Fish Spawning Aggregation Monitoring in the MARFish Network

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Results from the MARFish Workshop – Fish Spawning Aggregation Monitoring
21st-22nd November 2019, Hotel Marriot Courtyard, Cancun, Mexico

The document editors (Araceli Acevedo, Jacobo Caamal and Stuart Fulton) would like to thank all the people of contributed to the workshop, this document, and who provided feedback on the monitoring protocols. We would also like to thank MARFund, Fonds Français pour l'Environnement Mondial, and Summit Foundation for providing financial support for the meeting and MARFish initiative.

Citation: Acevedo, A., Caamal, J., & Fulton, S. (eds). (2020). Fish Spawning Aggregation Monitoring in the MARFish Network. Comunidad y Biodiversidad A.C. & MARFund. Cancun, Mexico.

Introduction

Many commercial fish species migrate long distances to spawn at specific times and locations each year. In the Wider Caribbean 37 species are known to form fish spawning aggregations (FSA), including the commercially important and heavily exploited grouper and snapper species. Top predators are key to marine ecosystem health. Species that form FSAs concentrate their whole annual reproductive effort into a small window a few days after the full moon of specific months of the year. Spawning occurs at the same site each year with several species often using the same area. The Mesoamerican Reef (MAR) is known to be overfished with human population increase and mass-tourism creating an insatiable demand for fish. The periodic nature of FSA, and the specific locations in which they occur, makes them easily exploited by fishers. For example, in 1964, at just one spawning site in Belize it was estimated that there were over 100,000 groupers, with two tonnes of fish being caught daily. By 2001, only 21 fish were seen by researchers. Similar events have occurred in Mexico where a grouper spawning site in Mahahual (Quintana Roo) disappeared in the early 2000´s.

Beginning in Belize, and posteriorly in Mexico, Honduras and Guatemala, efforts have been made to protect and monitor the FSA sites. FSA site detection often begins with documenting traditional ecological knowledge, before moving to fieldwork, including site characterization and underwater visual census surveys. Much of this work is based on similar methodologies (e.g. Heyman et al. 2004) but new techniques and technologies, plus changes over 15 years necessitate a review and standardization among those groups that continue to work on FSA conservation and monitoring.

Workshop Objectives

Validate a common monitoring strategy through a regional workshop: prioritization and validation of sites, protocol and partners, data sharing agreements
The main objective of this regional workshop was to bring together all of the region's partners (fishers, managers, CSO members, researchers, community leaders) working on fish spawning aggregation sites in the Mesoamerican Reef in order to reinforce the necessity of developing a regional monitoring network and to validate a common strategy (considering prioritization and validation of monitoring sites, protocols and partners, plus data collection, management and sharing).

Specific goals

- Map FSA site monitoring effort and current monitoring protocols.
- Discuss, develop and agree standardized procedures for use and implementation of traditional ecological knowledge, visual census, participatory science, new technologies, database management and data sharing for a regional network.

Workshop Agenda

Thursday 21st November 2019

Time	Theme
9:00	Introduction and Objectives What are FSA? Where are they? What are the regional tendencies? *Stuart Fulton - COBI*
10:00	How should we include traditional ecological knowledge and fisheries monitoring in FSA conservation? Presentation and discussion *Alfonso Aguilar - Universidad Autónoma de Yucatán*
11:00	Underwater visual census (UVC) for FSA monitoring. Presentation and discussion about the protocols current in use in the MAR *Jacobo Caamal – COBI, Myles Phillips – WCS, Marcio Aronne – Cayos Cochinos, Ana Giro - HRI*
13:00	Lunch
14:00	Activity mapping: who is doing what, where and when? Group exercise *Araceli Acevedo and Stuart Fulton – COBI*
16:00	Participatory monitoring for conservation success. Group discussion *Jacobo Caamal – COBI, fishers from Mexico and Belize*
18:00	Close

Friday 22nd November 2019

Time	Theme
9:00	Emerging technologies – passive and active acoustics, eDNA, ROV etc... *Stuart Fulton – COBI*
10:00	A new FSA, what should we do? Group exercise *Stuart Fulton, Jacobo Caamal - COBI*
11:30	FSA Database management. Discussion *Patricia Kramer – AGRRA*
12:30	Data sharing and collaboration. Discussion *Patricia Kramer - AGRRA*
13:00	Lunch (cont.) Discussion: Data sharing and collaboration
15:00	Recommendations for effective FSA monitoring and opportunities for future collaborations in the MAR and beyond
16:00	Agreements and conclusions for an effective MAR network of monitored FSA sites
18:00	Close

Summary of key points and agreements from the workshop

Technique	Notes and recommendations
Traditional Ecological Knowledge (TEK)	• Alfonso Aguilar (UADY) shared recommendations for effectively engaging with fishing communities. His chapter (Hamilton et al. 2011) should be read by all participants.

Underwater Visual Census (UVC)	• The group should continue to use Heyman et al 2004 as a visual census protocol as it is widely established.
	• The group should continue to collect visual size estimates until full deployment of laser caliper sizing. As other countries also acquire laser calipers, follow the same process and use the same datasheets as the Belize group.
	• The group recommends that all partners transition to using laser calipers for sizing. Sample size recommendations and best practices will be shared by partners already implementing this technique.
	• The *Belize SPAG Group* shared their data entry sheets and online portal, which could provide a model for the region.
	• Minimum recommended effort:
	  • Four divers (two buddy teams).
	  • Monitor during four peak abundance days (based on historic data), extending the monitoring so that on the final day you see less fish (to record max. abundance).
	  • Prioritise afternoon/evening surveys.
	  • Key indicators – fish diversity (# of species of grouper and snapper), total abundance by species, fish size (caliper or estimate), behaviour.
	  • Describe behaviour and colour changes as per seven categories in Heyman et al 2004
	  • Complete one Heyman et al 2004 data sheet per dive.
	• Purchase the following laser calipers: Mexico (3), Guatemala (2), Honduras (3), Belize (2).

