleback squid, covering most of their broad equatorial and subtropical region of range overlap in the Pacific during a period of 8 years.

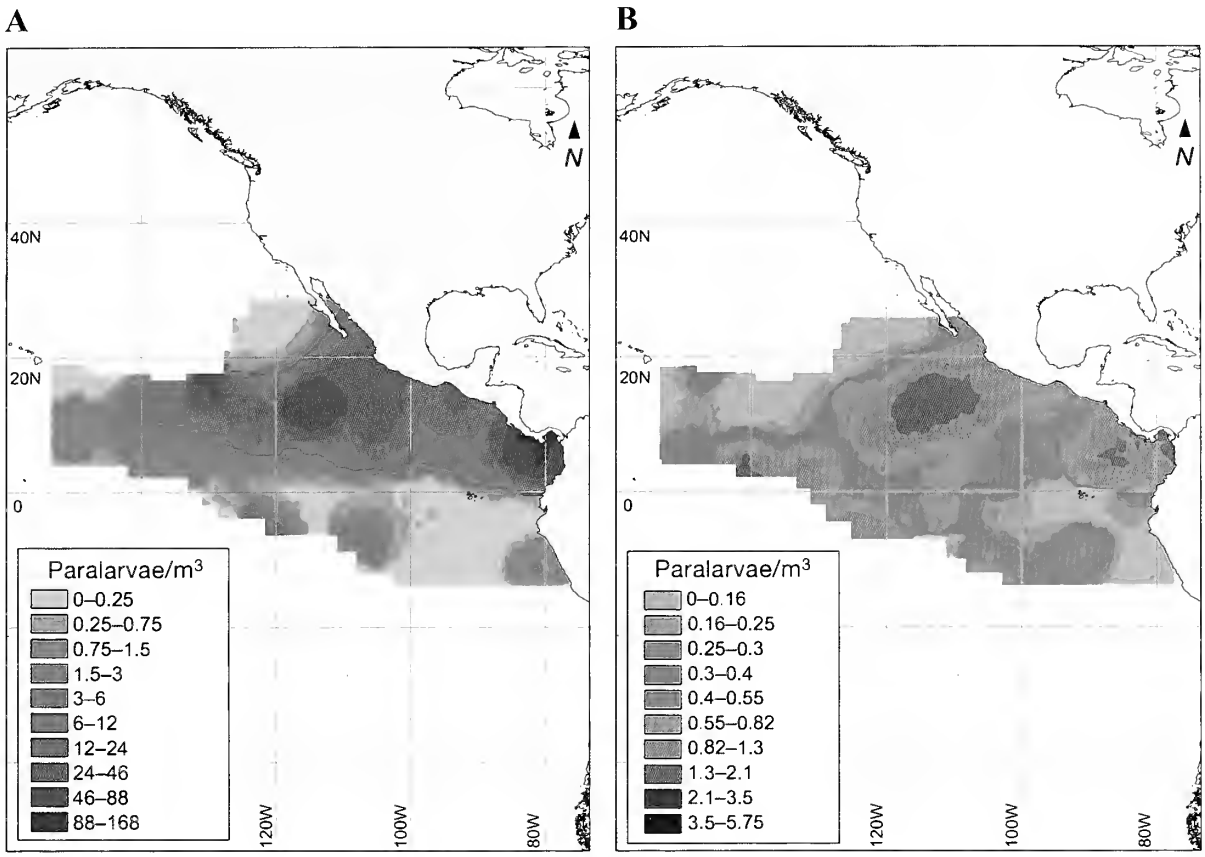

Figure 2

Abundance of paralarval purpleback squid (*Sthenoteuthis oualaniensis*) and jumbo squid (*Dosidicus gigas*) from all study years (1998–2006) for (**A**) manta (surface) and (**B**) bongo (oblique) tows conducted in the eastern tropical Pacific. Paralarval abundance was interpolated by using inverse distance weighting with a cell size of 1° and a fixed search radius of 5°.

Table 1

Generalized linear models used to relate the presence and absence of ommastrephid paralarvae in manta (surface) and bongo (oblique) tows conducted in the eastern tropical Pacific in 1998–2006 to 5 in situ oceanographic variables: sea-surface temperature (SST), sea-surface salinity (SSS), mixed-layer depth (MLD), temperature at thermocline (TT), and surface-concentration of chlorophyll-*a* (CHL). A stepwise approach selected SST, SSS, and TT for the final manta model; SST and MLD were selected for the final bongo model. Better-fitting models have a higher percentage of explained deviance and a lower Akaike's information criterion (AIC) value.

Manta			Bongo		
Model	Deviance (%)	AIC	Model	Deviance (%)	AIC
Null		913	Null		710.3
SST × *SSS* × *TT*	18.8	748.1	*SST* × *MLD*	12.2	627.7
SST × *TT*	18.5	748.5	*SST*	10.6	637.1
SST × *SSS*	18.4	749.6	*MLD*	0.2	711.1
SSS × *TT*	12.7	801.6			
SST	18.1	750.2			
SSS	4.6	873.1			
TT	11	814.9			

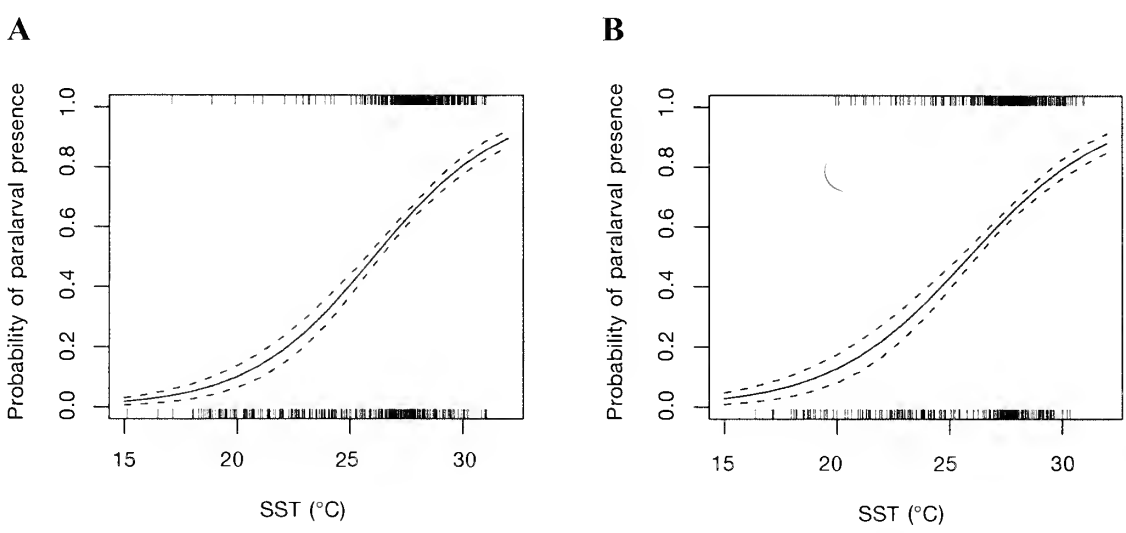

Figure 3

Probability of finding paralarval purpleback squid (*Sthenoteuthis oualaniensis*) and jumbo squid (*Dosidicus gigas*) as a function of sea-surface temperature in samples from (**A**) manta (surface) and (**B**) bongo (oblique) tows conducted in 1998–2006 in the eastern tropical Pacifc. All other variables were set to their median values. Dashed lines indicate standard error of the regression. Tick marks indicate raw binomial data.

Vertical distribution of paralarvae

We found no difference in the size of paralarvae between surface (manta) and oblique (bongo) tows, in agreement with Yatsu.[3] These observations are not consistent with an ontogenetic vertical migration to increasing depths within the paralarval stage of development, as proposed for *Todarodes pacificus* (Yamamoto et al., 2002; 2007). This feature, therefore, may not be common to all ommastrephids.

Although *incidence* of capture in surface and oblique tows was nearly identical (54% positive samples in both), *abundance* of paralarvae was much greater in surface tows. High abundance in surface tows also has been reported for other ommastrephids (Ueynagi and Nonaka, 1993), and extremely high numbers of SD-complex paralarvae have been captured in single surface tows: 819 off Jalisco, Mexico[4] and >10,000 in the

[4] Palomares-García, R., R. De Silva-Dávila, and R. Avendaño-Ibarra. 2007. Predation of the copepod *Oncaea mediterranea* upon ommastrephid paralarvae in the mouth of the Gulf of California. Abstract *in* Proceedings of the 1st international CLIOTOP symposium; La Paz, México, 3–7 December.

Table 2

Generalized linear models used to relate nonzero abundance of ommastrephid paralarvae in manta (surface) and bongo (oblique) tows conducted in the eastern tropical Pacific in 1998–2006 to 5 in situ oceanographic variables: sea-surface temperature (SST), sea-surface salinity (SSS), mixed-layer depth (MLD), temperature at thermocline (TT), and surface-concentration of chlorophyll-*a* (CHL). A stepwise approach selected SST, MLD, TT, and CHL for the final manta model and SST, MLD, and CHL for the final bongo model. The resulting percentage of explained deviance and the Akaike's information criteria (AIC) value for these models indicate that none of the oceanographic variables is a strong predictor of nonzero abundance.

Manta			Bongo		
Model	Deviance (%)	AIC	Model	Deviance (%)	AIC
Null		1341	Null		782
SST × *MLD* × *TT* × *CHL*	12.1	1303.8	*SST* × *MLD* × *CHL*	7.5	764.6
MLD × *TT* × *CHL*	11.3	1305	*SST* × *CHL*	6.9	764.8
SST × *MLD* × *TT*	11.1	1306	*MLD* × *CHL*	6.6	765.6
SST × *TT* × *CHL*	9.8	1310.9	*SST* × *MLD*	4.5	772.2
SST × *MLD* × *CHL*	9.5	1312.2			

