Research design for visual and acoustic survey to estimate vaquita abundance in 2015

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Objective: To provide the Government of Mexico an accurate and precise estimate of current vaquita (*Phocoena sinus*) abundance as soon as possible.
Important times: Survey dates anticipated as September 15-December 6, 2015. Report with new abundance estimate May 1, 2016.

Estimating the abundance of the most endangered marine mammal in the world is not easy. Vaquitas are notoriously difficult to detect. A complete count (a census) of vaquitas is not possible, so any method of determining vaquita abundance will be based on statistics and will contain some degree of uncertainty. Fortunately there is a history of estimating vaquita abundance, so it is known which methods work and which do not work (Appendix 1). The most accurate and precise estimate of 2015 vaquita abundance will require both visual and acoustic components covering the entire range of the vaquita in the northwestern Gulf of California. No form of aerial survey has been found to be suitable for estimating vaquita abundance, and mark-recapture methods with photo-identification are not feasible (Appendix 1).

The visual component of the vaquita survey utilizes a large ship to conduct transects in waters deeper than 20m (Appendix 2). The most effective method is to use high power (25x) binoculars at a height of at least 10m above the water. The powerful binoculars allow vaquitas to be detected up to 5km from the ship, which is important because vaquitas react to the ship at distances up to 1km. Accurate estimation of abundance requires detection before the animals react to the ship. For this reason, the visual part of the 2015 vaquita survey should utilize a ship that can support 25X binoculars on a covered observation deck 10m or more above the water (Appendix 3).

The acoustic component of the survey is necessary because part of the vaquita population lives in shallow waters that cannot be visually surveyed by the large ship. The acoustic data will allow the estimate of vaquita density from the visual survey area to be extrapolated into shallow areas (Appendix 4). To increase the precision of the estimate in shallow water areas, we recommend using an array of autonomous acoustic recording devices (CPODs) instead of the single towed hydrophone that was used in 2008. This will require maintaining the grid of 45 CPODs in their current locations throughout the Vaquita Refuge and adding 68 CPODs located evenly throughout the shallow water area (Figure 1).

Shallow-water locations are likely to be noisier than deeper-water areas because the CPOD is necessarily closer to the bottom with noise from snapping shrimp and sediment particle collisions and closer to the surface where breaking waves introduce noise. Dedicated experiments will be carried out to estimate the reduced capacity to detect vaquita clicks to correct for any bias in the abundance estimate (Appendix 5). *To avoid loss of the CPODs, no fishing of any kind (including trawling) can occur during the research period unless coordinated with the survey leaders.* This may require extra enforcement and coordination and potential restrictions on trawling activities. The expected 7,200 days of acoustic data should greatly increase abundance precision for the shallow water area (including 4,350 days within the shallow water area), which was a large contributor to imprecision in past estimates.

Another source of imprecision in past estimates was the estimate of the fraction of vaquitas missed on the trackline (analytically known as g(0)). Due to the turbid waters in the upper Gulf of California and the elusive behavior of vaquitas, a substantial fraction of vaquitas will not be detected even when they are close to the ship. An estimate of this fraction of animals missed is necessary for accurate estimation of abundance. The fraction is specific to the ship and specific to the sighting protocols (number of observers and type of binoculars used). For the *Ocean Starr* and the sighting protocols recommended in this proposal, the fraction missed was estimated to be 0.43. In other words, even with 3 observers searching with 25X binoculars and one observer with hand-held binoculars at a height of 10.2m above the water, only 57% of vaquitas were detected near the trackline. If the fraction of vaquitas missed is not taken into account, the abundance of vaquitas will be underestimated.

Because knowing the fraction of animals missed is so important, the 2015 vaquita cruise will use 2 teams of observers. One team will observe from the flying bridge level and an independent team will search from the bridge level. All observers will use 25X binoculars. This will allow the fraction missed to be estimated. Two specialized data recorders will take data from both teams and determine which vaquitas were seen by both teams in real time. Communications with the recorder will be continuously recorded. The number of total observers needed is 12, because the 6 observers on watch at any one time need to be rotated regularly to prevent fatigue. It is also critically important that all of the observers have previous experience with vaquita or harbor porpoise. For this very important study, we should use only the most experienced porpoise observers possible. Observers must be able to differentiate between vaquita and bottlenose dolphins (also found in small groups in the area and easily confused with vaquitas). We can obtain enough experienced observers from past vaquita surveys, plus some observers from the EU porpoise efforts to staff the cruise.

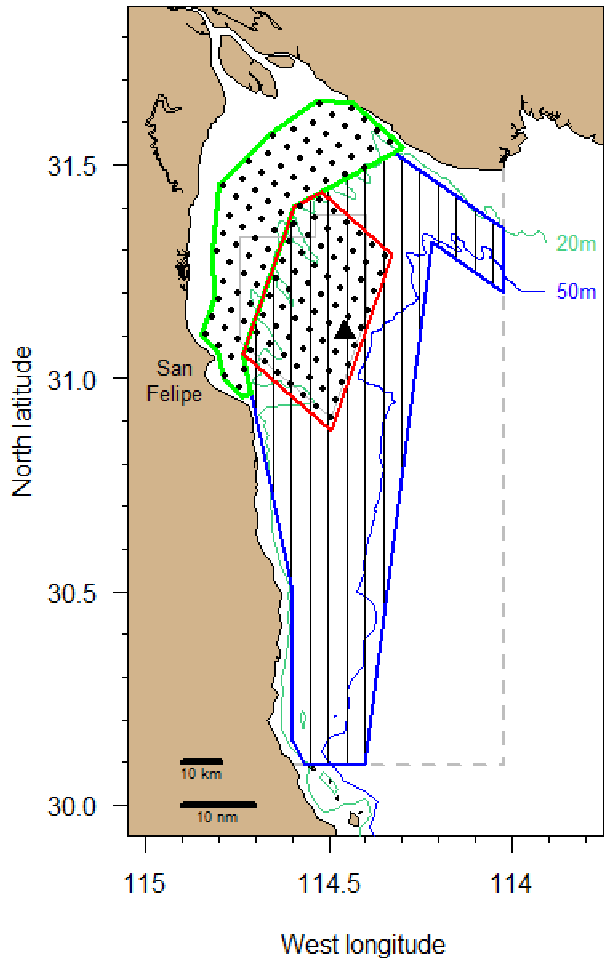


Fig. 1. Research design for the 2015 vaquita abundance study. The area to be sampled visually is outlined in blue, and visual transects are shown as black north-south lines. The area to be sampled acoustically is outlined in green, and acoustic sensor (C-POD) locations are shown as black points. The area to be sampled with both acoustic and visual methods (the calibration area) is outlined in red. The gillnet exclusion area is shown as a dashed gray line, the Vaquita Refuge Area as a thin gray line, and Consag Rocks as a black triangle. Depth contours of 20m and 50m are shown.