Passive acoustics	• One hydrophone per sentinel site. • Implement from December to March. Recording period to be defined. • Purchase the following Loggerhead SNAP sensors: Guatemala (from match), Honduras (3), Belize (2). • Investigate training opportunities. Ideally one person from each country as “train the trainer”.
Other techniques	• Additional techniques included bathymetry, tagging, fisheries monitoring, eDNA will be discussed in final report.
Data sharing	• Use Belize UVC excel data collection format across the region (ENG/ESP). • Laser caliper data sheet. • Maintain coordinates private – use grid coordinates for general public. • Review data sharing agreements. • Database ideally hosted by AGRRA. • Store photo and training resources on MARFund site.

Fish Spawning Aggregations Monitoring Protocols

Fish spawning aggregations (FSA) are critical sites in the life cycle of many commercial fish species. The sites have socio-culture importance, have specific biogeographical characteristics and they are sites of unique biological importance. Due to this, finding, characterizing and monitoring FSA sites should draw on all these components.

The MARFish project is the latest and largest iteration of coordinated FSA work in the Mesoamerican Reef (MAR). The MARFish monitoring workshop, held in November 2019 in Cancun, Mexico, brought together stakeholders from civil society, government, fisher, and academic communities to consolidate a vision for FSA monitoring in the MAR. The key output was that the group would agree on the use of standardized monitoring protocols and data sharing agreements to further FSA conservation science in the region.

At the basic level, FSA monitoring is simple. From over 10 years of experience of FSA site characterization, monitoring and protection in Mexico, the document editors highly recommend the use of the KISS[1] principle for FSA monitoring. Basic monitoring protocols that can be replicated across the region on a low budget are recommended. More elaborate protocols can be developed to answer specific questions, but it should be understood that all stakeholders may not have the resources, need, or context in which to apply these protocols in *their* sites. A regional FSA monitoring network will draw on basic, common indicators that are shared across the MAR, in much the same way that the Healthy Reefs Index has brought together key stakeholders under a common vision for coral reef health.

Across the following pages readers will find short, summarized monitoring protocols for each technique that could be applied at their FSA site. This does not mean *all* protocols need to be applied. This will vary on a case by case basis. Readers should also not forget the considerable work by researchers, both in the MAR and at the international level, to create protocols and methodologies for FSA research. This document and its protocols draw heavily on many of them. They are cited in each protocol. The following documents should be consulted by all readers and are found here.

Table 1 Key reference guides and protocols

Document Title	Authors	Year	Notes
Fish Spawning Aggregation Sites in the MBRS Region: Recommendations for monitoring and management	Heyman, Requena et al.	2003	Consultancy document for the MBRS Project
Spawning aggregation monitoring protocol for the Meso-American Reef and the Wider Caribbean	Heyman, Azueta, Lara et al.	2004	First extensive FSA monitoring protocol for the MAR
Reef fish spawning aggregations: Biology, Research and Management	Sadovy de Mitcheson & Colin	2011	Excellent reference material for FSA investigation
Protocolos de monitoreo e investigación participativa para agregaciones reproductivas de peces en México	Heyman, Fulton, Erisman & Aburto-Oropeza	2018	Adapted and updated FSA protocols for the Mexican Caribbean
Monitoreos pesqueros - Generalidades y protocolo	Rivera	2018	Summary of fishery monitoring protocols

[1] Keep It Simple, Stupid – a principle developed by the US Navy that states that systems work best when kept simple rather than being made complicated. Unnecessary complexity should be avoided.

1. Traditional Ecological Knowledge[1]

Introduction

Traditional ecological knowledge - TEK - (Drew 2005) refers to the accumulated knowledge of individuals or groups based on their experiences and interactions with natural world. While TEK does not follow the formal processes of Western Science, it has been documented, on numerous occasions, to be aligned with scientific theory. Perceptions are also dynamic and change with time, providing new sources of information to researchers. In data poor sites, TEK can be a critical data source for understanding changes in fishery or social conditions.

Figure 1 Identifying potential sites with fishers (Credit: COBI).

In the process of "discovering"[2] and investigating fish spawning aggregation sites (FSAs), documenting TEK is usually the first step (Hamilton et al. 2012). Subsequently, the information should be validated in situ through SCUBA diving or another methodology, ideally involving the same fishers as citizen scientists[3] (Fulton et al. 2018).

In principle, conducting interviews with fishers about FSAs is simple and inexpensive, but the researcher should always be cautious, interviewers well-prepared, and relationships of trust, confidentiality and social contexts should be considered. It is also important to gain permission from the community to perform the activities. Semi-structured interviews are recommended.

Materials and equipment

- Format 1 "Traditional ecological knowledge"
- Nautical chart, satellite images and other maps showing the bathymetry of the area and coastline
- Local fish species field guide
- Table of spawning times by species, season and lunar period
- Photos or videos of fish spawning behaviour
- Digital camera

Methodology

Before departure:

- Conduct a review of the scientific literature (published articles and grey literature) to identify potential spawning sites. This can be done online and in regional libraries.
- Identify fishers willing to be interviewed in the communities of the area in question. Interviews with free and cooperative fishers with a wide range of ages and experience

[1] Prepared by Stuart Fulton – COBI, with contributions from workshop participants "MARFish – Monitoring of Fish Reproductive Aggregations" November 2019, and information in the reference list.

[2] In parenthesis because it is more likely that fishers already knew the site before researchers arrived.

[3] Citizen science - the collection and analysis of data relating to the natural world by members of the general public, typically as part of a collaborative project with professional scientists.

are recommended.

In the field:

- Approach the fishers and explain the reasons for the interview, the confidentiality of the information and the ultimate goal of the project.
- Listen carefully and take notes.
- Complete the survey format.
- Request photographs and/or video to support anecdotal information.
- Where possible, identify the site with coordinates, if not, with a point on a map.
- Schedule future meetings with fishers to feedback the information generated.

On your return:

- Store all collected information in the TEK spreadsheet.
- Archive the original data sheets.
- Backup to paper and external hard drives.
- Provide feedback to local fishers who provided vital information by organizing workshops.