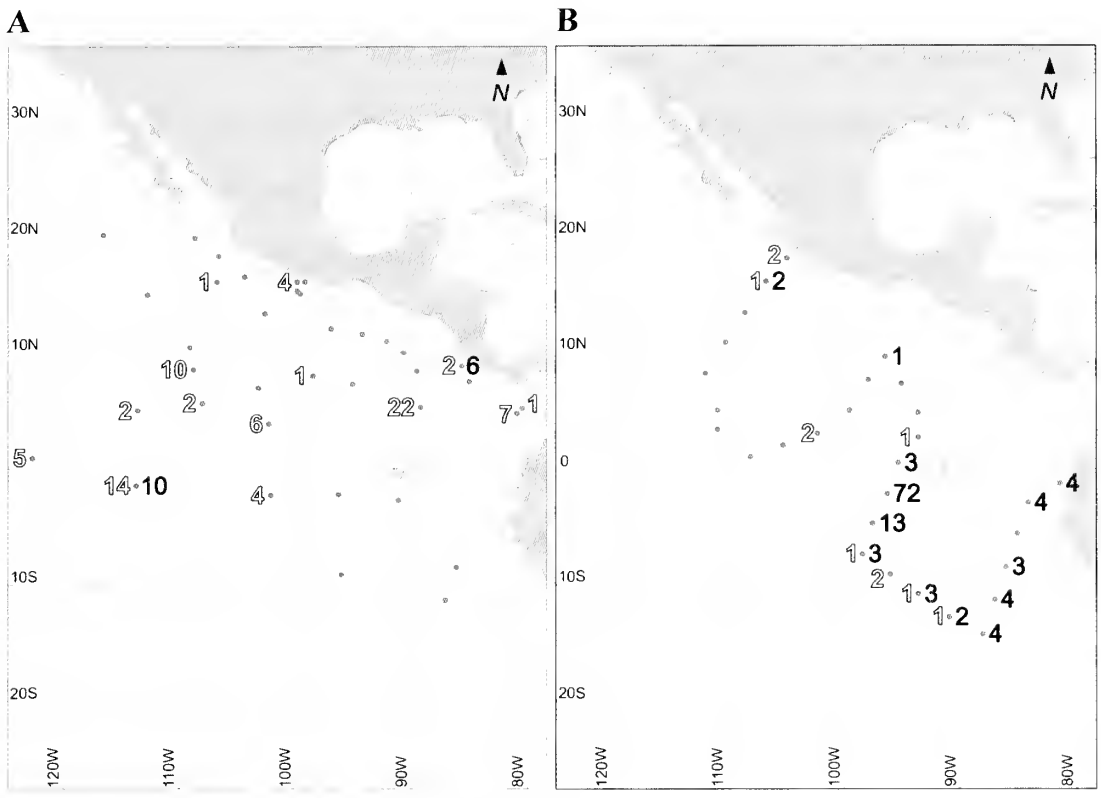

Figure 4

Geographic distribution and abundance of (**A**) genetically identified paralarvae and (**B**) morphologically identified adult ommastrephids caught in the eastern tropical Pacific during surveys conducted in 2006. The numbers at each station (small dot) represent the total number of individuals of purpleback squid (*Sthenoteuthis oualaniensis*) (outlined in black) and jumbo squid (*Dosidicus gigas*) (solid black) captured at that station. At stations where numbers do not appear, no squid were caught.

ETP (Vecchione, 1999). The consistency of this result seems surprising, because ommastrephid egg masses are thought to occur near the pycnocline, typically tens of meters deep, and not at the surface (O'Dor and Balch, 1985). The only reported observation of an in situ egg mass of jumbo squid was in the Gulf of California at a depth of 16 m near the pycnocline (Staaf et al., 2008). Presumably, this characteristic is common to purpleback squid, but we are unaware of descriptions of natural egg masses for this species.

Not only are egg masses of jumbo squid found at depth, but paralarvae are negatively buoyant. Paralarvae in the laboratory can swim to the surface but sink as soon as they stop swimming (Staaf et al., 2008); this negative buoyancy indicates that surface tension is insufficient for passive retention. We can only assume that purpleback squid paralarvae share this trait, and that tissue density of wild paralaravae is similar to laboratory-reared animals.

A preferred surface habitat, in which maintenance of position requires significant energy expenditure, strongly indicates that some benefit is derived from this behavior; the benefit may be access to increased food quantity or to food of higher nutritional value (Yamamoto et al., 2007). Nothing is known of the diet of jumbo squid paralarvae, but amphipods, copepods, and crab zoeae have been found in the digestive tracts of purpleback squid paralarvae (Vecchione, 1991); these and other zooplankton, as well as phytoplankton, also have been found in paralarvae of another ommastrephid, *Illex argentinus* (Vidal and Haimovici, 1998). Furthermore, a case has been made for the use of dissolved and particulate organic material by ommastrephid paralarvae (O'Dor et al., 1985). At certain times and in certain regions, oceanic surface waters may have high concentrations of these foods. The depth of the chlorophyll-*a* maximum in the ETP ranges from 60 to 90 m in open-ocean regions to near the surface in coastal boundary regions (Pennington et al., 2006).

It would be valuable to examine the vertical distribution of paralarvae with systematic oblique or horizontal tows at a series of discrete depths through the upper 100–200 m of the water column at a variety of times in a given area. This approach would give a more accurate picture of habitat use and of any association with the subsurface chlorophyll-*a* maximum or acoustic

scattering layers. To our knowledge, such a dedicated effort to address this problem has not been reported.

Oceanography

The number of both bongo- and manta-net tows that contained paralarvae increased as SST increased from 15°C to 32°C (Table 1, Fig. 3). This increased paralarval occurrence is consistent with the literature. Paralarvae of purpleback squid exhibit a preference for warm temperatures (28–31°C) in waters off Japan (Saito and Kubodera, 1993), and extremely large numbers of SD-complex paralarvae in the ETP were captured in individual tows coincident with the 29°C SST isotherm (Vecchione, 1999). In the Gulf of California, paralarvae of jumbo squid are more abundant during the warm months of June and September (SST of 27.7–29.4°C) than during the cooler season of February and April (SST of 15.3–18.1°C) (Camarillo-Coop et al., 2011). Because our surveys were conducted only between late July and early December, we were unable to assess seasonal variability in paralarval distribution.

We found no evidence for a decrease in paralarval occurrence at the highest SST values, despite the fact that embryonic development in vitro is optimal in the range of 17–25°C and fails to proceed at 30°C (Staaf et al., 2011). The idea that paralarvae may be better able than developing embryos to withstand warmer temperatures would be consistent with a upward vertical migration after hatching. If hatchlings promptly swim from near the pycnocline up to warmer near-surface water, where food may be more readily available, an ontogenetic increase in temperature optima would be advantageous. It also is possible that the upper thermal limit for successful development of wild embryos could be higher than the limit observed in laboratory studies. Embryos studied in the laboratory, particularly those embryos obtained through in vitro fertilization, may perish at high temperatures because of microbial infection, which could be inhibited in the wild by the presence of natural egg jelly (Staaf et al., 2011).

Peak abundances of SD-complex paralarvae observed in our study were an order of magnitude lower than the abundance levels reported during the 1986–87 El Niño (Vecchione, 1999). This discrepancy could be due to chance in sampling or a real difference in abundance. Among our study years, only in 2006 was an El Niño observed, and it was weaker than the one in 1986–87. The other years of our sampling were either in La Niña (1998, 1999, 2000) or neutral (2003) conditions (http://ggweather.com/enso/oni.htm). Year was included in our models as a potential explanatory discrete variable, but it was determined not to be an informative predictor of paralarval abundance or presence, indicating no difference between El Niño, La Niña, and neutral years. However, the strong positive relationship between paralarval occurrence and temperature found in our study is consistent with Vecchione's (1999) hypothesis that the extraordinarily high paralarval abundances in 1987 were related to the 3.5°C increase in SST during El Niño.

Reduced upwelling during the 1986–87 El Niño led to a 50% decline in chlorophyll-*a* in the region of highest paralarval abundance (Vecchione, 1999). Similarly, in our study, ommastrephid paralarvae were not associated with upwelling zones or their resultant high primary productivity. In general, zooplankton biomass in the ETP tends to be greatest in the 4 major upwelling regions—the Gulf of Tehuantepec, Costa Rica Dome, Equatorial Cold Tongue, and coast of Peru (Fernandez-Alamo and Farber-Lorda, 2006)—but ommastrephid paralarvae were not especially abundant in any of these regions (Fig. 2). Indeed, we found no relationship between SD-complex paralarvae and primary productivity, as measured by CHL or MLD (where the thermocline is shallow, primary productivity tends to be higher [Pennington et al., 2006]).

Species-specific spawning area

Molecularly identified jumbo squid paralarvae have been reported from the Gulf of California (Gilly et al., 2006), off the Baja California Peninsula (Ramos-Castillejos et al., 2010), off Peru (Wakayabashi et al., 2008), and now, in this study, from the ETP. We found that most molecularly identified paralarvae from the ETP were purpleback squid (Fig. 4A), but most adult squid captured by jigging were jumbo squid (Fig. 4B). Although jigging capture rates may have been biased, adult jumbo squid have also been found to outnumber purpleback squid as prey items of the Dolphinfish (*Coryphaena hippurus*) in the ETP (Olson and Galván-Magaña, 2002). Despite this abundance of adult jumbo squid, we found jumbo squid paralarvae in only 2 samples, and these samples also contained paralarval purpleback squid in appreciable numbers (Fig. 4A). Neither the geographic locations nor oceanographic features of these 2 sampling sites were distinct from sites where only purpleback squid was found. Therefore, we can say only that purpleback squid paralarvae appear to be far more abundant than paralarvae of jumbo squid because we have no way of assessing bias in the capture rates of the 2 species during plankton tows.

Species-level molecular identification of paralarvae was possible in this study only with material from oblique tows. If future work on material from surface tows were to find a similar predominance of purpleback squid, it would support the hypothesis that the purpleback squid is the primary ommastrephid that spawns in the ETP. Although jumbo squid can spawn in the ETP or subtropical fringes, its primary spawning grounds may actually lie farther to the north, off the Baja California Peninsula in both the Pacific (Ramos-Catellejos et al., 2010) and Gulf of California (Staaf et al., 2008; Camarillo-Coop et al., 2011), and farther to the south off Peru (Tafur et al., 2001; Sakai et al., 2008; Anderson and Rodhouse, 2001).

This view clearly contrasts with the one originally proposed by Nesis (1983) in which the jumbo squid spawns in the ETP and then migrates to feed at higher latitudes in both hemispheres. Available genetic analysis instead indicates 2 separate breeding populations, 1 in the northern hemisphere and 1 in the southern hemisphere (Staaf et al., 2010). If the preferred spawning habitat of jumbo squid is indeed subtropical to temperate, rather than tropical, it could explain the division into 2 populations, 1 breeding off Mexico and 1 breeding off Peru.

For future collections, we recommend preservation of material from both oblique and surface tows in ethanol. Although we were able to extract and identify paralarvae from frozen plankton samples, the technique has 2 drawbacks: 1) the difficulty of visual identification of individual specimens in the thawed slurry and 2), if the samples are to be sorted in more than one session, the damage done to the entire sample by repeated freeze-thaw cycles.

Conclusions

We found paralarvae in surface and oblique tows to be of equal size, indicating that paralarvae of the 2 ommastrephid species jumbo squid and purpleback squid do not engage in ontogenetic vertical migration at the paralarval stage. Ommastrephid paralarvae were much more abundant in surface tows than in oblique tows; this finding may indicate an ecological advantage of surface waters—perhaps, related to feeding. Models selected SST as the strongest predictor of paralarval presence in both surface and oblique tows; presence was more likely at higher temperatures. Therefore, warm surface waters appear to be the preferred habitat of ommastrephid paralarvae in the ETP. Molecular identification of specimens from a small subset of oblique tows showed that paralarvae of purpleback squid far outnumbered those of jumbo squid in this region. Adults of purpleback squid are broadly distributed in the tropics, whereas adult jumbo squid are abundant in tropical, subtropical, and temperate waters and occasionally present in boreal waters. Results from this study are consistent with the possibility that the purpleback squid spawns primarily in the tropics, and the jumbo squid spawns preferentially in subtropical or, perhaps, even temperate regions.