To obtain at least 40 vaquita sightings with 95% probability (Appendix 6) requires 64 days in the study area using the same ship (*R/V Ocean Starr*) as past surveys in 1993, 1997 and 2008 and assuming that about 100 vaquitas remain. Results from the 2013 monitoring season indicated a stronger decline (CIRVA, 2016) and as a consequence the design was changed to spend more time in calibration area (outlined in red, Fig. 1), which is the known area of highest vaquita density. The transect lines shown in Fig. 1 have a total distance of about 600nm. Based on past experience, this amount of transect effort can be completed in about half the total survey days. During the other half of the survey, we plan to sample intensively in the core area, where most sightings occurred during surveys in 1997 and 2008 (Fig. 2). Survey effort in the calibration area will consist of a high density of north-south transect lines that complement the primary survey lines. We anticipate that saturation sampling (>100% of surface area surveyed) of the core area can be achieved with a high density of survey effort during the 32+ survey days of effort. The two modes (primary tracklines and core area tracklines) will be alternated throughout the survey to strive for even spatial and temporal coverage. If weather is good and all primary tracklines are completed early, the remaining time will be devoted to increased coverage of the core area.

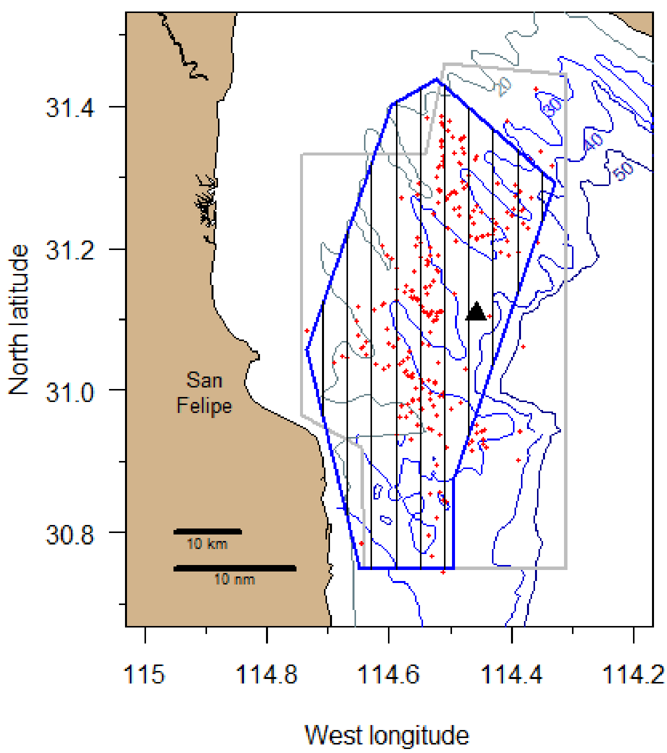


Fig. 2. Details of visual transects in the core area for the 2015 vaquita abundance study. The core area is outlined in blue, and visual transects are shown as black north-south lines. Vaquita sightings are shown as red points, based on uniform survey coverage during the 1997 and 2008 surveys within the area outlined in gray. Depth

contours of 20, 30, 40 and 50m indicate the diagonal ridges in the survey area. The black triangle shows the location of Consag Rocks.

Survey lines were chosen to be north-south to avoid glare and therefore maximize transect line covered so as to increase precision. Vaquita sightings from the 1997 and 2008 visual surveys (Fig. 2) suggest that vaquita densities are highest along the tops of underwater ridges running roughly NW-SE. The north-south transect lines cut through these densities in a manner allowing an even coverage of vaquita densities. Typically tracklines are set in a zig-zag pattern and avoid paralleling the coast to avoid inadvertent biases associated with density patterns that also parallel the coast. Because vaquita density patterns are known and do not parallel the coast, this bias is not a concern for vaquita transect lines. Many of the north-south transect lines are approximately one day's effort moving at 6 knots. Moving to the south during the night to begin the next day's transect is, therefore, easy to do so that no survey time is sacrificed. In contrast, some survey time will be lost in the 'corners' of zig-zag transect lines because of double coverage.

The survey will be conducted as two 32-day legs with a refueling and re-provisioning stop in Guaymas between them. Because the ship survey will use standard software and analytical methods, results from the area surveyed by the ship is anticipated approximately six weeks following the surveys. With acoustic analysts working throughout the survey, it is anticipated that acoustic CPOD data should be processed within six weeks of the end of the survey. Pooling the visual and acoustic data to make a total abundance estimate will take an additional six weeks such that abundance estimates should be ready 3 months after the conclusion of field operations.

One of the important components of a successful survey of this very rare porpoise is using a team of scientists and support staff already experienced in vaquita and porpoise research. A list of staff with brief skill descriptions are given in Appendix 7. Finally, because this work affects the management of a critically endangered species which, in turn, affects the livelihood of thousands of people, this design was reviewed by the best available experts in marine mammal visual and acoustic surveys. Reviews were requested from both the Society for Marine Mammalogy (the international scientific society for the study of marine mammals) and the International Whaling Commission's Scientific Committee. Reviews were also requested by Pablo Arenas, Director of INAPESCA. An online meeting was held and a Report was written and is available on the SEMARNAT and IUCN CSG websites. The research design specified here has greatly benefited from these reviews. As a result of the reviews of the research design, the expert panel assembled to analyze the acoustic monitoring data is being augmented by several experts in line-transect analysis who will be updated and consulted during the survey period and then afterwards as analyses are being completed.

The total estimated costs are: $42,299,439 (MXN). Details of the budget and payment times are given in Appendix 8. Anticipated cruise dates are from

September 15 through December 6, 2015 (depending on negotiations for ship time). The report with a new vaquita abundance estimate is anticipated in May 1, 2016.

Appendix 1: An Evaluation of Potential Abundance Estimation Methods for Vaquita

Executive Summary

A wide variety of methods have been developed and used to estimate the abundance of cetaceans (whales, dolphins and porpoises). Methods fall into three general categories: complete enumeration methods (census), density-based survey methods, and mark-recapture methods. Many of these methods have been tried for estimating vaquita abundance in the past. Here we review all available methods and evaluate their utility for estimating vaquita abundance in 2015. We conclude that ship-based visual density estimation complemented by acoustic surveys in shallow waters is the only practical method to obtain a precise estimate of vaquita abundance. Regardless of the method used, survey design and analysis methods should be reviewed by an international team of experts to ensure the credibility of the results. Pilot studies are recommended if new methods are used.