Reference protocols

Heyman, W., Azueta, J., Lara, O., Majil, I., Neal, D., Luckhurst, B., Paz, M., Morrison, I., Rhodes, K.L., Kjerve, B., Wade, B., Requena, N. (2004). Spawning aggregation monitoring protocol for the Meso-American Reef and the Wider Caribbean. Version 2.0. Meso-American Barrier Reef Systems Project, Belize City, Belize.

Heyman, W.D., Fulton, S., Erisman, B. Aburto-Oropeza, O. (2017). Protocolos de monitoreo e investigación participativa para agregaciones reproductivas de peces en México. Comunidad y Biodiversidad A.C., Guaymas, Sonora, Mexico & LGL Ecological Research Associates, Inc. Bryan, TX, Estados Unidos. 40 p.

2. Catch Monitoring[1]

Introduction

Fish spawning aggregations (FSAs) are highly vulnerable to overexploitation since fishers capitalize on their predictability and the high concentration of biomass (Sadovy and Domeier 2005). Thus, in places where FSAs are actively fished, catch monitoring can be used as a cost-effective method to assess the status of aggregating fish stocks (Graham et al. 2008). This technique allows researchers to collect information on fishing effort, costs and biological information (biometric and reproductive).

Figure 2 Community scientist surveying fish landings (Credit: CORAL).

Catch monitoring for FSAs is usually carried out at landing sites when fishers are processing their catches (Heyman et al. 2004) but can also be done aboard fishing vessels and in local markets (Environmental Defense Fund 2013). Regardless of the data collection method, building trust with local fishing communities is a key aspect for success. Enlist a community scientist (a respected member of the local fishing community with knowledge of local species), to aid or perform the fish landing monitoring is recommended (Rivera 2018). Catch monitoring should encompass the entire period that fishers are targeting the FSA, usually 15 days before and after the full moon.

The assessment of catches and earnings from FSAs will provide information on the status of fish stocks as well as the profitability of harvestings FSAs. This information will be useful to develop conservation policies that simultaneously protect local species and fishers' livelihoods.

Materials and equipment

- Fish landings data collection sheet
- Pencils
- Blue or green plastic bag
- 2 zip-lock bags
- Plastic gloves
- Plastic basket
- Scale with a precision of 0.1g
- Gonad scale with a precision of 0.01g
- Fish measuring board
- Knife
- Camera
- Local fish species field guide

Methodology

Before departure:

- Develop a relationship with local fishers through informal interviews where you introduce yourself, explain the objective of the work and detail the activities you will be undertaking. Take the opportunity to ask about the main species they harvest, local names, and the distribution of landing sites.

[1] Prepared by Antonella Rivera – The Coral Reef Alliance, with contributions from workshop participants "MARFish – Monitoring of Fish Reproductive Aggregations" November 2019, and information in the reference list.

- Collect anecdotal evidence on the targeted species spawning peaks in the area and determine a monitoring schedule.
- Review the literature to get information on the physical traits of local species so it is easier to identify them in the field.
- Ensure all batteries are charged and that the equipment is working correctly.
- Place the scales within zip-lock bags to ensure they will not suffer any water damage in the field. Place the basket on the scale and calibrate the scale to 0.

In the field:

- In a conversational manner ask fishers about their harvest effort (number of fishers on the vessel, location, depth, time, gears and fishing vessels) and their estimated expenses (fuel, ice, bait, gear repair and food).
- When possible collect biometric data of the entire catch (see Protocol 3). If this is not possible, collect information from a heterogeneous sub-sample of at least 30 fishes.
- Identify the species and indicate both common name and scientific name. If the species is unknown, place it on top of a blue or green background, extend its fins and take a picture of the whole fish and a close up of the head to identify it at a later time.
- Place fish in the basket and measure individually on the scale. Indicate if the fish has been eviscerated.
- Measure the fork length (tip of the snout until the bifurcation of the tail) for each fish. For species with a truncated tail, measure the total length (tip of the snout to the end of the tail).
- Obtain the gonads by making a shallow vertical cut in the fish's abdomen, extending from the anal orifice to the pelvic fin. You will find the reproductive organs in the upper back area of the abdominal wall; it is the only bilobed organ in the abdomen. Record the sex of the fish and the stage of maturity (i.e. indeterminate, immature, mature, gravid, spawning and spent).

On your return:

- Rinse with freshwater all the equipment that has come in contact with the fish.
- Remove batteries from the scales.
- Store all the collected information on a database.
- Archive original fish landings collection sheets.
- Back up digital information on at least two locations (these can be hard drives and online servers).

Reference protocols

Heyman, W., Azueta, J., Lara, O., Majil, I., Neal, D., Luckhurst, B., Paz, M., Morrison, I., Rhodes, K.L., Kjerve, B., Wade, B., Requena, N. (2004) Spawning aggregation monitoring protocol for the Meso-American Reef and the Wider Caribbean. Version 2.0. Meso-American Barrier Reef Systems Project, Belize City, Belize.

Rivera, A. (2018). Monitoreos Pesqueros: Generalidades y Protocolo. Tegucigalpa, Honduras: The Coral Reef Alliance.

3. Fish Biometrics[1]

Introduction

Many commercial fish species migrate long distances to aggregate to spawn at specific locations and times in coral reefs each year. In most cases, local fishers are the first to identify these spawning aggregations (Heyman & Kjerfve 2008) and either fish the aggregation site, or the migration routes. Fisheries monitoring programmes are effective tools for collecting biological information on key species (see Protocol 2). These programmes incorporate traditional ecological knowledge, involving trained fishers in data collection and engaging the community in making decisions to manage resources (Ramírez-Valdez et al. 2017).

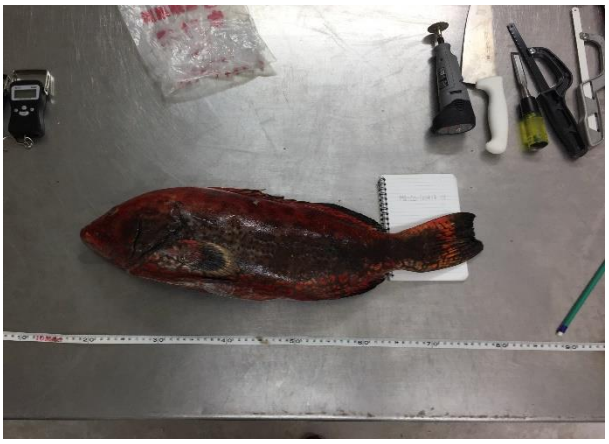

Figure 3 Tissue sampling (Credit: Arturo Ramírez-Valdez).