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Staging ovaries of Haddock (*Melanogrammus aeglefinus*): implications for maturity indices and field sampling practices

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Abstract—We build on recent efforts to standardize maturation staging methods through the development of a field-proof macroscopic ovarian maturity index for Haddock (*Melanogrammus aeglefinus*) for studies on diel spawning periodicity. A comparison of field and histological observations helped us to improve the field index and methods, and provided useful insight into the reproductive biology of Haddock and other boreal determinate fecundity species. We found reasonable agreement between field and histological methods, except for the regressing and regenerating stages (however, differentiation of these 2 stages is the least important distinction for determination of maturity or reproductive dynamics). The staging of developing ovaries was problematic for both methods partly because of asynchronous oocyte hydration during the early stage of oocyte maturation. Although staging on the basis of histology in a laboratory is generally more accurate than macroscopic staging methods in the field, we found that field observations can uncover errors in laboratory staging that result from bias in sampling unrepresentative portions of ovaries. For 2 specimens, immature ovaries observed during histological examination were incorrectly assigned as regenerating during macroscopic staging. This type of error can lead to miscalculation of length at maturity and of spawning stock biomass, metrics that are used to characterize the state of a fish population. The revised field index includes 3 new macroscopic stages that represent final oocyte maturation in a batch of oocytes and were found to be reliable for staging spawning readiness in the field. The index was found to be suitable for studies of diel spawning periodicity and conforms to recent standardization guidelines.

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An important component of the assessment and management of any fish stock is quantification of the stock's productivity, which is a function of survival, individual growth, and reproductive success of a fish population (Wootton, 1998; Morgan, 2008). There are several factors that can be used to estimate the annual reproductive potential of a fish stock, including but not limited to sex ratio, age and size at maturity, spawning stock biomass, fecundity, and stock recruitment estimates where egg and larval viability are taken into consideration (Jennings et al., 2001; Morgan, 2008). Regular monitoring and data collection on reproductive potential, including estimation of spawning stock biomass, age and size at maturity, and fecundity, are dependent upon the use of reproductive maturity indices from a sample of the population (Tomkiewicz et al., 2003).

Because the ability to accurately determine reproductive maturity by macroscopic examination of the gonads alone is fallible, the validity of field reproductive indices has been questioned (Hilge, 1977; Templeman et al., 1978; Saborido-Rey and Junquera, 1998; Vitale et al., 2006). Determination of maturation stages in the field has been criticized as not being dependable because different reproductive phases may appear similar during gross staging of the gonad. For example, estimates of spawning stock biomass or mean length at maturity will depend upon an accurate distinction between adult fishes with regenerating gonads and immature fishes (Forberg, 1982; West, 1990). Similarly, estimates of fecundity in determinate-spawning species, such as Atlantic Cod (*Gadus morhua*) and Haddock, require accurate identification of ovaries in prespawning stages (Murua et al., 2003). Therefore, it is important that the system used for determination of maturity stage is accurate and unambiguous (Brown-Peterson et al., 2011; Lowerre-Barbieri et al., 2011).

There have been considerable inconsistencies in the definitions of maturity stages of fishes among the existing indices in the literature. For example, O'Brien et al. (1993) defined

a female developing ovary as "a mixture of less than 50% yolked eggs and hydrated eggs"; however, according to Murua et al. (2003), the presence of hydrated oocytes indicate that the spawning process has begun and the gonad is in a "spawning" stage, where "oocytes are either in migratory nucleus stage or hydration stage." This discrepancy between indices in the definition of a developing ovary could result in different estimates of fecundity in determinate-spawning species for which prespawning, when the most advanced oocytes in an ovary are in the late vitellogenesis stage, is the optimal phase in reproductive maturity for the collection of samples for accurate estimation of fecundity. If sampling is conducted before this stage, all oocytes destined to be spawned may not be developed and would be left out, and, as a result fecundity would be underestimated. If samples are taken from females that have already spawned, the number of eggs that have already been released cannot be detected, an outcome that also would result in an underestimation of fecundity.

Another important difference between the maturation indices of Murua et al. (2003) and O'Brien (1993) is the description of a resting ovary. The definition of O'Brien (1993) was based on a description by the NMFS (1989) and Kesteven (1960) and was similarly defined by Waiwood and Buzeta (1989), Tomkiewicz et al. (2003), and Vitale et al. (2006). All these authors described the resting maturity stage as occurring after the spent maturity stage. Conversely, Murua et al. (2003) described the resting stage as an in-between batch state occurring before the spent stage, when some hydrated oocytes from the previous batch may remain and further batches of hydrated oocytes are still to be produced. Therefore, there was a need for greater consistency in definitions and standardization in terminology of reproductive maturity stages of fishes. In a recent work by Brown-Peterson et al. (2011), a great deal of effort was invested in providing such standardization.

Although certain reproductive traits, such as maturity phases, are universal among teleost fishes, the temporal patterns of these traits vary among species (Lowerre-Barbieri et al., 2011). Incorporation of temporal components into standardized indices potentially could produce more accurate staging results for each species studied, as well as provide additional information on the reproductive success of a species. A recent study by Tobin et al. (2010), published after our sampling was completed in 2006–07, identified the timing and microscopic changes in maturation events of female Haddock as they transition from immaturity to maturity between summer and winter. That study provided evidence that Haddock commit to maturation by October or November with the existence of cortical-alveolar–stage oocytes in the ovaries. Knowledge of this maturation commitment can allow researchers to confidently identify females as either immature, skipped-spawner, or mature after November, improving estimations of spawning stock biomass.

Haddock is a batch-spawning species with group-synchronous ovary organization and determinate fecundity (Clay 1989; Murua and Saborido-Rey, 2003). This collection of reproductive traits is common in demersal Northwest Atlantic fishes, including but not limited to Atlantic Cod, Yellowtail Flounder (*Limanda ferruginea*), and Atlantic Halibut (*Hippoglossus hippoglossus*; see Murua and Saborido-Rey, 2003). The standard number of yolked oocytes immediately before the onset of spawning in a determinate-fecundity spawner can be considered equivalent to the potential annual fecundity of that fish (Murua et al., 2003). After the onset of spawning, the individual will hydrate several batches of yolked oocytes throughout the spawning season.

The purpose of our study was to develop a standard field-proof, macroscopic ovarian maturity index for Haddock that is suitable for use in studies of diel spawning periodicity (Anderson, 2011) and conforms to the recent standardization guidelines of Brown-Peterson et al. (2011). Diel spawning periodicity has been widely studied in marine fishes (e.g., Ferraro 1980; Walsh and Johnstone, 1992; Wakefield, 2010) and provides details on the chronology of reproductive processes in species. It has been suggested that diel spawning periodicity maximizes fish survival and reproductive success (Ferraro, 1980; Lowerre-Barbieri, 2011). In addition to support for the collection of field data on reproductive stages, we also wanted the index to provide guidance on sampling techniques for the collection of samples for laboratory analysis. First, a staging method developed from unpublished observations and a review of data published before our sampling in 2006–07 was used to stage female Haddock ovaries in the field. The resulting maturity index was then revised compared with a laboratory histological staging method similar to that of Tomkiewicz et al. (2003) for Atlantic Cod in the Baltic Sea. New stages were assessed to determine whether they could be used in future studies to examine diel patterns in spawning (Anderson, 2011). Finally, the relative strengths and weaknesses of both the field and laboratory approaches were assessed.

Materials and methods

Initial field and laboratory indices

A new field macroscopic ovarian maturity index for female Haddock was developed by building on previous published indices (Homans and Vladykoy, 1954; Robb, 1982; Murua et al., 2003; Brown-Peterson et al., 2011) and unpublished observations made in the field (Table 1). The index consists of 8 stages, progressing from immature to regressing. To move toward use of standard phraseology, the terminology follows Brown-Peterson et al. (2011). It differs from previously published indices with the addition of 3 stages that represent early to late progression of oocyte maturation (OM; Brown-

Table 1

Field index developed and used to stage the reproductive maturity of female Haddock (*Melanogrammus aeglefinus*) caught in the Gulf of Maine in 2006–07 during this study in which macroscopic methods in the field were compared with histological methods in the laboratory. OM=oocyte maturation.

Stage	Abbreviation	Description
Immature	I	Ovaries small and firm, about 1/8 the volume of the body cavity. Membrane thin and transparent, gray to pink in color. Contents microscopic. Individual oocytes not visible to the naked eye.
Developing	D	Ovaries larger and plump, about 1/3 to 1/2 the volume of the body cavity. Membrane reddish-yellow with numerous blood vessels. Contents visible to the naked eye and consist of opaque eggs that give the ovaries a granular appearance.
Hydration stage 1	H1	Ovaries well developed, reddish-yellow in color, at least 2/3 volume of body cavity. Membrane opaque with blood vessels conspicuous. Contents consist mostly of yellow-looking oocytes with <25% of the ovary containing larger translucent oocytes. A batch of oocytes in the early stages of OM where oocytes start to hydrate.
Hydration stage 2	H2	Ovaries well developed, reddish-yellow in color, at least 2/3 volume of body cavity. Membrane opaque with blood vessels conspicuous. Visible surface of the ovary consists of 25–50% larger translucent oocytes. Further progression of a batch of eggs in OM.
Hydration stage 3	H3	Ovaries well developed, reddish yellow in color, at least 2/3 the volume of body cavity. Membrane opaque with blood vessels conspicuous. Visible surface of the ovary consists of 50–75% larger translucent oocytes. Ovaries may appear a little flabby, indicating the previous release of batch(es) of eggs. Final stages of the maturation of a batch of oocytes before a spawning event.
Ripe and running	RR	Ovaries very large, over 2/3 the volume of the body cavity. Contents consist of mostly large, translucent eggs. Eggs running freely with little to no pressure on the abdomen.
Regressing	S	Ovaries soft, and flabby, about 1/4 the volume of the body cavity. Membrane thick and tough, purplish in color, and bloodshot. Contents empty, few eggs remain, giving the gonad a patchy appearance.
Regenerating	RE	Ovaries small and firm, 1/6 the volume of the body cavity. Membrane thin but less transparent than an immature ovary, yellowish-gray in color. Contents microscopic, opaque.

Peterson et al., 2011) on the basis of the percentage of hydrated oocytes present (H1, H2, H3; Table 1, Fig. 1).

During observations of mature female Haddock ovaries, we noticed that many of them had varying numbers of hydrated oocytes. We did not find an ovarian maturity index in the literature that categorized the progression in percentage of hydrated oocytes in a gonad. We were interested in whether the increase in percentage of hydrated oocytes was detectable over time and whether these stages may aid in examination of diel reproductive periodicity (Anderson, 2011).

Hydration stage 1 (H1) is an ovary where a batch of oocytes is in the early phase of OM and when <25% of that ovary's visible surface contains translucent, hydrated oocytes (Table 1).

Hydration stage 2 (H2) is an ovary where a batch of oocytes is in the middle phase of OM and when 25–50% of that ovary's visible surface contains translucent, hydrated oocytes (Table 1).

Hydration stage 3 (H3) is an ovary with a batch of oocytes in a late phase of OM and when 50–75% of the visible surface of that ovary contains translucent, hydrated oocytes (Table 1).