Survey Methods

Complete Enumeration Methods (Census)

The size of human populations in many countries is determined using a census or complete enumeration of all individuals. This works well for humans if the vast majority of individuals can be identified by a permanent address, but even in developed countries some individuals are missed. Outside of zoos and aquaria, the only cetacean population in the world that is censused by complete enumeration is southern resident killer whales in Puget Sound. That method works for them because all individuals are identifiable in a good photograph and because all individuals are seen and photographed every year. Complete enumeration methods will not work for vaquita because the vast majority of animals are not individually identifiable in a good photograph (see mark-recapture methods, below).

All other abundance estimation methods are statistically based and are subject to random sampling variability. For vaquita, there is no method that can estimate abundance without some degree of statistical uncertainty. The best method is one that minimizes this uncertainty for a given survey budget.

Density-based Survey Methods

Density-based survey methods are based on estimating the density of individuals (number of individuals per square kilometer) and then extrapolating that density to a study area. Abundance is estimated as animal density times the size of the study area. This method does not require that all individuals be seen and it doesn't matter if some individuals are seen more than once. Distance-sampling survey methods (Buckland et al, 2001) are a sub-set of density-based methods and require recording the distance between the survey platform (ship or plane) and the animals that are seen. Distance-sampling survey methods include line-transect surveys from boats, ships and aircraft and point-transect surveys from fixed locations. Strip and quadrat surveys are also density-based survey methods but are less efficient and are more typically used for plants and slowly moving animals. Three key requirements for all density-based survey methods are: 1) the survey must be random with respect to the distribution of the animal within the study area, 2) the probability of detection must be estimated (as a function of distance from the survey platform for line- and point-transect surveys), and 3) animals must be detected before they move in reaction to the survey platform.

Visual Line-transect Survey Methods from Boats & Ships

Visual line-transect surveys have been used for many species of cetaceans in many populations around the world. The method has been used successfully on ship-based surveys for vaquita in 1993, 1997, and 2008 and has generated the most precise estimates of vaquita abundance (Barlow et al. 1997; Jaramillo et al. 1999; Gerrodette et al. 2011). Previously, vaquita abundance was crudely estimated from data collected from small boat surveys in 1986-1988 (Barlow et al. 1997), but those estimates were not rigorous because the data were not collected using line-transect methods and because detection probabilities were crudely estimated by guesswork and comparison to other surveys for a different species. The 1997 survey used two independent teams of visual observers on the same ship, and a comparison of data from these two teams showed that vaquita started avoiding the vessel at distances of greater than 1km. High-powered, pedestal-mounted binoculars are required in order to detect vaquita before they move away from the transect line in reaction to the ship. Fujinon 25X150 binoculars were used on each of the three ship-based vaquita surveys. This requirement means that the ship must be large enough (>50 meters length) and stable enough to allow use of such binoculars. The vessel must also be large enough to carry two teams of observers to allow estimation of the probability of detection at zero distance.

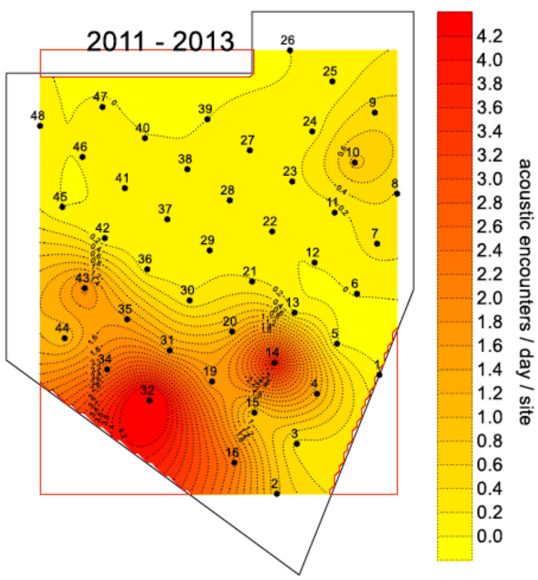
One drawback of using a large ship for visual line-transect surveys of vaquita is the need to survey portions of the vaquita distribution that are too shallow to survey by large ship. During the 1997 survey the Mexican research ship BIP XI was used for the shallow water areas. This converted shrimp trawler did not have a configuration (height and stability) suitable for the 25x binoculars, so hand-held binoculars were used. Only 6 sightings were made on-effort in the shallow-water area when the estimated abundance was approximately 600 vaquitas. Because many fewer vaquitas were expected to remain in 2008 (because of ongoing unsustainable deaths in gillnets between 1997 and 2008) a different approach was used: a small sailing vessel towing a hydrophone array (see acoustic line-transect survey methods, below). This was reasonably successful, but again there was sparse sampling and few detections in shallow waters (see below). Because a significant portion of vaquita's range extends into shallow waters, an alternative survey method would be needed to extend the survey into these shallow waters if a ship survey is done in 2015.

Visual Line-transect Survey Methods from Aircraft

Aerial surveys are commonly used to estimate the abundance of harbor porpoise in the U.S. and in Europe. An experimental aerial survey for vaquita was attempted in 1991 (Barlow et al. 1993). Less than two vaquitas were seen on this survey per 1000 km of transect line. Given the extreme turbidity of waters in the upper Gulf of California and the rapid changes in turbidity over short distances (Barlow et al. 1993), no method was found to effectively estimate the probability of detecting vaquitas on the transect line during an aerial survey. The same problem would exist with fixed-wing aircraft, helicopters and drones, so no form of aerial survey will be effective for vaquita abundance estimation.

<u>Land-based surveys</u>

For coastal migrations of gray whales and humpback whales, data are collected from land-based viewing platforms using binocularsObservers change effort to avoid fatigue. Observations are conducted continuously in sea state 2 or less. In 1996 reserachers from the Instituto Nacional de Pesca proposed to build in a viewing platform from Rocas Consag to survey vaquitas. However the project was cancelled after a review with experts and the results of the vaquita 1997 cruise. The cost-benefit ratio is very poor for such a land-based survey because it can only survey from a single point in an area of very low density of vaquitas. Besides, during the 1997 cruise (and subsequent ones) it is clear that the closest vaquitas to these rocks are more than 2 nmi. Low densities near Rocas Consag have been confirmed with the passive acoustic monitoring. The figure below shows vaquita densities using data from 3 years of summer monitoring effort. Rocas Consag lies between CPOD locations 5 and 13, which are near zero in acoustic detections. Vaquitas near the high density site 14 would not be visible from Rocas Consag. Hence the platform has limited value for a proper survey.