Landed fish permit biometrics to be obtained for each fish, such as total length, standard length, fork length, head length and weight. In addition, tissue samples (e.g., muscle, liver, or fins), scales and otoliths can be collected. The information generated can allow researchers to better understand population dynamics and structure, spawning periods, and collect samples for age and genetic analysis. Fish can be obtained as part of a systematic monitoring programme, or opportunistically at landing sites or markets.

Materials and equipment

- Hammer
- Chisel
- Adhesive tape
- Permanent marker
- Dissection tweezers
- Dissection scissors
- Digital scale
- Scalpel
- Saw
- Knife
- Dremel
- Saw blade for Dremel
- Safety glasses
- Latex gloves
- Tape measure

Methodology

Obtaining biometrics

- Lay the fish out sideways on a flat surface. The tape measure must be flat and straight.
- Total Length (TL) = From the tip of the snout to the tip of the longest lobe of the tail.
- Standard Length (SL) = From the tip of the snout to the posterior boundary of the last vertebra, where a notable fold is made at the beginning of the tail.
- Head Length (HL) = From the tip of the snout to the rear limit of the operculum, at the opening of the gills.
- Weight (W) = The weight assessment is reported in kilograms (kg) and it should be

[1] [1] Prepared by Araceli Acevedo – COBI, with contributions from workshop participants "MARFish – Monitoring of Fish Reproductive Aggregations" November 2019, and the information in the literature.

specified if the fish is whole or eviscerated.

Tissue sampling

- Samples the size of grains of rice can be collected from cuts on either the pectoral or pelvic fin, the gills or muscles.
- Tissues should be deposited in a vial with 95% alcohol. The entire sample should be covered by the alcohol.
- Vial labels should correspond to same label of fish biometrics taken previously.

Otoliths extraction

- With the fish on its side, cut with a scalpel into the area of the gills to gain access to the inner wall of the skull, locating a bone structure (capsule) with a tubular shape. Remove the soft tissue that covers it.
- Make two diagonal cuts, then two parallel cuts that join them, forming a square. Remove this square with the hammer and chisel.
- With dissection tweezers, carefully extract the otoliths. They are fragile. Once the two otoliths are removed, one from each orifice, they should be rinsed in water and placed in a vial.

Obtaining scales

- Two or three scales can be pulled with the dissection tweezers from behind the pectoral fin. The scales are placed in the same vial as the otoliths.

Sample labelling

- Ensure vials and bottles are well sealed and clearly labelling. The labelling of each sample should be duplicated on the vial or bottle, first with permanent marker and then with tape glued to the bottle. All samples from each individual should be stored in a Ziploc bag, which is also labelled with the sample code.

Reference protocols

Ramírez-Valdez, A. Sgarlatta, M.P. Villaseñor-Derbez, J.C. Cota-Nieto, J.J. Rowell, T.J. Gómez-Gómez, A. Domínguez-Guerrero, I. Domínguez-Reza, R. Hernández-Velasco, A. Santana-Morales, O. Ruiz-Campos, G. Erisman, B. (2017). Manual para monitoreo biológico del Mero gigante (*Stereolepis gigas*) en aguas mexicanas: Proyecto Mero gigante del Pacífico mexicano. SIO-UCSD, UABC, COBI A.C., ECOCIMATI A.C., 42 pp.

Ramírez-Valdez, A. Caamal-Madrigal, J. Fulton, S. Domínguez-Guerrero, I. Rowell, T.J (2018). Ficha técnica para el monitoreo biológico de peces gigantes del Caribe mexicano. COBI A,C., Proyecto Mero gigante, Kuali Comunicación

4. Bathymetric Mapping[1]

Introduction

A complete site characterization involves creating maps and temporal-spatial use descriptions for fish species at the aggregation site. Among indirect evidence of aggregations are increased catch during spawning periods, high fish density and signs of courtship behaviour. If fishing exists on site, information on landings can be collected by measuring effort and biological sampling (Protocol 2-3). Underwater observations can also be made using different methodologies (see Protocol 5). Landing locations and underwater observations can be overlaid on bathymetric maps to create accurate site maps and help characterize the sites (Heyman et al. 2017).

Figure 4 Fishers and researchers undertaking bathymetric transects (Credit: COBI).

The geomorphology of fish spawning aggregation sites is often very similar, i.e. given structural characteristics and conditions that species prefer when aggregating (Erisman et al. 2018). Common features are depths ranging from 20-40 m, near a shelf edge with deep water, and on underwater pinnacles (elbows) on the reef. This information can be valuable for efficiently searching for and locating the spawning sites. The cost for generating bathymetric maps is highly variable and depends greatly on the equipment used. We describe a low-cost method.

Materials and equipment

- Boat and engine with fuel
- 12-volt battery
- Safety equipment including VHF radio, flares, life jackets, anchor and long rope.
- Lowrance Fish Finder with internal GPS and recording capability (e.g. HDS7)[2]
- Dual frequency sonar or TM260 Airmar[3] transducer
- Removable SD card with at least 16 GB of storage capacity
- Dry box to transport sonar and transductor
- Tools and power supply
- Computer (not needed at sea)
- Mapping program (for example, QGIS or ArcGIS)

Methodology

Before departure:

- Activities should be coordinated using the traditional ecological knowledge of local fishers as well as open access digital mapping programmes.
- The surveys should be executed with the help of quadrants covering the study. Under

[1] Prepared by Jacobo Caamal – COBI, with contributions from workshop participants "MARFish – Monitoring of Fish Reproductive Aggregations" November 2019, and the information in the literature.
[2] https://www.lowrance.com/lowrance/type/fishfinders-chartplotters/hds-7-gen3-insight-noxd/
[3] http://www.airmar.com/productdescription.html?id=39

favourable conditions a 3 km by 2 km quadrant is covered 4-6 hours of continuous work. The quadrant is plotted on the computer and then transferred to the sonar device via the SD card. The quadrant should begin at approximately 10 m and continue to 250 m.