We hypothesized that H1, H2, and H3 occur with each batch of oocytes before it is spawned (Fig. 1). The index also includes for each stage: 1) a macroscopically derived ratio of ovary volume to body cavity volume, similar to the ratio of gonad cavity length to body cavity length that Robb (1982) included for some stages; 2) a physical description of the ovary membrane, as Homans and Vladykoy (1954) included for some of the stages; and 3) a grossly assessed oocyte development description, included by Homans and Vladykoy (1954), Robb (1982), and Murua et al. (2003) (Table 1).

The histological staging method was derived independently of the macroscopic ovarian maturity index (i.e., during analysis, field-based stages were not used by laboratory personnel in development of histological stages and vice versa), and it was based on previous work of Tomkiewicz et al. (2003), Roumillat and Brouwer (2004), and Brown-Peterson et al. (2011) (Table 2). To differentiate the processes of early versus later vitellogenic activity, 2 histological index stages (2.1 or 2.2) were used to define developing ovaries (Table 2). Because Haddock are classified as possessing determinate fecundity (Murua et al., 2003), all oocytes that will be spawned during the upcoming season develop during these 2 stages, leaving a group of primary oocytes as a reserve for the successive spawning season. However, the developing stages in the histological index (2.1 and

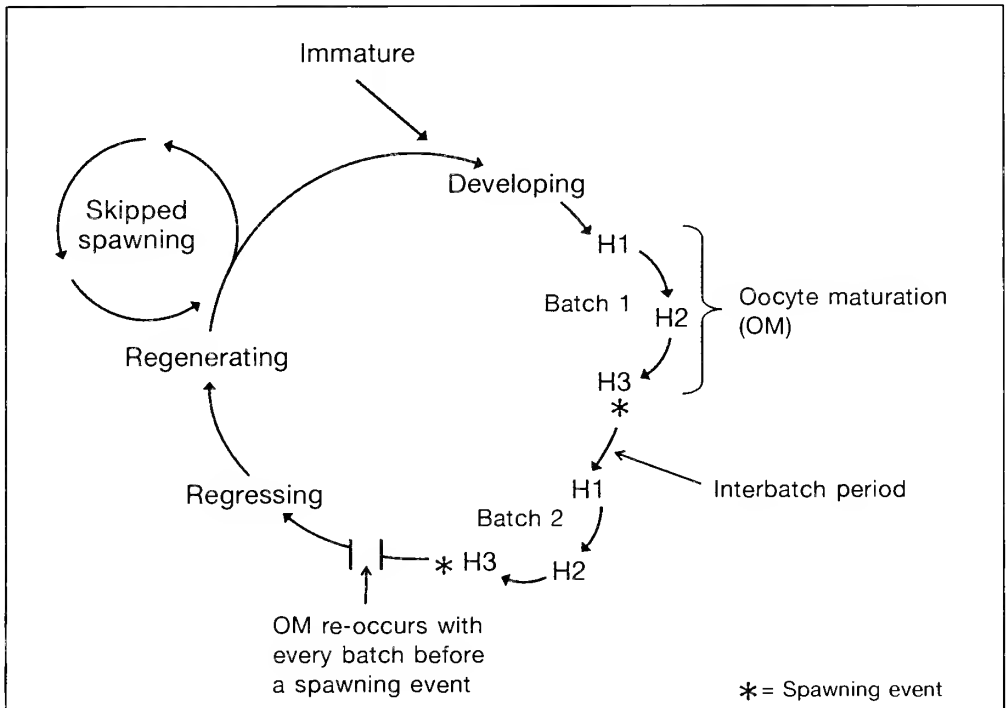


Figure 1

The maturation cycle of the female Haddock (*Melanogrammus aeglefinus*), including 3 hydration stages and an interbatch period, introduced and used during this study of methods for staging the reproductive maturity of Haddock sampled in the southwestern region of the Gulf of Maine in the spring of 2006 and 2007. Hydration stage 1 (H1), hydration stage 2 (H2), and hydration stage 3 (H3) represent early-to-late progression of final oocyte maturation (OM) of a batch of oocytes, based on the percentage of hydrated oocytes present. *=spawning event.

2.2) were grouped together as one developing stage (2.0) when the histology results were compared with the field results because those stages could not be differentiated by macroscopic examination. Three phases of spawning-capable (SC) ovaries were assigned in the histological index as 3.1, 3.2, and 3.3 to differentiate the process of early, middle, and late phases of OM: early germinal vesicle migration (GVM) and germinal vesicle breakdown (GVBD) (Table 2). The gross assessments of H1, H2, and H3 are based on morphologically distinct criteria that are corroborated by the histological sections that effectively separate these stages from each other (Table 2). Two histological index stages (4.1 and 4.2) were defined to categorize SC ovaries that showed evidence of recent ovulation with the presence of recent (4.1) or old (4.2) postovulatory follicles (POFs; Alekseyeva and Tormosova, 1979; Saborido-Rey and Junquera, 1998). POFs are ruptured empty oocyte casings left in the ovary after a spawning event (Table 2; Alday et al., 2010; Saborido-Rey and Junquera, 1998). If a sample contained POFs but also exhibited characteristics of another stage, the alternative stage was assigned with a note that the sample contained POFs (e.g., if a sample primarily contained oocytes in stage 3.1 but also contained POFs, it was assigned to the 3.1 stage).

Field sampling

Commercial fishing vessels were chartered for 25 dedicated survey trips in the spring of 2006 (15) and 2007 (10) to collect biological samples of Haddock in the southwestern Gulf of Maine (National Marine Fisheries Service Statistical area 514; Fig. 2). Surveys were based on a fixed station design with sampling where Haddock aggregations were known to previously exist. Sampling was conducted during the known spawning season of Haddock in the Gulf of Maine, between January and June (Brown, 1998). Haddock were identified in the manner used by Collette and Klein-MacPhee (2002).

Longlining was the preferred collection method for samples because few discards would result. Approximately 19 m of longline was set and retrieved 3 times at each sampling location over a 12-h period with the objective of having 2 consecutive trips represent sampling over a 24-h period (0100–0000 h; Table 3). Sets were conducted within specific 4-h time bins

Table 2

The reproductive maturity index developed and used in this study of staging methods for female Haddock (*Melanogrammus aeglefinus*) during histological analysis with analogous stages from the macroscopic field index. Histological definitions were based on criteria of Brown-Peterson et al. (Table 2 in 2011) CA=cortical alveolar; GVM=germinal vesicle migration; GVBD= germinal vesicle breakdown; NA=not applicable; OM=oocyte maturation; POF=postovulatory follicle; SC*=spawning capable, actively spawning subphase; Vtg1=primary vitellogenic; Vtg2=secondary vitellogenic; Vtg3=tertiary vitellogenic.

Histology	Stage	Macroscopic	Histological description
Immature	1.0	I	Small ovaries, only oogonia and primary growth oocytes present. Ovary wall thin, no muscle bundles evident.
Developing (early developing subphase)	2.1	D	Only primary and cortical alveolar oocytes present.
Developing	2.2	D	Primary growth, CA, Vtg1 and Vtg 2 oocytes present.
SC* early GVM	3.1	H1	Predominance of Vtg3 and early OM and beginning of GVM, yolk coalescence beginning. Few germinal GVBD oocytes observed, although some hydrated oocytes present.
SC* GVM	3.2	H2	Both early and late stages of GVM oocytes, obvious yolk coalescence occurring. Greater abundance of GVBD oocytes seen. Increased number of hydrated oocytes present.
SC* GVBD	3.3	H3	Predominance of GVBD oocytes, many with complete yolk coalescence. Many hydrated oocytes present—immediately before ovulation.
SC recent POF	4.1	NA	Many recent POFs present, showing few signs of degeneration. Otherwise advanced oocytes consist most noticeably of Vtg1–Vgt3 oocytes.
SC older POF	4.2	NA	Only older POFs present with advanced structural degeneration. Advanced oocytes consist of Vtg1–Vgt3 oocytes.
Regressing	5.0	S	Only spawning residue (old POFs) and primary growth oocytes remain in the ovary. Spawning effort for season ceased.
Regenerating	6.0	RE	Only primary oocytes remain in small ovary. Ovarian wall thickened, muscle bundles present.

(0100–0500 h, 0500–0900 h, 0900–1300 h, 1300–1700 h, 1700–2100 h, 2100–0000 h EST) to examine diel periodicity in reproductive maturity (Anderson, 2011). Each longline was fished with 150 to 400 circle hooks set 2 m apart for an average soak time of 2 h. The number of hooks fished per line on each trip was dependent on the success of catching Haddock that day. With the intent of sampling at least 50 Haddock from each longline set, the number of hooks was increased if the sample size was not reached or decreased if more fish than were needed were caught.

All Haddock were measured by fork length (FL, ±1 mm) and examined externally for signs that indicated if they were in the ripe and running maturity stage (classified RR; Table 1). Ovaries were classified as RR when eggs were observed to be running freely from females with little pressure applied to the abdomen. The first 50 Haddock in each set were sacrificed to determine the stage of development of the gonads. If a fish ovary was observed to be ripe and running, its sex and maturation stage could be determined without excisions, and it was automatically classified as RR in the field. A subsample of the 50 sacrificed female Haddock that represented all reproductive stages from each longline set was labeled and reserved on ice. Fish from each of the following length bins were collected from each set if possible to have representation from as many cohorts as possible: 30–40 cm, 40–50 cm, 50–60 cm, and >60 cm FL.

Laboratory methods

Samples were processed in the laboratory within 24 h of the end of each trip. Total weight (±0.1 kg) and ovary weight (±0.01 kg) of each individual were recorded. Macroscopic maturity stage of all samples was re-examined by the same field examiner. Digital photographs of whole ovaries were taken from a random subsample of each stage in the field index. To determine the accuracy of macroscopic maturity staging performed with our maturation index, histological analysis was conducted on tissue samples of a subsample of 169 ovaries from 1706 macroscopically classified fish representative of all 8 stages.