<u>Acoustic Line-transect Survey Methods from Boats & Ships</u>

Towed hydrophone arrays have been used in the past to detect the echolocation signals of harbor porpoises, Dall's porpoises, finless porpoises, and vaquitas. Like other porpoises, vaquita produce distinctive echolocation clicks that are easy to distinguish from the lower-frequency clicks made by dolphins in the northern Gulf of California. It is very difficult to estimate porpoise abundance using acoustic methods alone, and this has been done only for harbor porpoises in a few instances and at great expense. One of the difficulties is that hydrophone arrays are towed behind a vessel and porpoises are detected only after they may have reacted to the presence of the vessel. This is a particular problem for vaquita, which respond to ships by moving away from them. Detection probability and avoidance bias are particularly hard to estimate for vaquita. Acoustic survey data are be more easily used as a measure of relative vaquita density rather than as a measure of absolute density.

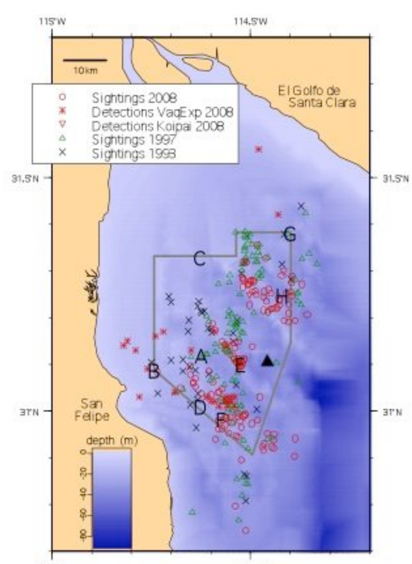
A towed array was used for the 2008 vaquita survey to measure the relative density in a calibration area that overlapped with the visual ship survey and in the shallow water areas where the ship could not survey (Rankin et al. 2009). These data were used as estimates of relative density to extrapolate estimates of absolute density from the calibration area into the shallow-water areas (Gerrodette et al. 2011). A small, 7.3-meter sailing trimaran was used to minimize the avoidance problem and to allow the vessel to survey in very shallow water. There were total of 29 acoustic detections of vaquitas. However, the small sailboat struggled to cover the survey area due to its slow speed, lack of accommodations for multi-day trips, and safety concerns. A larger sailing vessel was recommended for future surveys of this type (Rankin et al. 2009). On the 2008 survey, the acoustic trackline detection probability could only be estimated by comparison with the visual estimates of vaquita density in the area of overlapping survey methods (Gerrodette et al. 2011).

Acoustic Point-transect Survey Methods from Autonomous Instruments

In many cases, acoustic data can be collected much more cost-effectively with stationary instruments than with a towed hydrophone array. Instruments (called CPODs) have been developed that can be anchored on the sea floor and collect porpoise acoustic data continuously for 5-6 months. A network of 48 CPODs has been deployed in the vaquita refuge over the past four summers.

A larger network of CPODs have recently been used to estimate the density and abundance of harbor porpoise in the Baltic Sea. The probability of detecting a porpoise as a function of its distance from a CPOD cannot be estimated from a sparse array of single instruments. In the Baltic, a dense array of CPODs was deployed in an area of very high harbor porpoise density, thus allowing CPODs to be used to make absolute estimates of porpoise density. Because there are no areas of high vaquita density, this approach will not work for vaquitas. However, CPODs can be used to estimate relative vaquita density much more precisely than towed hydrophones because many CPODs can be deployed at the same time and collect data day and night for months.

Mark-recapture Methods



Mark-recapture is a commonly used, non-density-based method to estimate wildlife abundance. This method requires that individuals are individually recognized. It is not possible to tag cetaceans in large numbers, so individuals are recognized using distinctive marks seen in high-resolution photographs. This so-called photo-identification method works well with humpback whales and killer whales because every individual in the population has distinctive marks. An intensive photo-identification study was conducted during the 2008 vaquita survey, and only two photographs were obtained of distinctively marked individuals (Jefferson, pers. comm.). No other individuals had marks that were sufficiently distinct to allow them to be recognized if seen at a later date. For this reason, mark-recapture by photo-identification will not work to estimate vaquita abundance. Also, vaquita cannot be physically tagged or genetically tagged (by biopsy) because they cannot be approached. Although Rocas Consag apprears to be in the middle of the vaquita territory (black triangle in the figure), vaquitas appear to avoid this rock and the idea of visual studies from this location, including photographic identification studies, have been abandoned. Mark-recapture methods can be ruled out based on these experiences.

Conclusion

We conclude that visual line-transect surveys from ships are the most robust method to estimate vaquita abundance. The ship must be large enough to accommodate multiple teams of observers and be stable enough to mount 25-power, pedestal mounted binoculars. Such a ship will not be able to survey in shallow-water parts of the vaquita distribution. Acoustic surveys are a cost-effective method to measure relative vaquita density and thereby extrapolate density from the area surveyed by the ship to areas that are too shallow. Autonomous acoustic recorders (CPODs) are much more cost-effective than towed hydrophones for the acoustic component of such a survey.

Pilot Surveys

The use of pilot surveys is strongly recommended whenever an inexperienced team first applies a survey method to estimate cetacean abundance (Dawson et al. 2008).

Expert Peer-review

It is extraordinarily important for a survey plan to be reviewed by a team of experts. No survey plan is perfect and all plans benefit from the careful review by experienced experts in survey design, execution, and analysis. For credibility and transparency, reviews and responses should be in writing.

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Appendix 2: Physical setup.

The configuration of the flying bridge would be similar to that shown below from the 2008 survey, which had 3 full-time 25x binocular observers plus a recorder using the same software (WinCruz) that generates data in the format that can be immediately used in abundance estimation software.

Configuration of the flying bridge with 4 25x binocular positions (photo from 2008 vaquita survey). Two additional pairs of 25x binoculars would be added one deck below (bridge deck) to allow 2 independent teams to estimate the proportion of vaquitas seen on the trackline with greater precision.

Vaquitas can only be seen in the calm conditions shown here and with 25x binoculars at a height above the water of at least 10m.

Appendix 3: Ship requirements

Because vaquitas tend to avoid boats, a ship used for a visual vaquita survey should allow observers to detect vaquitas before they react to the vessel. Based on past experience, this requires 25X binoculars at a height of 10m or more. Hand-held binoculars (7, 8, or 10X) detect fewer vaquitas and are not recommended as the primary method of detecting vaquitas visually.