- The 12-volt battery should be charged, and all equipment tested.

In the field:

- Connect the sonar to the transducer and battery. The sonar should be programmed to record the information to the SD card.
- Start navigation from the shallowest point, moving deeper. Navigation speed will depend on the sea state and depth.
- Once the depth boundary of the quadrant is reached, return in the opposite direction, navigating in parallel 50 - 100 m from the previous transect.
- Perform this operation as many times as necessary, until the entire quadrant is covered. One person should direct the captain along the route.
- If the sonar loses signal, stop the vessel and wait until the signal returns, before resuming navigation.
- Data should be saved every hour, to safeguard against equipment failure and data loss.
- Once the quadrant is completed there will be four or five files on the SD card. Save this information to a computer on return to port.

Figure 5 Example of transects to collect bathymetric data (Credit: COBI).

On your return:

- The information should be converted to a .csv file using the SonarViewer[1] programme.
- Filter the data to remove invalid points using provided R-script.
- Remove points with depths less than the minimum or greater than the maximum target depth.
- Load data into a GIS program and use Inverse Distance Weighted (IDW) interpolation to generate a digital elevation model.
- If necessary, additional bathymetric information should be collected to fill the empty spaces information and increase accuracy of the map. New information can be added to the database and re-interpolated to create a new bathymetric surface. The more data is collected, the more detailed the map will be.
- Equipment should be cleaned thoroughly, especially the terminals of the connections.

Reference protocols

Heyman, W. D., Ecochard, J. L. B., Biasi, F. B. (2007). Low-cost bathymetric mapping for tropical marine conservation—a focus on reef fish spawning aggregation sites. Marine Geodesy, 30(1-2), 37-50.

Heyman, W.D., Fulton, S., Erisman, B. Aburto-Oropeza, O. (2017). Participatory monitoring and research protocols for fish breeding aggregations in Mexico. Comunidad y Biodiversidad A.C., Guaymas, Sonora, Mexico & LGL Ecological Research Associates, Inc. Bryan, TX, United States. 40 p.

Heyman, W.D., Kobara, S., Olivares, M. (2013). Creating a TIN from Sonar. Internal document.

[1] Contact sfulton@cobi.org.mx for the programme and cleaning scripts

5. Underwater Visual Census[1]

Introduction

Underwater visual censuses (UVC) allow researchers to identify species and quantify abundance and size of fish at spawning aggregation sites located within safe SCUBA diving limits. UVC can be used to verify times and locations of the aggregation, document courtship and spawning behaviour, and evaluate changing site use patterns.

Figure 6 Divers recording data in an aggregation of Epinephelus striatus (Credit: COBI).

Important considerations that impact data quality are observer skill and their ability to identify and quantify fish, and the visibility during the dive. To minimize biases before each survey, a refresher course is recommended for participants to standardize data collection. Each survey team should also have a monitoring detailed plan, with clearly defined roles to ensure that the entire area is systematically inspected.

Even if divers are well trained and with considerable experience, fish size estimation from UVC can be of variable quality as many factors are involved, including distance between diver and fish, perspective, and number of fish at the site. However, this information can be calibrated by using more modern methods involving greater accuracy, such as laser calipers (Heppell et al. 2012, see Protocol 6).

UVCs on spawning sites are ideally conducted in the late afternoon (3-6 pm), between 30 and 60 min before sunset to record courtship and spawning behaviour.

Materials and equipment

- Boat and engine with fuel
- Depth meter, GPS, VHF radio, flares, life jackets, anchor and long rope
- Complete diving equipment for each SCUBA diver: mask, fins, snorkel, BCD, regulator with octopus, weights and belt, watch, depth meter, pressure gauge and compass
- Dive computer (one per diver)
- Diving safety equipment with diving flag, safety buoy, whistles, flashlight or strobe
- DAN oxygen equipment
- First aid kit
- GoPro camera, charger, extra fully charged battery, extra memory card, USB cable, lens cleaning cloth and lens cleaning liquid
- Handheld GPS, charger, extra battery fully charged, USB cable
- Slates and pencils or underwater markers
- Laminated copies of protocols
- Sketch maps and GIS maps with coordinates and graticules, preferably laminated
- Underwater measuring tape or marked rope (50-100m)

[1] Prepared by Araceli Acevedo – COBI, with contributions from workshop participants "MARFish – Monitoring of Fish Reproductive Aggregations" November 2019, and the information in the literature.

- Datasheet 5 "Underwater Visual Census"

Methodology

Record environmental conditions

- Record atmospheric temperature, wind speed and direction.
- Record surface temperature and temperature at the depth at which the aggregation is located. The temperature can be constantly monitored with an on-site temperature logger such as the HOBO TidbiT® V24 or other thermistor. A dive computer can give an estimation.
- Estimate the speed and direction of surface currents. Experienced fishers can accurately estimate the speed and direction of the current.
- Use GPS distance and position functions to determine the speed and direction of the current from the boat (mark your site and then accurately record the time the boat takes to drift 250 metres. This will allow you to approximate your drift speed).

Underwater Visual Census

- Prepare SCUBA equipment carefully, performing all safety reviews for a deep dive.
- Carry out survey dives at the spawning site to estimate the number and size of all the fish in the aggregation. Record the start and end time, and the location of each dive.
- One buddy team of divers should collect abundance counts and another pair size estimates (ideally with the laser caliper).
- A diver should collect video footage.
- Record observed courtship and spawning behaviour.

Processing underwater visual census information

- As soon as the dive is complete, divers will need to work together to compile all the data collected during the dive. Use video to help quantify visual estimates.
- Move all measurements and diagrams from underwater tables to paper data sheets.
- Store all data from datasheets in spreadsheets and generate digital backups.

Reference protocols

Heyman, W., Azueta, J., Lara, O., Majil, I., Neal, D., Luckhurst, B., Paz, M., Morrison, I., Rhodes, K.L., Kjerve, B., Wade, B., Requena, N. (2004) Spawning aggregation monitoring protocol for the Meso-American Reef and the Wider Caribbean. Version 2.0. Meso-American Barrier Reef Systems Project, Belize City, Belize.