All histological tissue samples were taken from the forward right lobe of each ovary. It was assumed that this approach was appropriate because, according to Robb (1982), Haddock ovaries are homogeneous in structure throughout both lobes with oocytes present in various stages from the walls to the center of the ovary. Samples of 10-g tissue sections were fixed for at least 14 days in 10% neutral buffered formalin before they were transferred to 50% isopropyl alcohol. Samples were processed with standard histological procedures

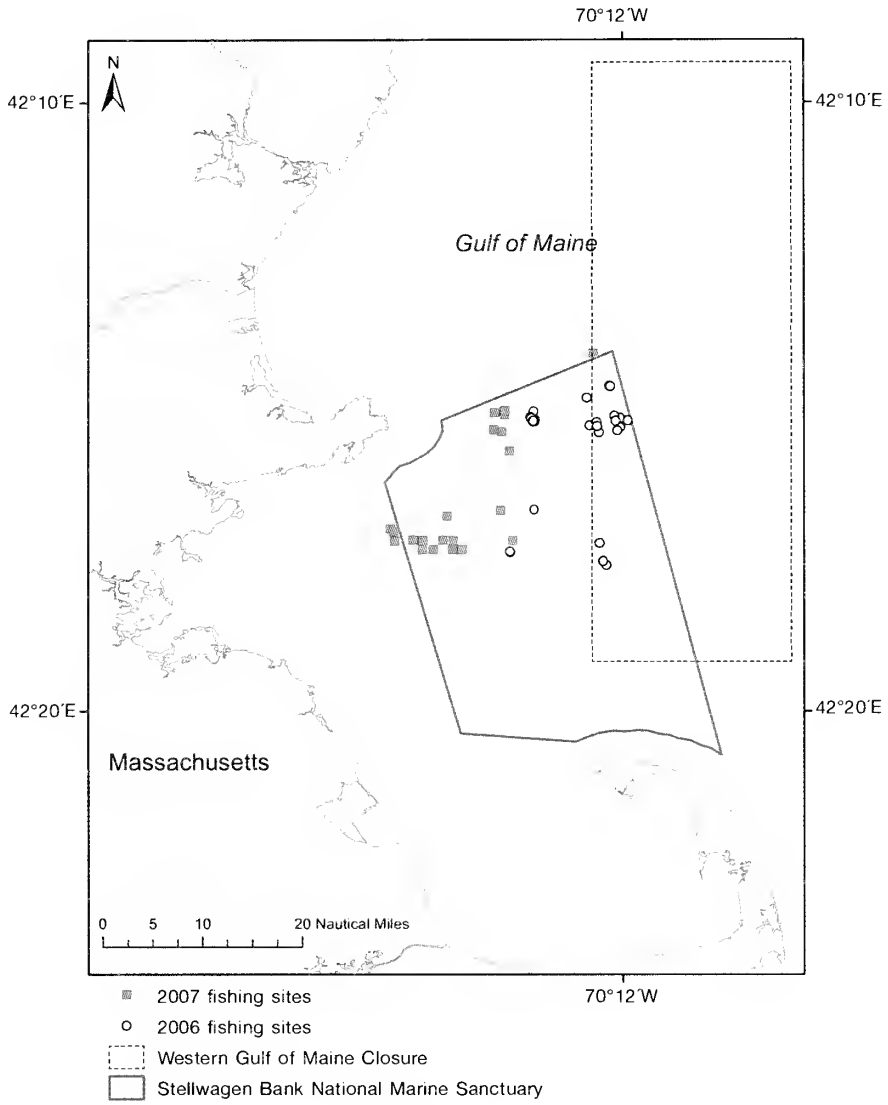


Figure 2

Map of the locations where mature female Haddock (*Melanogrammus aeglefinus*) were sampled in the southwestern region of the Gulf of Maine in the spring of 2006 and 2007 for for staging reproductive maturity.

(Humason, 1972) through a graded ethanol series, embedded in paraffin, and sectioned at 6 µ. Tissues were stained with Gill's hematoxylin and counterstained with eosin-Y. Ovary samples were classified by the occurrence of specific histological features that represent progressive oocyte maturation stages (Brown-Peterson et al., 2011) (Table 2). The most progressive feature observed in each sample was used to assign the appropriate stage. Photomicrographs were taken of a random subsample of stained tissue for each field index stage.

Statistical analysis

A contingency table was used to compare the results between the macroscopic staging methods used in the field and the histological staging methods used in the laboratory (Table 4). The table cell where the 2 equivalent stages cross shows the number of samples for which the data from the 2 methods agreed. Because the 2 indices were developed independently, 2 different types of percent agreement were calculated. One type was derived by dividing the number of samples for which the 2 methods agreed by the field stage sample size (last row in Table 4). The second type of percent agreement was calculated by dividing the number of samples for which the 2 methods agreed by the histological stage sample size (last column in Table 4). We did not have enough observed frequencies in each cell to perform a chi-square statistical analysis.

Table 3

Dates of trips during which longlines were set and retrieved in the southwestern region of the Gulf of Maine in the spring of 2006 and 2007 to collect samples of female Haddock (*Melanogrammus aeglefinus*) over a 12-h period with the objective of having 2 consecutive trips represent sampling over a 24-h period.

24-h period	Year	Sampling dates
1	2006	3/12, 3/28, 3/31
2	2006	4/7, 4/10, 4/28
3	2006	4/30, 5/4, 5/8
4	2006	5/8, 5/16
5	2007	3/26, 3/31, 4/10
6	2007	4/10, 4/21, 4/24
7	2007	5/1, 5/22
8	2007	5/24, 5/30

Results

The results of each stage are formatted to explain both types of percent agreement as a function of each of the two staging methods. For each stage, the results of the macroscopic field staging method are presented first, followed by the results of the histological laboratory staging method.

All 6 ovaries classified as immature *(I)* with the field index were also classified as the equivalent histological stage (1.0) in the laboratory. In contrast, all but 2 of the 8 samples classified as I (1.0) with the laboratory staging method were also classified as I with the field index (Table 4). Two samples classified as 1.0 in the laboratory were classified as regenerating (RE) with the field index.

Only 4 of the 9 ovaries classified as developing (D) with the field index were also classified as developing (2.0) with the laboratory staging method (Table 4). Two of the remaining ovaries classified as D with the field index were classified as the adjacent histological stage 3.1, and 2 samples contained early POFs (stage 4.1) and 1 sample contained late POFs (stage 4.2). In contrast, 7 of the 12 ovaries classified as 2.0 in the laboratory were classified as the adjacent H1 with the field index, and 1 sample was classified as RE.

Twelve of the 32 ovaries classified as HI with the field index were also classified as the equivalent histological stage 3.1 (Table 4) in the laboratory. Seven of the ovaries classified as H1 with the field index were

Table 4

Contingency table showing the results from the cross classification between the histological maturity stages (columns) and the field maturity stages (rows) in the indices used in this study of methods for staging the reproductive maturity of female Haddock (*Melanogrammus aeglefinus*). The gray squares represent where the cross classification is expected to have the highest frequencies of agreement. *n*=sample size; PA=percent agreement; NA=not applicable. If NA was used in place of PA, then that stage was not expected to agree with any of the opposing index stages.

Maturity-index stages based on histological examination	Maturity-index stages based on field examination									
	I	D	H1	H2	H3	RR	S	RE	n	PA
1.0	6	0	0	0	0	0	0	2	8	75%
2.0	0	4	7	0	0	0	0	1	12	31%
3.1	0	2	12	0	1	0	1	0	16	75%
3.2	0	0	2	21	2	0	4	0	29	72%
3.3	0	0	5	9	22	17	2	2	57	39%
4.1	0	2	1	1	0	0	0	0	4	NA
4.2	0	1	5	2	0	1	0	0	9	NA
5.0	0	0	0	0	0	1	4	16	21	19%
6.0	0	0	0	0	0	0	1	12	13	92%
n	6	9	32	33	25	19	12	33		
PA	100%	44%	38%	64%	88%	NA	33%	36%		

classified as the adjacent histological stage 2.0, 2 ovaries were classified as 3.2, and 5 ovaries were assigned as 3.3. One H1-classified ovary contained early POFs, and 5 H1 ovaries contained late POFs. In contrast, 2 of the 16 samples classified as 3.1 in the laboratory were classified as the adjacent D stage with the field index, 1 sample was classified as H3, and 1 sample was assigned as regressing (S).

Twenty-one of the 33 ovaries classified as H2 with the field index were also classified as the equivalent histological stage 3.2 in the laboratory (Table 4). Nine H2-classified ovaries were classified as the adjacent histological stage 3.3. One ovary contained early POFs, and 2 ovaries contained late POFs. In contrast, 4 of the 29 ovaries classified as the 3.2 stage in the laboratory were classified as the adjacent field stages (H1 and H3), and 4 of those ovaries were classified as S.

The H3-classified samples were most frequently classified as the equivalent histological stage 3.3 (n=22; Table 4). Two H3-classified ovaries were classified as the adjacent histological stage 3.2, and 1 ovary was classified as 3.1. In contrast, 35 of the 57 ovaries classified as the histological stage 3.3 were classified differently with the field index, with most ovaries classified as H2 (n=9) or RR (n=17).

All but 2 of the ovaries classified as RR (n=17) in the field were classified as the histological stage 3.3 (Table 4). The 2 remaining ovaries were classified as the histological stages 4.2 and 5.0.

Four of the 12 ovaries classified as S with the field index were assigned the equivalent histological stage 5.0 (Table 4). Four additional ovaries classified as S with the field index were classified as the histological stage 3.2, and 2 ovaries were assigned as 3.3, 2 ovaries as 3.1, and 1 ovary as 6.0. In contrast, most of the 21 ovaries assigned to the histological stage 5.0 in the laboratory were classified as RE with the field index (n=16, 76%); however, 1 ovary was assigned as H3 (Table 4).

Twelve of the ovary samples classified as RE with the field index were classified as the equivalent histological stage 6.0 (Table 4). Sixteen samples classified as RE with the field index were classified as the adjacent histological stage 5.0 in the laboratory. Two additional samples classified as RE in the field were classified as histological stage 3.3, and 2 samples were classified as 1.0, and 1 sample was assigned as 2.0. In contrast, all but 1 of the 13 ovaries classified as histological stage 6.0 in the laboratory were also classified as RE with the field index.

A final composite ovarian maturity index was created on the basis of the findings from this study (Table 5). Visual characteristics for both the whole ovary and tissue sample were emphasized as was similarly done by Tomkiewicz et al. (2003) for Altantic Cod in the Baltic Sea. The final index consists of 7 stages of ovary reproductive maturity distinguishable at sea. Table 5 includes for each maturity stage an image of the whole ovary, a photomicrograph of equivalent histological tissue, and both a macroscopic and microscopic physical description of the ovary. Notes are included to aid the user in correct macroscopic identification of each stage. Sampling techniques for collection of tissue samples are also included for problematic stages. On the basis of comparison with the histological data, we concluded that H3 and RR field stages are identical and grouped them together as a single stage (H3). When we used this revised H3 field stage, 39 of the 44 ovaries assigned as H3 were assigned the equivalent 3.3 histological stage.

Discussion

The utility of the field-based staging method for the classification of fish reproductive maturity for fisheries management is dependent on its biological accuracy. The findings from this study highlight the problems of development of an accurate error-proof field ovarian maturity index on the basis of macroscopic observation. However, a comparison of field-based and histology-based staging methods of Haddock ovaries presented in this study revealed the need to revise the field staging methods to increase the accuracy of both staging methods. Although laboratory staging done on the basis of histology is inherently more accurate than any macroscopic field staging method, there was indication that field observations can reveal weaknesses in the laboratory approach because samples of the ovary taken for histology are not always going to be representative of the whole ovary. The strengths and weaknesses of both approaches for each maturation stage are discussed in the next sections, followed by recommendations for correct identification of each stage and a description of helpful sampling techniques for collection of tissue samples of problematic stages.

Immature stage

The I stage in the field index was equivalent to the 1.0 histological stage (Tables 1 and 2). The only stage mistaken for immature in the field was RE (Table 1). In both stages, the ovary was small and firm. The RE ovary appeared to be a little larger, less transparent, and grayer in color in comparison with the pink color of an immature ovary. However, in a young mature fish or late immature fish, these differences were less detectable. The imprecision in separation of immature and regenerating mature females also has been encountered in staging Atlantic Cod ovaries (Tomkiewicz et al., 2003). Comparison of the current mean length at maturity for Haddock with the size of the specimen may help support either maturity stage in the field, but this criterion should not be relied on because length at maturity can change over time (Saborido-Rey and Junquera, 1998; Tobin et al., 2010).