The minimum ship requirements for conducting a vaquita line-transect survey in 2015 are a ship that has:

(1) at least 15 berths for scientists; and
(2) an observation deck (usually the flying bridge) which:
- (a) is at least 10m above the surface of the water;
- (b) is large enough to accommodate at least 3 25X binoculars at a time and a data recorder position (approximately 10m x 5m);
- (c) has an awning to protect observers from the sun;
- (d) has steel plates with bolts for attaching the 25X- binocular pedestals

(3) an independent deck (likely the bridge) for the independent team with 2 25X binoculars.

Appendix 4: Acoustic component

Estimating the density of vaquitas in the shallow-water areas will depend on continued monitoring of the Vaquita Refuge plus adding CPODs to the shallow water area (see Figure 1, body of document). The grid pattern used in the acoustic monitoring effort is extended in the same density into shallow-water areas (about 36 sites per 1000 Km^2). There are 136 acoustic sensors (C-PODs), which is our financial and logistical limit. The sensors are in 2 groups: (1) in shallow water where the ship cannot survey, approximately between the 10m and 20m depth contours in the northwest part of the study area; and (2) in a calibration area where there will be simultaneous visual and acoustic sampling. Data from the calibration area will be used to estimate abundance from click rates in shallow water. In contrast to the 2008 abundance estimate, which used a sailboat to obtain acoustic data, the acoustic methods in this proposal will generate data continuously throughout the shallow area and calibration area throughout the survey period.

Vaquita population trend has been estimated using the acoustic grid inside vaquita refuge since 2011, including annual sampling periods (June to September) until 2014 (CICESE, 2010, 2011, 2012, 2014). The estimate using sampling periods from 2011 to 2013 resulted in an annual 18.5% decrease, which addressed to an estimate of abundance of less than 100 individuals (CIRVA, 2014). Adding the 2014 data increase precision and that rate of decline to an annual 31%/year decrease (CIRVA, 2015).

To accomplish this goal it will be required to. To assure that all acoustic detectors (C-PODs) are operational and with memory available, it is planned to interchange equipment during the survey at least twice. At the interchange time data will be downloaded and memory cards and batteries replaced. It also will allow having raw data to analyze during the survey, rather than waiting until the end of the whole survey.

Retrieving the acoustic equipment is not a trivial task. For the acoustic monitoring project, pangas are used locate each sight and use a hook trawled behind the boat to locate the mooring because CPODs were bottom mounted (no surface buoy) to avoid theft or vandalism. Three methods can be used to retrieve equipment depending on the expectations of fishing activities in the area during the survey:

a) If it can be assured that no fishing will occur, every mooring can be marked in surface with a buoy, which would allow a very fast retrieval of equipment, reducing greatly funds needed to construct more complicated moorings, personnel and fuel.
b) If it is anticipated that fishing could occur, moorings could be deployed using longlines as in the fishing techniques. A boat like Unicap 16, equipped with a

stern winch could be used to deploy and retrieve the lines. It could reduce retrieval times, as it will be needed to locate only one extreme of the longline per time.

c) If a boat with a reliable winch is not allocated, the traditional method of individual moorings would be applied, which would require the hiring of several field teams, every one composed out of a panga and three operators. Given past experience, it will be required to have at least 8 teams to assure effective deployment-retrieval operations, but 10 would be better.

Data analysis would occur as data become available after retrieval periods. It is anticipated with the current low number of vaquita clicks that the acoustic monitoring team can handle analysis as data come in. As many days of data will be generated in a short time period, analysts will be focused into review a subset of data that can be compared in performance with the GENENC algorithm. It is anticipated from past data that GENENC performs very alike to analysts (CIRVA 2014), which would reduce greatly analysis time and analysts required. It would be required to test GENENC performance over different acoustic conditions, in order to assure reliable analysis. In case GENENC do not perform well under some conditions, analysis will be completed by the analysis team.

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Appendix 5. Acoustic research to estimate detectability of vaquitas by CPODs in shallow and deep waters.

Odontocetes (dolphins, porpoises and river dolphins) have the ability to produce sonar signals. It means that they are able to emit sound pulses in a defined direction and receive the echoes. The analyses of these echoes in the brain allow them to perceive the characteristic of the objects from where sonar signals rebound (Au, 1993; Richardson *et al*., 1995). This ability is commonly known as echolocation, first discovered in bats (Au, 1993).

Echolocation signals are transient pulsed sounds of short duration, typically named "clicks" (Au, 1993; Richardson *et al*., 1995). Acoustic characteristics of clicks vary greatly among odontocete species, ranging from tens to about 160 KHz in predominant frequency, bandwidths (frequency content from predominant one) of few up to about 70 KHz, and durations from about 50 to around 160 μs (Au, 1993). Porpoise (family to which vaquita belongs) clicks are characterized by high frequencies and narrow bands (Kamminga *et al*., 1996; Chappell *et al*. 1996). Vaquita emits clicks with fundamental frequencies around 135 KHz, band with around 17 KHz and duration between 79 and 193 μs (Silber, 1991).

Clicks are not emitted alone by dolphins. Instead, they are produced in series of clicks, separated by regular intervals (Au, 1993; Richardson *et al*., 1995). It is because a click is emitted after the previous one has been received (Au, 1993). Series of clicks are commonly named as click trains.

C-PODs are tonal click loggers, identifying and storing clicks with relatively narrow band (http://www.chelonia.co.uk/downloads/CPOD.pdf, Chelonia Limited, manufacturer of C-PODs). The dedicated program, CPOD.exe, uses a stochastic algorithm named KERNEL, to identify porpoise like click trains based on individual click frequencies, durations, bandwidth, waveform and levels, as well as click intervals, average levels and train envelope form. Hence, a reliable sampling of acoustic detection rates of vaquitas depends on the ability of the C-PODs to identify and store vaquita like click trains, as well as the reliability of KERNEL algorithm to identify trains, under different background noise conditions

The perception of an acoustical signal of interest, in this case vaquita click trains, depends on the levels of background noise, which can be produced by physical or biological sources. Levels of the signal of interest must be higher than the noise levels to be adequately discriminated, a factor known as signal-to-noise ratio (SNR). SNRs could be enhanced by placing acoustic detectors at points where noise level is reduced. After experimentation in sampling sites inside Vaquita Refuge (average depths between 20-30m), this was done by placing C-PODs at middle depth between bottom and surface, which reduces reception levels of sediment knocks on the hydrophone and clicks emitted predominantly by snapping shrimps, as well as clicks emitted by the action of explosion of air microbubbles in the surface by wave forcing (Medwin and Clay, 1998).