Heyman, W.D., Fulton, S., Erisman, B. Aburto-Oropeza, O. (2017). Protocolos de monitoreo e investigación participativa para agregaciones reproductivas de peces en México. Comunidad y Biodiversidad A.C., Guaymas, Sonora, Mexico & LGL Ecological Research Associates, Inc. Bryan, TX, Estados Unidos. 40 p.

6. Laser Sizing[1]

Introduction

Laser calipers are a simple video-based apparatus which use the known distance between two laser points to allow in-situ measurement of fish species. Laser calipers have been used successfully by researchers to more accurately estimate fish size underwater at grouper and snapper spawning sites.

Figure 7a Laser caliper array used by WCS Belize (Credit: A.Tewfik/WCS).

The apparatus features two submersible lasers mounted under a handheld underwater camera system. Properly calibrated and operated by an experienced diver, in-situ fish measurements using these devices can be up to 98% accurate. The equipment is relatively inexpensive (roughly $200USD for lasers and mounting components), with the underwater camera representing the main cost. Laser calipers can be built from store-bought components or assembled from PVC and other multipurpose materials. Video lights are not required for this application; an advantage as the light causes distraction to the spawning fish.

The camera operator must position themselves perpendicular to as many fish as possible, placing the two laser points on the lateral surface of the animals while recording video. This must be achieved during a limited "bottom time", all while maintaining buoyancy, taking note of their surroundings, and remaining steady in the water for high quality video capture. After the survey, data collectors review the video footage, and use the known distance between the two laser points on each animal's body to extrapolate the actual size of the individual.

Figure 7b A camera operator casting the laser points onto a male tiger grouper. Sediment in the water has made the beams visible (Credit: A.Tewfik/WCS).

Materials and equipment

- Underwater camera tray x1
  - Attachment points for cameras, lasers
- Submersible laser x2
- Underwater camera (a second camera is optional)
- Lanyard (to attach to diver wrist)
- Carabiner (to attach to diver BCD)

[1] Prepared by Myles Phillips – Wildlife Conservation Society, Belize Program, with contributions from workshop participants "MARFish – Monitoring of Fish Reproductive Aggregations" November 2019, and information in the reference list

Methodology

Before use:

- Inspect components and replace where necessary.
- Charge cameras and replace laser batteries where necessary.
- Assemble laser calipers and calibrate the position of the lasers for the beams to approximate a predetermined distance from each other (e.g. 20 cm).
- Calibrate the orientation of the lasers. The lasers should be pointed at marks of known distance from each other (e.g. 20 cm apart) with the operator standing 2 m, 3 m and 5 m away from the marks to ensure that the distance between the beams is consistent.
- Upon calibration, the operator should ensure that the lasers are properly fastened in place so their position and orientation will not change.

Figure 8 A Nassau grouper with laser points on its lateral surface. Still frames are extracted from video footage and are used to extrapolate fish size. (Credit: A.Tewfik/WCS).

In the water:

- The operator should turn on the lasers within the first 5 m of the descent, confirming that they are on and working by passing a hand in front of the beams. The operator must then then descend onto the dive site with lasers pointed down and away from other divers. The lasers should be calibrated again at survey depth using a slate or sheet of underwater paper to ensure that the distance between beams has remained true. The lasers will not be turned off again until ascent to 5 m is completed after the dive. This reduces the risk of water infiltration.
- Upon confirmation of laser calibration, the camera can be turned on and prepared for recording at the aggregation site.
- The operator should approach target individuals slowly and indirectly to avoid startling the fish, being certain not to chase target fish. Best results are obtained when the operator can position the laser calipers perpendicular to the animal while it swims at ease in a single direction and is not swimming evasively.
- Both laser points should be placed on the lateral line of as many individual fish as possible, with the operator being mindful to avoid the eyes of the fish and of fellow divers. The operator should try to use the device within 5 m of the target fish (the effective range of the apparatus).
- The operator should attempt to capture high quality of footage of each individual to allow the sex of individual fish to be assessed using gravid state as well as size and colour.
- The operator should attempt to collect sizes of 30 fish per dive.

After use:

- Rinse all apparatus with freshwater, then disassemble and soak to ensure that all salt is removed from small apertures.
- Spray all metal components, particularly moving parts, with WD40 to prevent corrosion and ensure long life.
- Clear all camera and laser seals of residual salt or debris and reapply silicone grease where necessary.

Data recording and archiving:

- A data collector should review the video footage on a computer screen, pausing the video to obtain still frames for measurement of individual fish. The animal should be in frame, perpendicular to the viewer with both laser points as close to the lateral line as possible, and stretched out to its total length, not curved. The data collector must be able to clearly see the anterior point of the head, and the end of the tail.
- The data collector must then use a ruler on these still frames to measure:
    - The distance between the laser points on screen (a)
    - The total length of each fish's body on screen (b)
- The data collector will then use the known distance between the two laser points (c) as a relative scale to extrapolate the actual size of the animal (d) using the formula (d=(b/a)*c).
    - For example, where the size of the laser on screen (a) is 5 cm, the size of the fish on screen (b) is 15 cm, and the known distance between laser points is 20 cm, then the actual size of the animal (d) = (15/5)*20 = 60 cm.

7. eDNA Sampling[1]

Introduction

Genetics can provide important information for fisheries management. At spawning aggregation sites, genetics can provide information on cross-site connectivity, sources and sinks of larvae, population health, and larval displacement from given areas (Burgess et al. 2014). Genetic surveys require collection of tissue samples at landing sites. Endangered fish present a problem since stocks are low, and catches can be infrequent or prohibited. FSAs represent a unique opportunity to collect DNA samples without causing negative impacts on the population.