In this study, the smallest Haddock caught was 35.5 cm FL, larger than the mean length at maturity re-

corded for this species in the Gulf of Maine (34.5 cm; Collette and Klein-MacPhee, 2002). The gear type used in this study selected for larger fish, and we suspect that smaller fish avoided the longline hooks. Although to our knowledge skipped spawning (when a mature individual skips a year of spawning) has not been observed in Haddock, it is not uncommon in long-lived iteroparous fishes, including Atlantic Cod (Jørgensen et al., 2006; Rideout et al., 2006; Fig. 1). Therefore, we could not have assumed that a female was immature if it lacked signs of sexual maturity during the spawning season, as was assumed by Waiwood and Buzeta (1989) because there is the possibility that the fish had skipped spawning that year.

The use of microscopic analysis or histological examination of a tissue sample of the ovary was a reliable way to determine whether the ovary was immature or regenerating. Immature ovaries could be distinguished histologically from regenerating ovaries by the diameter of the primary oocytes (W. Roumillat, personal commun.). Immature ovaries contained primary oocytes that were equal in diameter, but regenerating ovaries had primary oocytes that varied in diameter. Additionally, the RE phase can be differentiated from the I phase by the following features: RE ovaries 1) have a thicker ovarian wall, 2) have more space, interstitial tissue, and capillaries around primary oocytes, and 3) have the presence of late-phase atresia and muscle bundles (blood vessels surrounded by connective and muscle tissue) (Brown-Peterson et al., 2011). Because of the selectivity of the fishing gear for larger-size fish and our limited sampling period, our study did not provide adequate data to fully resolve macroscopic differences between the RE and I stages. Further work should focus on differentiation of a regenerating ovary from an immature ovary with sampling conducted further into the summer with less size-selective gear. Proper identification of immature ovaries would greatly reduce the error in calculation of spawning biomass estimates and improve accuracy of estimates of length at maturity.

Developing stage

There was disagreement between D and early OM phase, H1 (Table 1). We observed that when a Haddock ovary began OM, some oocytes in the initial batch completed the process before others within the same ovulating batch. Although Haddock ovaries have been reported to be homogeneous in structure throughout all phases of maturity (Templeman et al., 1978; Robb, 1982), our observations indicate that it is not homogeneous in structure during this very early phase of OM (H1). This result is supported by Alekseyeva and Tormosova (1979), who reported that formation of batches occurs through asynchronous maturation of individual groups of oocytes. The histological staging method sometimes resulted in H1 ovaries being misclassified as D, likely because they were sampled during initial OM of the first batch of oocytes for the season, when there were no histological characteristics present to indicate that prior batches had been spawned. Initial spawning H1 ovaries had so few fully hydrated oocytes (because of the asynchronous maturation of the batch) that collection of a small tissue sample from a central location was sometimes unsuccessful in representing all phases of oocytes present. As a single batch progresses through OM, evidence that spawning has been initiated becomes more obvious with GVM and yolk coalescence beginning in oocytes (Table 2; Lowerre-Barbieri et al., 2011). As the season progresses and the ovary initiates OM in later batches of oocytes, a H1 tissue sample could be distinguished from a D tissue sample by the presence of POFs.

The agreement between macroscopic and histological staging for D and H1 ovaries could be improved if the method used to take tissue samples from the ovary were modified. When ovaries are classified as H1 in the field, a larger tissue sample or samples should be taken from multiple places in the ovary to improve the accuracy of the histological results. Our observations demonstrate that determination of the maturation of an ovary based on histological examination alone may not always be accurate. To reduce staging errors based on histological analysis in future studies, it is recommended that each tissue sample be documented with a photograph of the whole ovary from which it was extracted and with an estimate of the percentage of hydrated oocytes observed on the visible surface of the ovary.

Three ovaries classified as D in the field contained POFs when analyzed histologically, and, by our definition, a D ovary could not have previously spawned that season (Table 1; Fig. 1). Therefore, those specimens had spawned at least one batch of eggs but had not yet hydrated oocytes for the next batch, and the decrease in volume of the ovary after spawning a prior batch of eggs was not evident in field observations. A closely related species, Atlantic Cod, begins to hydrate a batch of oocytes 1–2 days before spawning (Kjesbu, 1991). Final oocyte maturation in cold-water marine fishes with pelagic eggs generally lasts 1–2 days (Thorsen and Fyhn, 1996). Trippel and Neil (2004) reported that Haddock had a mean interval of 5.4 days between batches of released eggs, and Hawkins et al. (1967) and Alekseyeva and Tormosova (1979) reported an interval of 26–40 h. These findings combined indicate that there is an interbatch period between the spawning of a batch and the next batch that is beginning to hydrate, a period described by Murua et al. (2003) as the resting stage (Fig. 1).

Consequently, there was the possibility that a mature ovary could be incorrectly classified as D in the field if it was between ovulation events during this interbatch period. Therefore, we concluded that it is not always possible to be certain that an individual has begun spawning for the season on the basis of macroscopic observation alone and this uncertainty can pose

a problem for fecundity studies where ovary weight is used as a factor in determining fecundity. For the same reason, we also concluded that it is not possible to accurately stage an ovary as D by macroscopic observation alone. This issue poses a problem for studies that use gravimetric counting of vitellogenic oocytes and oocyte density to determine fecundity. The D stage, when the most advanced oocytes in the ovary are in the late vitellogenesis phase, is the optimal stage from which samples should be taken to determine fecundity. Therefore, we recommend that ovary samples be collected from fishes classified as D on the basis of macroscopic observations to confirm through microscopic or histological analysis that the ovary is in a prespawning state.

Hydration stages

A challenge in the use of the field index was the subjective evaluation of the percentage of hydrated oocytes in an ovary that was used to assign the consecutive H1, H2, and H3 stages. Therefore, histological samples were often assigned to a stage adjacent to the stage that was reported in the field. There were 5 instances where an ovary was macroscopically classified as H1 with the field index but microscopically classified as the histological stage 3.3. This difference in staging was likely due to some variation in individual and temporal batch fecundity (Trippel et al., 1998). However, this error was rare and the hydration stages were correctly staged consistently enough that we do not consider this misclassification problematic in identification of the correct hydration stage for the purpose of assessing diel reproductive patterns.

The histology-based laboratory staging method underestimated the H1 stage because the ovary typically appears to be heterogeneous during this stage and, therefore, was not adequately represented in the tissue samples. An H1-classified ovary could be incorrectly identified as D based on histological examination under these conditions. However, as an ovary matured further, the oocytes appeared to hydrate in unison and evenly throughout the ovary and nuclear migration and globule yolk coalescence became more evident. These criteria reduced the bias in the sampling method in later phases of H1 and eliminated it in later stages H2 and H3.

Histological analysis verified that H3-stage ovaries were in a state where the next batch of oocytes to be spawned were in final OM phase (GVBD), with most oocytes fully hydrated. This consistent result is important because both the field H3 and histological 3.3 stages can be confidently used to identify spawning readiness, and, therefore, we concluded that they will be well suited for use in studies of diel spawning periodicity in Haddock (Anderson, 2011) and other fishes.

Ripe and running stage

When the ovaries of RR females were examined macroscopically, they exhibited characteristics of the H3 stage. Furthermore, the tissue samples from these ovaries were classified as 3.3 (SC GVBD; Table 2) with histology-based methods. On the basis of results from the histological analysis conducted on ovaries classified as RR in the field and from the portion of the RR ovary full of hydrated oocytes during macroscopic observation, we decided to combine the RR and H3 field stages into a single stage in the final index (H3; Table 5).

Use of the RR field stage proved problematic because of the sampling method, and we recommend caution in its use in future studies. Homans and Vladykoy (1954) reported that female Haddock stop feeding during spawning—behavior that would make it difficult to catch actively spawning fish with baited gear and possibly result in an underestimation of RR females in the population. In addition, RR may be overestimated because of premature ovulation induced by stress or barotrauma. It is hypothesized that the barotrauma caused by forcing specimens to ascend to the surface from an average depth of 90 m during sampling can cause premature ovulation of hydrated oocytes. An increased level of cortisol in fishes is an indication of severe stress, but it is also involved in the natural process of ovulation (Billard et al., 1981; Wendelaar Bonga, 1997). The 2-h average soak time of the hooks in this study could have been enough time for the stress response to induce ovulation in an H3-stage fish before it landed on board the fishing vessel.

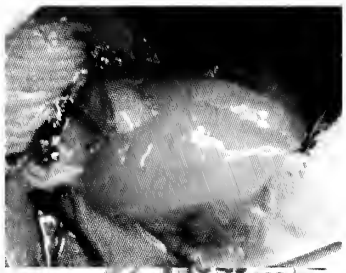
For the same reason, histological stage 4.1 may be overestimated, because the premature ovulation caused by barotrauma results in POFs appearing before they normally would. We concluded that it is difficult to catch a Haddock in the act of spawning, especially with baited hooks; therefore, use of H3-stage fish to estimate spawning readiness would be more accurate. However, the practice of macroscopically staging a RR Haddock through application of pressure to the abdomen and observation of the excretion of hydrated oocytes is a method that can be used to classify a female as spawning ready without need to sacrifice the fish.

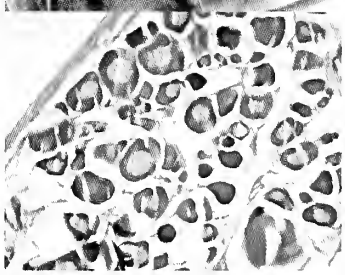
Regressing stage

The S ovary stage was the most problematic for macroscopic identification. The regressing condition is particularly difficult to detect in a species such as Haddock with asynchronous development, where batches of eggs are spawned multiple times over a prolonged season (Hickling and Rutenberg, 1936; West, 1990). Species with determinate fecundity complete a spawning season by the maturation and spawning of the entire cohort of oocytes developed that year. When only a single batch of oocytes was left in the ovary to be spawned, it was termed "last spawn." This stage was evident only during histological analysis. Of the ovaries classified

Table 5

The final female reproductive maturity index developed from findings with the macroscopic and microscopic method for staging the maturity of female Haddock (*Melanogrammus aeglefinus*).



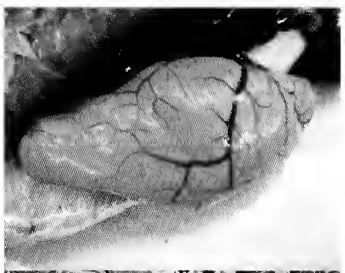


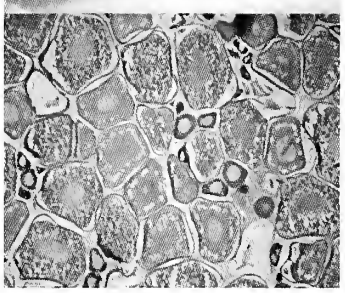
Immature (I)

Macroscopic: The ovary is small and firm, and approximately 1/8 the volume of the body cavity. The membrane is thin, transparent, and gray to pink in color. Individual oocytes are not visible to the naked eye.