In shallower waters, with average depths around 10m, noise levels could prevent reliable performance of C-PODs, as all described noise sources would be closer to the hydrophone. In order to assess this impact, an experiment will be performed to analyze the rate of detection of artificially generated signals under different noise conditions (over the planned sampling area in the Upper Gulf of California, Appendix 4).

A signal generator and a hydrophone with transmission capabilities will be used to generate sinusoidal signals resembling vaquita clicks. In an implementation of a calibration facility in Ensenada, B.C., it was determined that a 10 cycles of a 135 KHz sinusoidal signal is reliably identified as a single click by the C-POD (Oceanides, 2013). It is known that clicks emitted by vaquita contain around this number of cycles (Silber, 1991; Kamminga *et al*., 1996). Noise levels and frequency spectra will be recorded with an oscilloscope.

The harbor in San Felipe will be used to test the signal and determine the voltage required for the C-POD to detect clicks at distances of 100 m and greater. A Reson TC4013 hydrophone is able to emit a 135 KHz signal with a level about 152 dB_{RMS} re 1μPa at 1m, nearly the same emission level reported for harbor porpoise in a tank (162 dB_{p-p} that converts to 153 dB_{RMS}).

Test sites will be selected from sampling sites depicted in Figure 1, trying to cover representative portions of all the study area. At every site an array of C-PODs will be deployed, with detectors at different depths and the shallower one at a depth that avoids the C-POD surfacing at low tide. At every depth two C-PODs will be used with different settings. One will have regular settings and the other will be adjusted for high noise settings (use an 80 KHz high pass filter instead the 20 KHz to limit the number of low frequency clicks stored). The array will be deployed from an anchored boat. The arry will have a buoy at the surface and a weight in bottom that will not touch the bottom so it can freely move with currents. Distance from the emitting hydrophone on the anchored boat to the array will be measured with a rope attached to the buoy that is marked at regular intervals.

Trains with 10, 20 and 30 clicks, at click intervals of 0.05 seconds, at the voltage determined in harbor, will be generated with the C-POD array at different distances from the hydrophone. Precise time of signal generation will be recorded and C-PODs will be synchronized with generator time.

Data stored in C-PODs will be downloaded to generate CP1 files (the basic file created by C-POD, containing all clicks identified and stored) and KERNEL algorithm will be applied to generate CP3 files (which contain only click trains identified as porpoises, dolphins or sonar).

The experiment will continue by quantifying the degree to which the proportion of trains stored in CP1 files and the proportion of clicks agree with the number of generated clicks and trains. Also, the proportion of trains stored in CP3 files will be quantified. Finally, the assessment will compare these proportions between distances from hydrophones as well as between sampling sites representing different noise conditions, as determined by spectrograms registered with the oscilloscope.

Significant lower proportions of clicks and trains detected in C-PODs would be an indication of reduced abilities under the prevalent noise conditions. In this case, the proportions measured could be used as a parameter to correct acoustic detection rates, hence avoiding underestimation problems.

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Appendix 6: Number of sea days needed

In order to estimate abundance based on line-transect data reliably, a minimum of 60-80 sightings is recommended (Buckland et al, 1993). The number of days of ship survey effort required to obtain 60 vaquita sightings in 2015 cannot be predicted exactly, but it can be estimated probabilistically. We use data from the 2008 vaquita visual survey to estimate the number of vaquita sightings that will occur in 2015, if the same methods are used. This exercise calculates recommended sea days for both the *Ocean Starr* and another comparable ship that has not been used previously for a vaquita survey and would require more sightings to make a reliable estimate. The research design for the main proposal assumes use of the *Ocean Starr* because it is the only ship where a reliable estimate can be obtained even if few vaquita are sighted because numbers have declined further.

We know from past experience that vaquitas can be detected effectively only in calm wind conditions of Beaufort sea states 0-2. We also know that vaquita sightings do not occur evenly, but tend to be highly clustered. Figure 1 shows the daily survey distance on effort and number of sightings during the 1997 and 2008 studies to estimate vaquita abundance. In both years there were many days during which no effective survey effort was possible because of wind (Beaufort>2). On days when effective survey effort was possible, often no vaquitas were seen. The clustered pattern of vaquita sightings was particularly evident in 1997, when 81% of the sightings occurred during the last 5 days of the survey, and 53% of the sightings occurred on a single day.

Another consideration is that the number of vaquitas in 2015 is likely to be substantially less than in 2008. The best estimate of 2008 vaquita abundance, using all available data in a population model, is 214 vaquitas (Gerrodette and Rojas-Bracho, 2011). The best current (2014) estimate of vaquita abundance, using the 2011-2013 acoustic data, is 94 animals (CIRVA, 2014). This means that the number of vaquita sightings per day for a survey in 2015 is likely to be lower than in 2008.

To estimate the number of sightings that could be expected during a survey in 2015, the 2008 data were sampled with replacement by day for various numbers of sea days. The sighting rate was reduced by the ratio 94/214 to account for the estimated decline in vaquita abundance since 2008. It was assumed that wind conditions, vaquita group size, and vaquita spatial distribution in 2015 would be similar to 2008. It was also assumed that the same data-collection methods (ship speed 6 knots, 3 observers with 25X binoculars and one recorder at an observation height of 10m) used on previous vaquita line-transect surveys would be followed.

The results indicate that 85 days in the study area will be required to achieve 60 sightings with 95% probability (dotted line in Figure 2). This means 85 working days in the study area, excluding days in transit and days in port. Similarly, 64 days in the study area will be required to achieve 40 sightings with 95% probability (dashed line in Figure 2).

If the 2015 vaquita cruise uses a vessel that has not been used for previous vaquita surveys, such as *BIPO INAPESCA* or *Ocean Rover*, a target of at least 60 sightings is recommended, which will require 85 days of ship time in the study area. On the other hand, if the 2015 vaquita cruise uses the *Ocean Starr*, the vessel used for vaquita surveys in 1997 and 2008, a target of 40 sightings and 64 sea days would be sufficient. Because the *Ocean Starr* was used for previous studies, some key parameters are already known, and fewer sightings and fewer sea days are necessary. In addition, *BIPO INAPESCA* and *Ocean Rover* are both larger than the *Ocean Starr*, and the draft is greater. In 2008, the *Ocean Starr* was able to carry out transects in 59% of the vaquita range (Gerrodette et al, 2011). The rest of the vaquita range was too shallow, and acoustic sampling from a catamaran was used. If a larger ship is used in 2015, it will be able to cover less than 59% of the vaquita's range. This means that the 2015 vaquita abundance estimate is likely to have greater uncertainty if a larger ship is used.