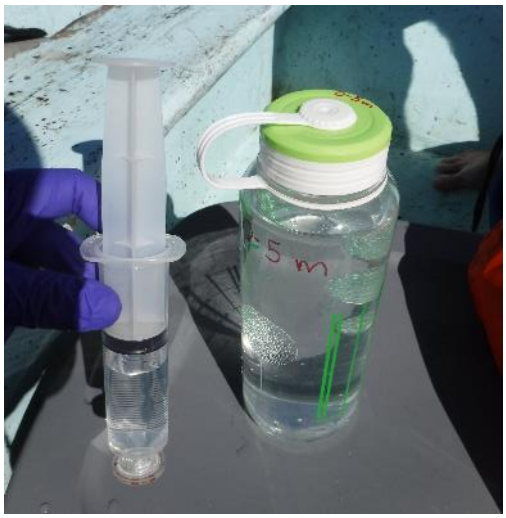

Figure 9 Water samples with eDNA (Credit: COBI).

Environmental DNA (eDNA) is a non-invasive method that allows to obtain genetic material directly from a water sample, capturing the cells that detach from the surface of the fish, or released organic matter (Thomsen and Willerslev 2015). In FSAs many individuals of the same species are concentrated in high abundances, releasing genetic material into the water column.

A genetic connectivity study from eDNA samples at various spawning sites would allow empirical estimate of larval retention, larval dispersion, and adult movements between sites. This information could result in the design of marine reserve networks at the regional level and provide information on the state of the population.

It should be noted that the collection and processing of eDNA in FSAs is a new and innovative technique, and any researcher using this method should consider that analytical methodologies continue to evolve.

Materials and equipment

- 1 or 1.5 litre plastic bottles
- 50 ml syringe and/or vacuum pump
- Cellulose acetate VWR filter with 0.45-micron pore
- DNA preservation buffer
- 0.5 ml test tubes
- Parafilm

Methodology

Before fieldwork:

- Disinfect plastic bottles with chlorinated water for 20 minutes. Rinse three times and leave the bottle full of water. The bottles must be properly marked and transported in a mesh bag. Maintain high hygiene standards to prevent contaminating the bottles once

[1] Prepared by Stuart Fulton - COBI, with contributions Dr. Adrian Munguía, workshop participants "MARFish – Monitoring of Fish Reproductive Aggregations" November 2019, and the information in the literature

disinfected.
- Take the samples during the peak days of spawning activity.

During fieldwork:

- Transport the sealed sample bottles to the study site.
- Water samples can be taken at different depths, depending on site conditions. One near the surface and one at the depth of the highest concentration of fish.
- Three replicates will be taken from each collection point, so it will be necessary to have the bottles marked with numbers 1 to 3.
- Once in the water and at the required depth, open the bottle (without touching the neck with your hands), put them upside down, and flush air into the bottle with the regulator octopus until the water is pushed out. Once the bottle is emptied of water, turn it 180º to fill it with water from the site. Once full, cover the container. Repeat this same operation with each of the bottles at the different depth. Try to approach to within five meters of the fish before taking the sample.
- You can combine the activity with a visual census.
- If ice is available on the boat, place the bottles on ice to preserve the sample.
- When returning to the coast, filter the contents of each bottle through a filter. For each depth you will have three filters. The filtration will be done with the help of the syringe or vacuum pump.
- Once the water is filtered from each container, place the identifier code on the filter, with date, site and bottle number.
- Preserve the filter in preservation buffer solution and place cover with parafilm.
- The process can be repeated during the number of dives required.
- The maximum time to filter samples after they are collected is four hours.

After fieldwork:

***IMPORTANT NOTE**: As this is a developing field, look for the most relevant and up-to-date methodologies, working in collaboration with trained researchers. For this reason, we only include a summary of the steps required to process and analyse the samples.*

- In the laboratory, process the filtered samples with DNeasy Kits to extract the genetic material from each filter. Run an electrophoresis analysis on an agarose gel to validate the presence of genomic DNA molecules. Amplify DNA using PCR with specific primers for the species of interest.
- Acquiring tissue samples from the species of interest helps make direct comparisons and validate eDNA samples.

Reference protocols

Munguia-Vega, A. (2016). Reporte de Viabilidad para la Realización de un Estudio de Conectividad Entre Sitios de Agregaciones Reproductivas de Peces en el Caribe Mexicano. PANGAS

8. Passive Acoustic Monitoring[1]

Introduction

Figure 10 Hydrophone installed at FSA site (Credit: COBI).

Some fish emit sounds during courtship and spawning. Underwater hydrophones have been used for recording spatial and temporal dynamics of aggregating species (Heyman et al. 2017). Hydrophones are usually instruments placed in fixed stations at the aggregation site and are considered a passive monitoring method. The autonomy of hydrophones provides advantages over a visual monitoring programme. Depending on the settings, battery charge and storage space, they can operate from one to 180 Days. Underwater hydrophones constantly monitor spawning areas along the year and only need battery changes and information downloads. Passive acoustic receivers can become a key component of long-term monitoring of fish spawning aggregation sites (Schärer et al. 2012) as they permit year-round all-weather monitoring.

Materials and equipment

- SNAP Acoustic Sensor
- Three type D alkaline batteries
- 64GB MiniSD Card
- Installation base and anchor system
- Case for hydrophone storage
- Stainless steel lock/clip
- Fastening screws
- Plastic cable ties
- Field data sheets

Methodology

Before deployment:

- A temporary anchor system must be constructed of corrosion resistant materials and installed at the FSA site before hydrophone deployment.
- The design of the anchor system will depend on the availability of materials in the area and conditions such as the depth or bottom type. It must be a system that allows the installation operation to be carried out within no-decompression dive times.
- The recommended anchor base design consists of three concrete blocks and a stainless-steel support. The steel support has a PVC structure, within which the acoustic sensor is placed, and which serves as a protective housing. On top of the PVC housing, a latch is placed to keep the hydrophone secure.
- The sensor has a display screen and three buttons that activate the configuration functions. They are shown below in Table 2. The recommended configuration is 20 seconds of recording and 300 seconds of rest.

[1] Prepared by Jacobo Caamal – COBI, with contributions from workshop participants "MARFish – Monitoring of Fish Reproductive Aggregations" November 2019, and the information in the literature.