*Note: This stage can look similar to a resting-stage ovary. Use of microscopic analysis or histology on a tissue sample of the ovary may be the only way to determine that the ovary is immature and not resting.

Microscopic: The ovary contains germ cells, oogonia, and primary oocytes. The ovary wall is thin and the primary oocytes vary little in diameter. No muscle bundles can be seen. The nucleus is relatively large with the most advanced oocytes having peripheral nucleoli (magnification 100×).





Developing (D)

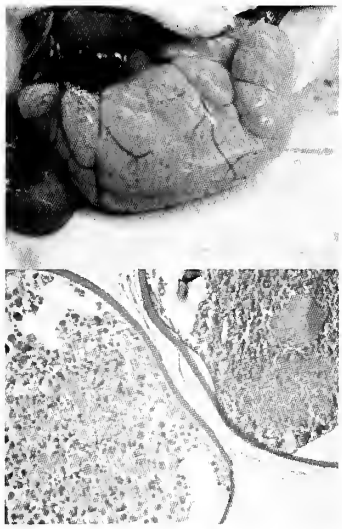
Macroscopic: The ovary is plump and approximately 1/3 to 1/2 the volume of the body cavity. The membrane is reddish-yellow and has numerous blood vessels. The contents are visible to the naked eye and consist of opaque eggs, giving the ovaries a granular appearance.

*Note: If hydrated oocytes are visible, the ovary should be classified as H1 (see the next stage below). Hydrated oocytes will be large in diameter and translucent in color. A large tissue sample should be taken from all ovaries macroscopically classified as developing and analyzed microscopically to confirm that postovulatory follicles are not present and that the ovaries are in a prespawning state. It may be helpful to document the tissue sample with a photograph of the whole ovary.

Microscopic: Primary and cortical alveoli oocytes, and primary and secondary vitellogenic oocytes are present. There is no evidence of postovulatory follicles (magnification 40×).

in the field as S, 58% (N=7) were classified as being in 1 of the 3 OM histological phases. The most plausible explanation for this result, other than observational error, is that these particular specimens were maturing the last batch of eggs to be spawned that season (last spawn) and the ovary at this point had lost its rigidness and, therefore, looked as though it was in the S stage. Last spawn was observed in 8 (5%) of the histological samples, 5 of which were classified as S in the field. Last spawn also was observed in Haddock in the North Sea (Alekseyeva and Tormosova, 1979). Near the end of the spawning season, the ovary can lose its rigidness, although it still has 1–2 batches of oocytes to spawn and appears as S. The outside membrane thickens, which increases the difficulty of staging the ovary through examination of just the outside (Templeman et al., 1978). Staging on the basis of the flabbiness of the ovary alone is not recommended, and the inside of the ovary should be examined for hydrated oocytes. If any oocytes during final oocyte maturation (OM) remain, the ovary is most likely not in the S stage and could be in last spawn. Histological examination of a sample of an ovary can be an effective way to determine if an ovary is regressing.

Table 5 continued

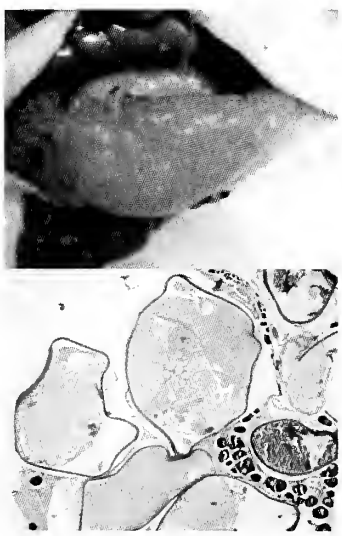


Hydration stage 1 (H1)

Macroscopic: The ovary is well developed, reddish-yellow in color, and approximately 2/3 the volume of the body cavity. The membrane is opaque and has prominent blood vessels. The contents consist mostly of yellow-looking oocytes and <25% of the ovary contains large translucent (hydrated) oocytes.

*Note: In the early phase of the H1 stage, the ovary is not visually homogeneous and hydrated oocytes can be unevenly scattered throughout. If microscopic analysis will be conducted on a subsample, take care to obtain a representative tissue sample that includes translucent, hydrated oocytes. Document with a photograph of the whole ovary if possible.

Microscopic: There is a predominance of tertiary vitellogenic oocytes, with many oocytes showing oocyte maturation, germinal vesicle migration and germinal vesicle breakdown. A small percentage of oocytes (<25%) will have completed oocyte maturation and are hydrated. Postovulatory follicles may be present (magnification 100×).



Hydration stage 2 (H2)

Macroscopic: The ovary is well developed, reddish-yellow in color, and approximately 2/3 the volume of the body cavity. The membrane is opaque with blood vessels conspicuous. The visible surface of the ovary consists of 25–50% of large translucent oocytes.

*Note: There are gradients between the consecutive H1 and H2 stages as well as the H2 and H3 stages, where it is difficult to assign one or the other stage. In these cases, the ovary is at a state where it is either close to entering the H2 stage or close to advancing to H3. In both cases the ovary is near if not in an intermediate phase of final oocyte maturation and may be accurately classified as H2.

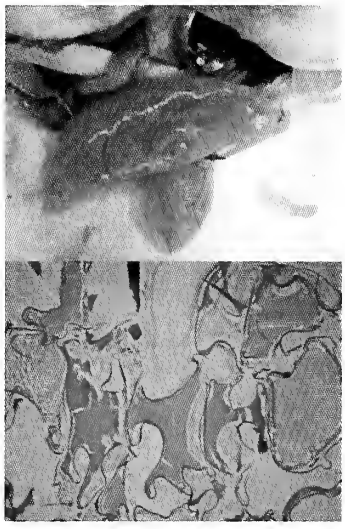
Microscopic: There is a predominance of oocytes showing germinal vesicle migration and germinal vesicle breakdown. Approximately 50% of the advanced oocytes are hydrated. Postovulatory follicles may be present (magnification 40×).

Regenerating stage

The histological results for RE stage ovaries reflected the difficulty in distinguishing between a regenerating and regressing ovary in the field, with 46% of the ovaries classified as RE in the field assigned as S during histological analysis. The plausible explanation for this result is observational error. As the ovary progressed into the RE stage, it became easier to differentiate from the S stage, but, because of the short sampling period, it was difficult to differentiate between the 2 stages during the time when regenerating fish were captured. For future studies, we recommend that sampling be conducted from well before to well after the known spawning season and that a photograph of each ovary be taken for comparison with histology-based staging results. Such documentation of the changes observed in different phases, from spent to regressing, could improve the ability to distinguish between these 2 stages. However, extension of the sampling period too far into the fall and winter may make it more difficult to distinguish the D and RE stages from spawning stages (Tomkiewicz et al., 2003). Histological examination of a sample of an ovary was an effective way to determine if an ovary was in the RE stage.

If a regenerating ovary was observed from a fish near or larger in size than the mean length at maturity during the peak spawning period, it is possible that

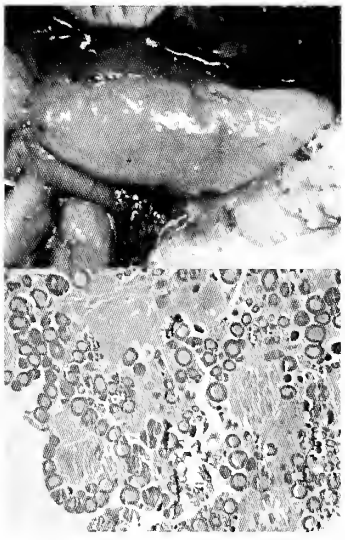
Table 5 continued



Hydration stage 3 (H3)

Macroscopic: The ovary is well developed, reddish-yellow in color, and approximately 2/3 the volume of the body cavity. The membrane is opaque with blood vessels conspicuous. Greater than 50% of the visible surface of the ovary consists of large translucent oocytes.

Microscopic: There is a predominance of oocytes showing germinal vesicle migration and germinal vesicle breakdown. Greater than 50% of the advanced oocytes are hydrated. Postovulatory follicles may be present (magnification 40×).



Regressing (S)

Macroscopic: The ovary is soft and flabby and approximately 1/4 the volume of the body cavity. The membrane is thick and tough, purplish in color, and bloodshot. The inside of the ovary is almost empty and few oocytes remain, giving the gonad a patchy appearance.

*Note: Toward the end of the spawning season, the ovary loses its rigidness although it still has 1–2 batch(es) of oocytes to spawn. Staging should not be based only on the flabbiness of the ovary, and the ovary should be inspected internally. The ovary is likely not yet spent if any hydrated oocytes remain.

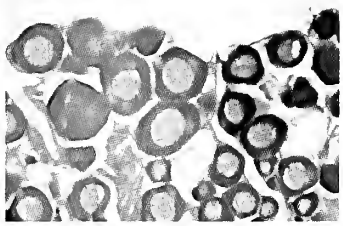
Microscopic: An abundance of postovulatory follicles are present. Oogonia and primary oocytes are evident. The ovary wall is thick, and muscle bundles are visible (magnification 40×).

it spawned much earlier that season or skipped that year's spawning season (Fig. 1). One mature regenerating female was observed during the peak of the spawning season. Skipped spawning is a response to various physiological and ecological conditions (Jørgensen et al., 2006) and often a trade-off between present reproduction and survival for future reproduction (Bull and Shine, 1979; Rideout et al., 2005). Because it is not possible to determine the existence and frequency of skipped spawning and its effect on recruitment, it is difficult to determine spawning stock biomass and, hence, difficult to conduct stock assessments and manage such species (i.e., stock-recruitment models may overestimate recruitment and underestimate survival; Rideout et al., 2005).

Postovulatory follicles

POFs were commonly found in ovary samples classified as H1, H2, H3, and S in the field, but these POFs often were in various phases of atrophy. The observation of early and late phases of POFs in the same ovary indicated that POFs from the 2 previous batches still existed during the OM of the next batch to be spawned (Table 2). Evidence indicates that the complete atrophy of a POF in Haddock could take up to 10 days, considering that Haddock have an average interval of 5.4 days between spawned batches (Trippel and Neil, 2004), and that final oocyte maturation in marine fishes with pelagic eggs generally lasts 1–2 days and ends with ovulation (Thorsen and Fyhn, 1996). The atrophy

Table 5 continued



Regenerating (RE)

Macroscopic: The ovary is small and firm, and approximately 1/6 the volume of the body cavity. The membrane is thin but less transparent, yellowish-gray. Contents are microscopic, opaque.

Microscopic: The ovary wall is thick. There is often indication of past spawning with remnants of unabsorbed material. The ovary contains primary oocytes that vary largely in diameter (magnification 100×).