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Gerrodette, T. and L. Rojas-Bracho. 2011. Estimating the success of protected areas for the vaquita, *Phocoena sinus*. Marine Mammal Science 27:E101-E125.

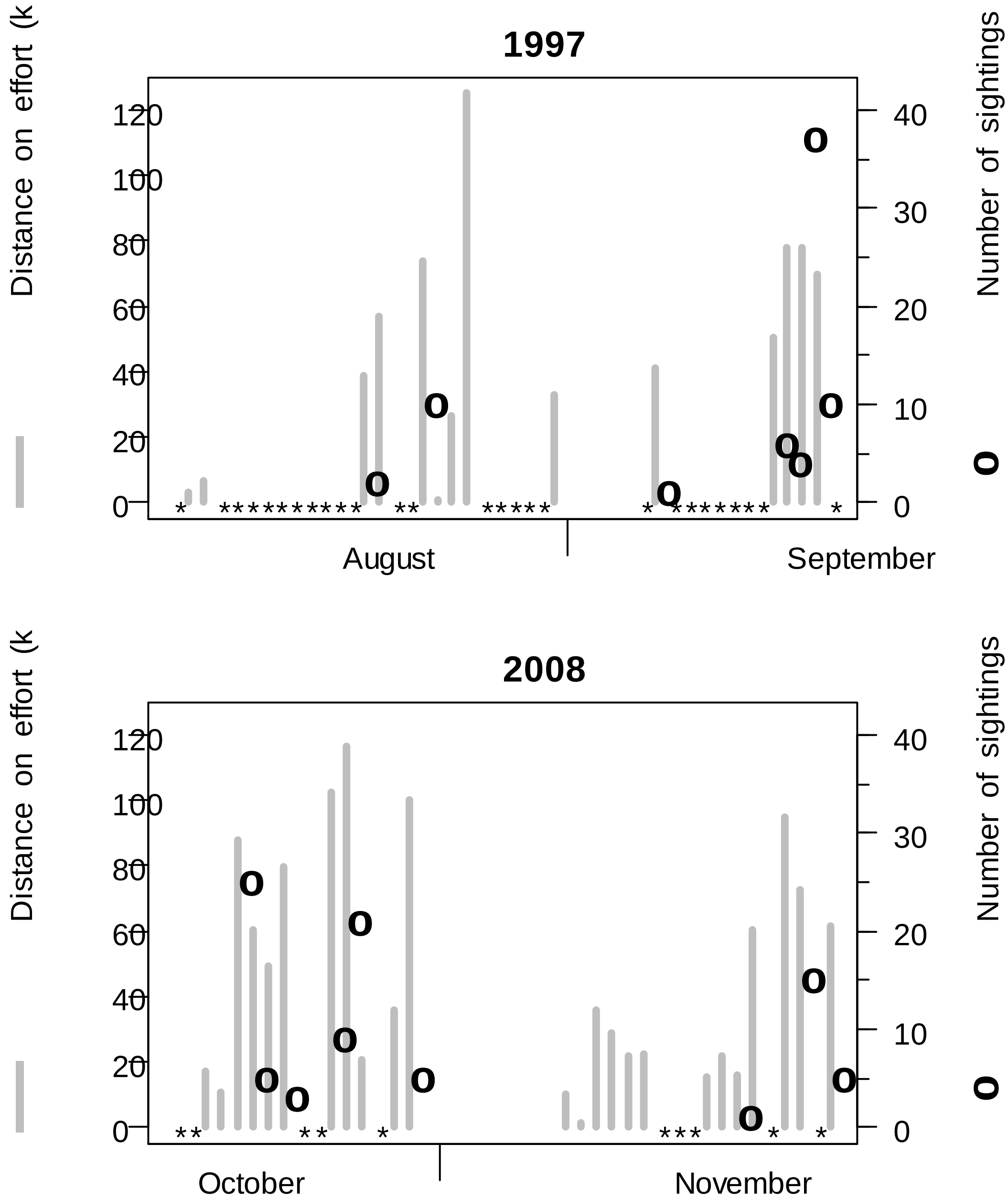

Figure 1. Distance on transect effort in conditions of Beaufort ≤ 2 (gray bars) and number of vaquita sightings (circles) on each day during cruises in 1997 and 2008. An asterisk (*) indicates days when the ship was in the study area, but Beaufort sea state was > 2 for the entire day and no survey effort was possible. In both years the gap in the middle represents time the ship was in port.