Table 2 Hydrophone setup process for the Loggerhead SNAP hydrophone

Function	Screen	Press
On	Move the switch to ON	
Settings	UP+DN-> Rec	ENT to change settings
Recording time(s) Rest interval(s) Year Month Day Time Minute Second	Rec: Xs Slp: Xs Year: 20XX Month: XX Day: XX Hour: XX Minute: XX Second: XX	UP or DN to change value ENT to accept change
Home	UP+DN-> Rec	Press the UP and DN buttons at the same time to start
End	Move the switch to OFF	

During deployment:

- Prepare SCUBA equipment carefully, performing all safety reviews for a deep dive.
- Descend with the hydrophone and place it in the PVC base. Close the latch.
- Record installation time in the log.

After deployment:

- Dive to remove the hydrophone once it meets its estimated battery/SD card time limit.
- At the same time, the hydrophone can be replaced with another sensor, charged and with fresh memory.
- Once out of the water and on dry land, the hydrophone should be rinsed with fresh water, dried and the MiniSD card removed to download the information to the computer.
- Two data backups are recommended, and a selection of files should be opened randomly to verify that they reproduce smoothly.
- Provide relevant data on the programming, installation, retrieval and download of data in field and electronic logs.

Reference protocols

Comunidad y Biodiversidad A.C. (2017). Reporte de actividades del proyecto de conservación de agregaciones reproductivas de peces en el Arrecife Mesomericano II: Capacitación en monitoreo comunitario de agregaciones reproductiva de peces (batimetría, buceo y monitoreo acústico).

Heyman, W.D., Fulton, S., Erisman, B. Aburto-Oropeza, O. (2017). Protocolos de monitoreo e investigación participativa para agregaciones reproductivas de peces en México. Comunidad y Biodiversidad A.C., Guaymas, Sonora, Mexico & LGL Ecological Research Associates, Inc. Bryan, TX, Estados Unidos. 40 p.

9. Data Sharing

Effective data sharing is critical for a regional collaboration. Sharing information about FSAs can be a sensitive subject, as some FSAs are closely guarded secrets, whilst some parties have concerns about sharing site information in case the information motivates illegal fishing. MARFish partners should strive to provide standardized data to the project, whilst maintaining a certain level of privacy over key aspects (particularly with data such as coordinates of FSA sites).

During the MARFish workshop, the participant organizations agreed to use standardized protocols for monitoring. These protocols are generally based on existing methodologies (e.g. Heyman et al. 2004) but also incorporate new elements (e.g. laser sizing). The data entry formats are found in the accompanying Dropbox folder.

The workshop participants recommended developing a formal Data Sharing Agreement and developing a specialized regional database for the project, ideally hosted in the AGRRA ecosystem, following the example set by the Belize Spawning Aggregation Working Group.

10. Key site indicators

Indicators allow progress to be measured. In the case of FSA sites in the MARFish network, we recommend collecting the following basic indicators at each site, as these are basic indicators. Additional data can also be collected, however this information will allow MARFish partners to measure progress over time.

Table 3 FSA site indicators

Indicators	Methodology	Units
Species spawning at FSA	UVC	# of species seen spawning/with spawning behavior
Maximum abundance	UVC	Maximum # of individuals (each species) seen during reproductive cycle
Species size distribution	UVC/laser	Binned cm size categories
Spawning period	UVC/passive acoustic/catch monitoring	Days after full moon Months of year
Site depth	Bathymetry	Metres
Site protection status	-	Protected/Not protected

11. Sentinel site recommendations

Each MARFish sentinel site should have the following:

- 3D bathymetric map.
- Operational hydrophone that is operational during the entire spawning period.
- HOBO (or similar) temperature sensor.
- Regular UVC monitoring, with the following components:
    - Sizing with laser calipers.
    - If no laser calipers are available, binned size estimates should be made (as per monitoring data sheet) until lasers are acquired.
    - Ideally, monitoring should cover the entire spawning period (from the day the fish arrive, to the day they leave), however considering budget restrictions, UVC monitoring should be prioritized to capture the maximum abundance at the FSA site. In this case, to be sure the maximum abundance is captured, monitoring should occur until the observers view a decrease in the number of fish (Figure 11, day 7). If this reduction is not seen, observers can not be sure they captured the maximum abundance.
    - Optimal monitoring periods can be found during the site characterization process.

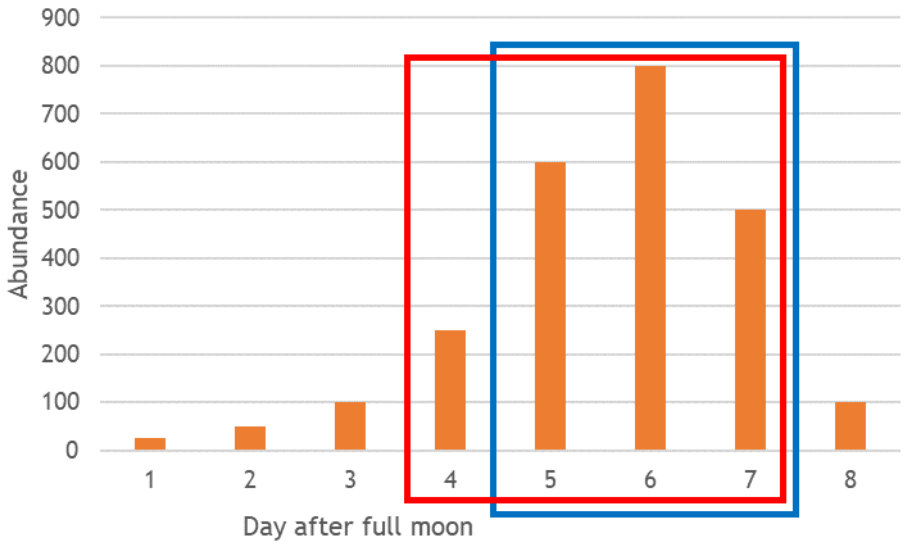

Figure 11 UVC monitoring prioritization. Red = four-day budget, Blue = three-day budget.

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Annexes

1. Presentations
2. Field datasheets
3. Database formats
4. Participants in the November 2019 MARFish Monitoring Workshop

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