*Note: If a resting ovary is observed from a fish greater in size than the mean length at maturity during the peak spawning period, then it is probable that the fish skipped that year's spawning season.

of POFs occurs for the Spotted Seatrout (*Cynoscion nebulosus*) in 24–36 h in water temperatures >2°C (Roumillat and Brouwer, 2004) and for the Northern Anchovy (*Engraulis mordax*) in 48 h at 19°C (Hunter and Macewicz, 1985). The atrophy of Haddock POFs may take much longer because this species prefers to spawn in cold temperatures (4–7°C; Overholtz, 1987)—an actuality that may be widespread in boreal fishes. The slow degeneration of POFs in cold-water species is supported by Brown-Peterson et al. (2011) and noted by Saborido-Rey and Junquera (1998).

Aging of POFs has been used in other species to determine spawning frequency or duration of time since the female last spawned a batch of eggs (Hunter and Macewicz, 1985; Roumillat and Brouwer, 2004). No definitive information on diurnal timing of spawning was clear from our inspection of Haddock POFs because none of them appeared to have been very recently created. Fish collections were concentrated in an area where active spawning took place, and those Haddock that had finished spawning may not have been available for capture. Observation of many ovaries in spawning condition that also showed many phases of POF atrophy indicated that these residual tissues had a very slow rate of atrophy and were of little use in making accurate assessments of diel timing of ovulation. A more advanced study of aging POFs in cold-water species similar to the studies done for clupeiforms by Alday et al. (2010) and Haslob et al. (2012) is needed and would increase our knowledge on the timing of spawning in cold waters.

There were no equivalent field index stages for the histological stages 4.1 and 4.2. Samples classified as 4.1 or 4.2 were typically assigned to an ovary in a state between the last batch of oocytes spawned and the next batch to be spawned, a state that we did not attempt to identify in the field. In ovaries of this state, no oocytes for the next batch had yet progressed to OM and the only oocytes present were in a vitellogenic developed phase equivalent to the resting stage described by Murua et al. (2003). We found that this stage was not easily or accurately ascertainable through macroscopic observation of the ovary. A trained eye may be able to recognize a degree of flaccidity of an ovary that has spawned already. Many of the ovaries assigned as 4.1 or 4.2 exhibited characteristics of an ovary that was classified as the D stage in the field. The overestimation of the D stage in this study indicates the need to conduct histology on a subsample of ovaries classified as D stage in the field to assure there is no indication, on the basis of the presence of POFs, that females thus classified have started spawning that season.

Conclusions

Working independently, we came to the same conclusion as Brown-Peterson et al. (2011): standardization of maturation staging methods and terminology are needed. Our study confirms the importance of these efforts but extends them with the development of a new ovarian maturity index specifically for examination of diel spawning periodicity while using the maturation terminology established by Brown-Peterson et al. (2011).

Comparison of macroscopic and microscopic observations of ovaries helped us to improve the initial field index and sampling methods, as well as to provide useful insight into the reproductive biology of Haddock.

Noting the apparent longevity of POFs helped us understand the duration and cyclical process of OM in this species and potentially other boreal or cold-water fishes. Because reproductive maturation occurred over a prolonged period of time, OM occurred throughout 3 distinct field stages (H1, H2, and H3) and histology stages (3.1, 3.2, and 3.3). This finding supports the conclusion of Alekseyeva and Tormosova (1979) that Haddock exhibits asynchronous maturation of individual groups of oocytes. We believe that the asynchronous maturation of oocytes in a batch results in heterogeneous ovaries during early phases of OM and can lead to misclassification of H1 ovaries as D stage in the field. However, Robb (1982) and Templeman et al. (1978) previously reported that Haddock ovaries are homogeneous in structure throughout all phases of maturity. Studies of follicle size-frequency distributions throughout OM are needed to confirm our observation of apparent heterogeneity of ovaries during early maturation to clarify how future studies should be modified to ensure accurate staging in the field and laboratory.

Additional work should be focused on differentiation of a regenerating ovary from an immature ovary. This differentiation is the most important distinction in determination of maturity or reproductive dynamics of a stock because of the use of these numbers in estimation of spawning stock biomass.

The timing of the sampling in this study, although restricted, was focused around the known spawning season of Haddock in the Gulf of Maine. This focus likely increased the reliability of staging SC fish because the closer in time to the spawning season the more developed the ovary becomes, as was observed by Tomkiewicz et al. (2003). Alternatively, reliability in staging SC fish in the fall and winter is tenuous because ovary development is just beginning (Tomkiewicz et al., 2003). Therefore, the optimal time to collect data to be used to estimate spawning stock biomass should span across the spawning season, and we caution against the use of SC data collected off season in estimation of spawning stock biomass.

It is anticipated that the revised ovarian maturity index (Table 5) presented in our study will be useful to Haddock resource managers. The H2 and H3 stages appear to be useful indicators of spawning readiness for Haddock ovaries in the field. We suspect that the progression of OM is detectable in other boreal species with the same reproductive traits as Haddock and that the later stages could also be used to examine diel periodicity in these species. Although this index was developed for studies on diel reproductive periodicity, we feel it would also be useful for study of other short-term temporal reproductive patterns related to tidal, lunar, or solar zenith cycles. The revised field index includes pointers to help users stage ovaries and take appropriate samples (Table 5). Although this revised field index will improve accuracy in the determination of the maturity stage of Haddock in the field, evidence has shown that field indices alone may not be enough to correctly classify a fish in problematic stages. However, the observations in our study also demonstrate that determining the maturation of an ovary by histological examination alone may not always be accurate, highlighting the importance of field staging. In addition to field staging with the index presented here, appropriate tissue samples should be collected and analyzed microscopically or histologically to verify problematic stages, especially when field data are used in assessment and management of a fish stock.

Acknowledgments

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Errata

***Fishery Bulletin* 110:344-360 (2012).**

Barlow, Paige F., and Jim Berkson

Evaluating methods for estimating rare events with zero-heavy data: a simulation model estimating sea turtle bycatch in the pelagic longline fishery

Corrections:

Page 354. The last paragraph in the right column should read as follows:

The GLMs only outperformed the delta-lognormal methods in the fully uniform scenario ($Turtles_{uniform}$, $Sets_{uniform}$). In this spatial scenario, the GLMs were the most accurate estimation method, but they produced more positive outliers. The co-occurrence clumping scenario ($Turtles_{clump}$, $Sets_{clump\text{-}turtles}$) was the only spatial scenario in which the GLMs did not produce more outliers than the delta-lognormal methods. The GLMs were biased lower than the delta-lognormal methods in the co-occurrence clumping scenario ($Turtle_{clump}$, $Sets_{clump\text{-}turtles}$) and sets-only clumping scenario ($Turtles_{uniform}$, $Sets_{clump\text{-}sets}$). No substantial difference was seen between GLM-P and GLM-NB performance in any spatial scenario.

Page 357. The third paragraph in the right column should read as follows:

The GLMs were more accurate than the delta-lognormal methods in the fully uniform scenario ($Turtles_{uniform}$, $Sets_{uniform}$) because this spatial scenario was the only one that did not violate the GLM-P assumption that counts are independent and randomly distributed in space (McCracken 2004, Sileshi 2006).

Page 355: Figure 4 should read as follows:

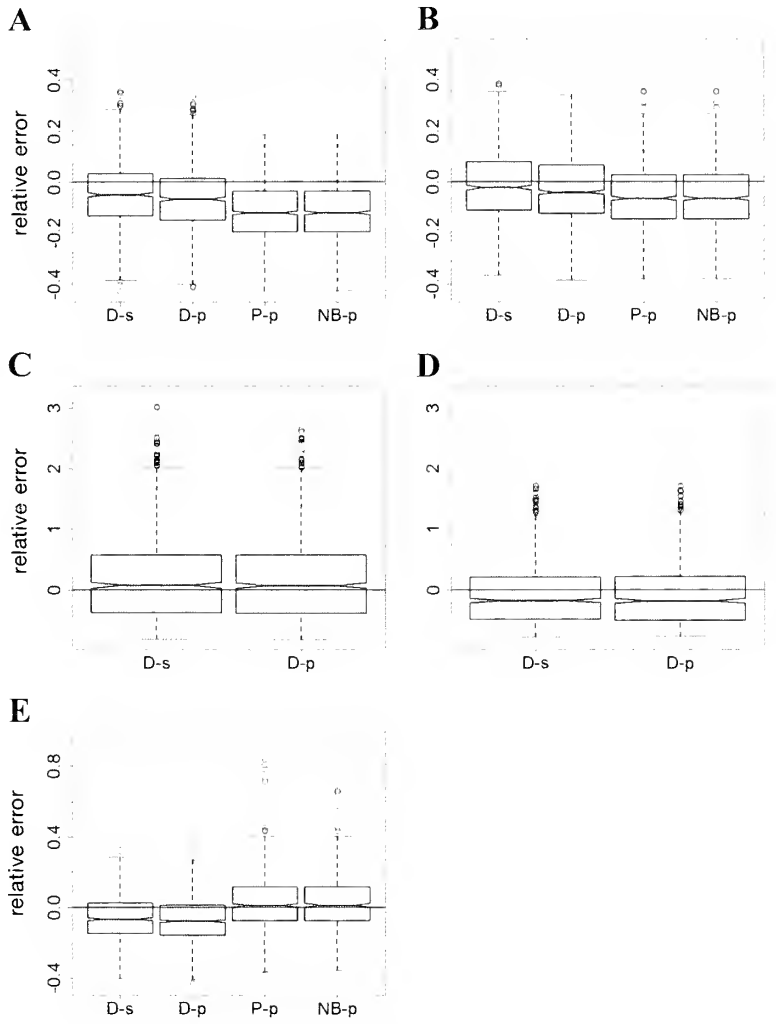

Figure 4

Comparison of bycatch estimates to the total amount of bycatch simulated to evaluate performance of estimation methods. The stratum-level delta-lognormal method (D-s), delta-lognormal method for all sets pooled (D-p), generalized linear model with Poisson error distribution for all sets pooled (P-p), and generalized linear model with negative binomial error distribution for all sets pooled (NB-p) were evaluated. Each of the 5 panels corresponds to one of the spatial scenarios: (**A**)=co-occurrence clumping ($Turtles_{clump}$, $Sets_{clump\text{-}turtles}$), (**B**)=sets-only clumping ($Turtles_{uniform}$, $Sets_{clump\text{-}sets}$), (**C**)=independent clumping ($Turtles_{clump}$, $Sets_{clump\text{-}sets}$), (**D**)=turtles-only clumping ($Turtles_{clump}$, $Sets_{uniform}$), and (**E**)=fully uniform ($Turtles_{uniform}$, $Sets_{uniform}$). Each of the plots within a panel corresponds to an estimation method. The scale of the *y*-axes varies by rows of panels for display purposes. The horizontal line at a relative error of zero marks where the median of an unbiased estimation method should fall. Notches are placed around the medians, and if the notches of 2 plots do not overlap, there is strong evidence that those medians differ. The box of each plot includes the first through third quartile. Whiskers extend to the most extreme data point that is no more than 1.5 times the interquartile range from the box. Small circles represent outliers. For purposes of display, in the panel for the sets-only clumping scenario ($Turtles_{uniform}$, $Sets_{clump\text{-}sets}$), one outlier was removed from each of the P-p and NB-p box plots.

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