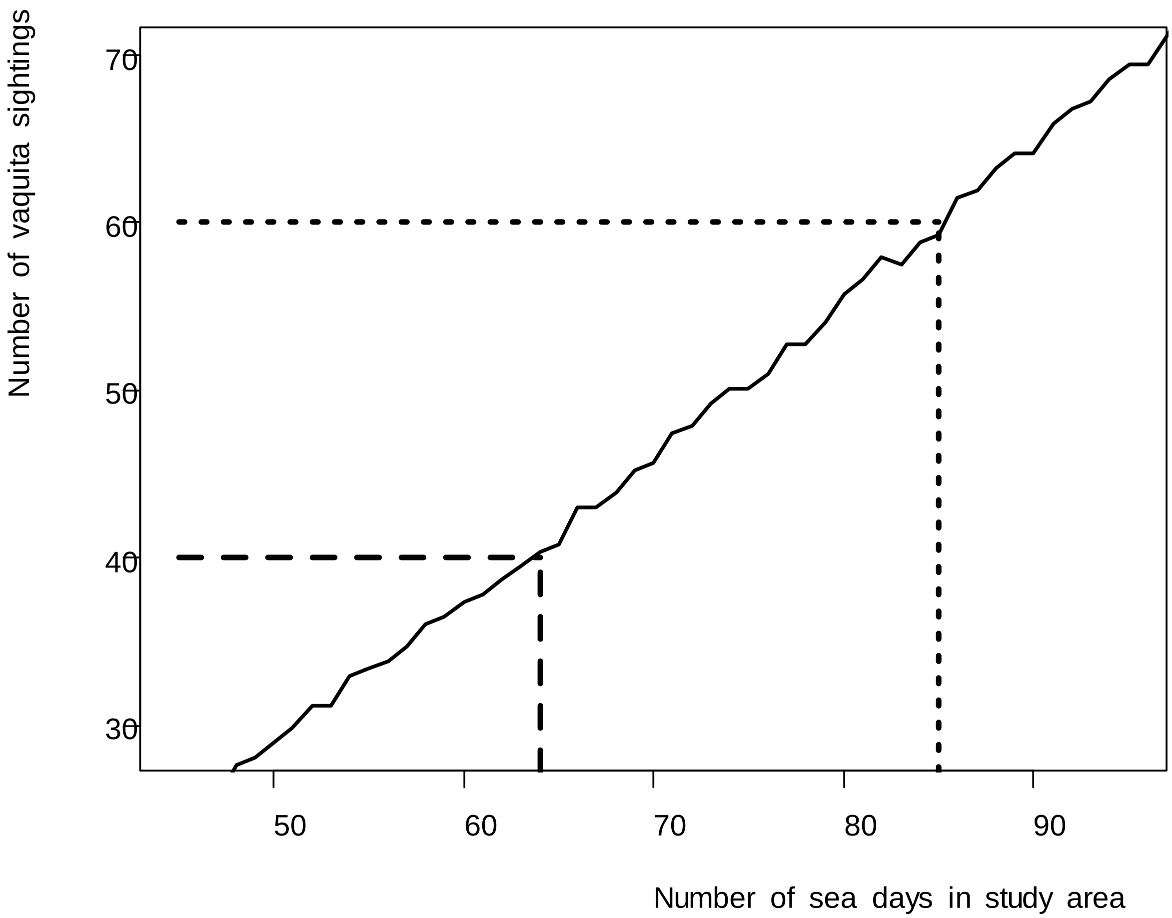

Figure 2. Number of sea days and number of vaquita sightings expected in 2015, using the same methods as surveys in 1997 and 2008. The figure shows the number of vaquita sightings (or more) that is expected with 95% probability for a given number of sea days in the study area. The dotted line shows that 85 days will be required to achieve 60 vaquita sightings with 95% probability. The dashed line shows that 64 days will be required to achieve 40 vaquita sightings with 95% probability.

Appendix 7. Personnel description

With so few vaquita remaining, there will be no opportunity to train observers to be able to identify vaquitas versus other dolphins present in the area. Also, because vaquitas are so hard to see, it is imperative that the very best available observers be used to increase the number of sightings. Similarly, an experienced crew is critical for deployment and retrieval of CPODs and rapid and accurate analysis of the acoustic data. Both teams are listed below with a brief account of their qualifications.

Visual Team (only 12 visual observers needed at one time, but we expect some will be available for only half of the cruise)

Lorenzo Rojas (Mexico) co-cruise leader 1997, 2008
Barb Taylor (USA) observer 1997, co-cruise leader 2008
Tim Gerrodette (USA) co-cruise leader 1997, analyst 1997, 2008
Ernesto Vazquez (Mexico) vaquita observer 1997, 2008
Juan Carlos Salinas (Mexico) vaquita observer 1997, professional observer on many cruises with harbor porpoise
Jay Barlow (USA) vaquita observer 1997, 2008
Robert Pitman (USA) vaquita observer 1997, 2008
Dawn Breese (USA) vaquita observer 1997, 2008
Karin Forney (USA) vaquita observer 1997, professional observer on many cruises with harbor porpoise
Sarah Mesnick (USA) vaquita observer 2008
Paula Olson(USA) vaquita observer 2008, professional observer on many cruises with harbor porpoise
Lisa Ballance (USA) professional observer on many cruises with harbor porpoise
Suzanne Yin (USA) professional observer on many cruises with harbor porpoise, expert data recorder
Jeff Moore (USA) professional observer on many cruises with harbor porpoise, expert panel on acoustic analysis
Susie Calderan (UK) professional observer on many cruises with harbor porpoise
Adam Ü (USA) professional observer on many cruises with harbor porpoise
Todd Pusser (USA) vaquita observer 2008, professional observer on many cruises with harbor porpoise
Cornelia Oedekoven (Germany) vaquita observer 2008, professional observer on many cruises with harbor porpoise
Melody Baran (USA) recorder with experience with WinCruz software and multiple observer teams
Andrea Bendlin (USA)) recorder with experience with WinCruz software and multiple observer teams

Acoustic Team

Armando Jaramillo-Legorreta (Mexico) acoustic lead for vaquitas 1997-2015
Edwyna Nieto-García (México) field researcher and acoustic analyst 2006-2015
Gustavo Cárdenas-Hinojosa (México) field researcher and acoustic analyst 2010-2015.
Francisco Valverde-Esparza (México) field operations manager
Alan Valverde-Esparza (México) field operations
Javier Valverde-Márquez (México) field operations
Rafael Sánchez-Gastelum (México) field operations
Alejandro Sánchez-Gastelum (México) field operations
Ramón Arozamena-Osuna (México) field operations
Juan Osuna-Romo (México) moorings assembly

Appendix 8: Budget

The budget presented here is an approximate estimate based on preliminary data on ship costs, which would need to be negotiated with the company. The costs here are for ships from Stabbart Maritime and were given in USD and converted using 1USD/15.44MXN (conversion on Apr 22, 2015).

Description	Payment Date	Amount (MXN)	Subtotal (MXN)
C-PODs, Moorings, and Batteries	01-May-15	$3,137,640	
Deposit for vessel	01-May-15	$2,871,840	
Storage and Pick-up truck	01-May-15	$524,960	
			$6,534,440
Contracted observers, travel funds, per diems	01-Jun-15	$4,647,440	
Deployment of moorings with C-PODs at 113 sites; fuel, meals and salaries	01-Jun-15	$105,224	
Field coordination, salary, per diem, fuel	01-Jun-15	$62,300	
			$4,814,964
Acoustic data analysis	01-Jul-15	$968,860	
			$968,860
C-POD retrieval, fuel, meals, and salaries	01-Aug-15	$486,978	
			$486,978
Supplies/Equipment/Shipping Expenses	15-Aug-15	$216,160	
Vessel insurance	15-Aug-15	$386,000	
			$602,160
Line-transect communications	01-Sep-15	$30,880	
Acoustic communications	01-Sep-15	$30,880	
Deployment of moorings with C-PODs at 113 sites; fuel, meals and salaries	01-Sep-15	$105,224	
In house observers	01-Sep-15	$404,914	
Modifications to shop to meet survey protocols	01-Sep-15	$46,320	
Travel to/from ship (government vehicles)	01-Sep-15	$30,880	
			$649,098
Field coordination, salary, per diem, fuel	15-Sep-15	$62,300	
			$62,300
1/2 payment for vessel	01-Oct-15	$11,966,000	
Ship/equipment contingency expenses	01-Oct-15	$200,720	
Supplies/Equipment/Shipping Expenses	01-Oct-15	$77,200	
			$12,243,920
C-POD retrieval, fuel, meals, and salaries	15-Oct-15	$487,055	
Final payment for vessel	01-Nov-15	$15,449,665	
Total			$42,299,439