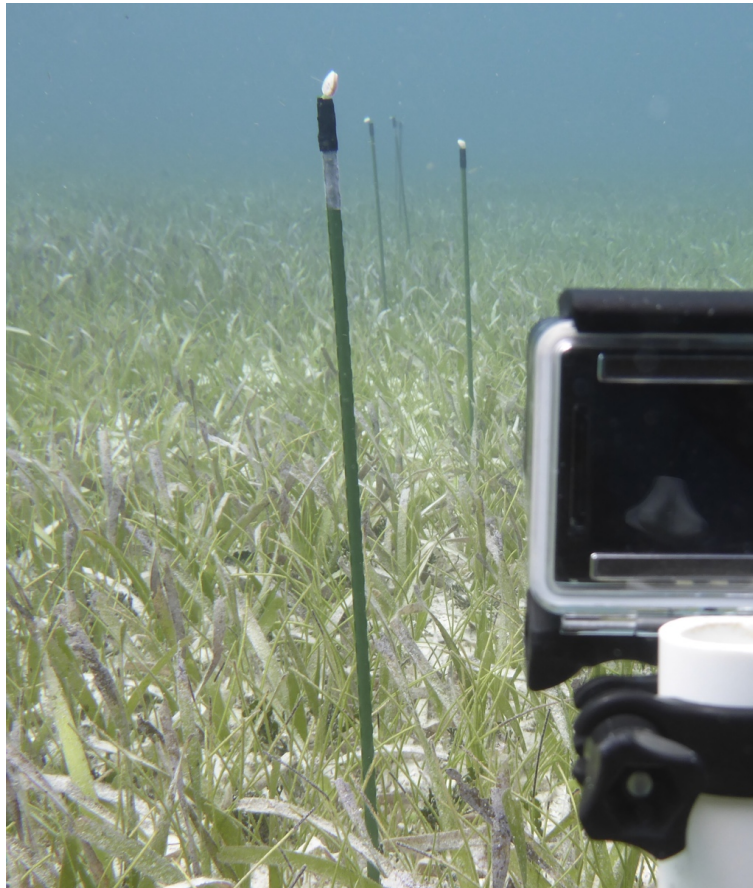




Protocol: Squidpop predation assay

V 1.0.0



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1. Introduction

This protocol provides a simple, quick, standardized assay of fish predation intensity. Fish predation is important in nearly all marine ecosystems as it structures communities and is how production moves up the food web. The goal of this protocol is to establish a simple, standard method to quantify the relative intensity of feeding by generalist predators that is comparable across a wide range of sites and conditions. This protocol has been used to address a variety of questions (see ‘literature’ on MarineGEO squidpops page) and when coupled with camera deployments can also be part of a Baited Remote Underwater Video (BRUV) sampling of the fish community. Additional copies of this protocol, field datasheets, excel data entry templates, tutorial videos, literature, and more can be found on the squidpop section of the MarineGEO protocol website: <https://marinegeo.github.io>.

2. Assay design

This assay measures the rate of fish predation as the loss of standard bait deployed for 24 hours in the field. Dried squid (Figure 1) is cut into pieces of standard size (~13 mm in diameter), attached to stakes (squidpops) with monofilament fishing line, and deployed in the field in groups of 25. Baits are scored as present or absent after 1 hour and again after 24 hours.

- **Assay frequency:** At least annually, preferably quarterly.
- **Measured parameters:**
 - Bait loss after 1 hour
 - Bait loss after 24 hours

3. Requirements

- **Materials:**
 - Dried squid mantle
 - 13 mm diameter auger punch or cork borer
 - Scissors
 - Sewing needle (or other thin, sharp tool)
 - Thin monofilament line (1 m)
 - Electrical tape (1 roll)
 - Fiberglass garden stakes (25)

Material specifications:

Squid: We use dried squid as bait because most marine predators will readily eat it, it is widely available, and the dried bait can be shipped and stored without refrigeration. It is important to get the type of squid in which the mantle remains whole, resembling a sheet. MarineGEO uses Hang Tai Marine Products Co. Dried Squid in 7oz. packages

Fishing line: Clear monofilament 2-10 pound test

Cork Borer: Cork borers, biopsy punches, and hollow punches will all work. Size of 13 mm \approx 05 inches.

Fiberglass Plant Stakes: 30-50cm in length. Do not use white, brightly colored, or florescent stakes.

- ***Required Personnel:*** 2
- ***Time:***
 - *Preparation:* 1person x 2 hours.
 - *Field work:* 2 people x 2 days (roughly two half days, depending on travel time and field conditions).
 - *Data curation:* 1 person x 1-2 hours.

4. Field preparation

- 1) **Cut 25 discs from dried squid mantle** using auger punch or cork borer to (Figure 1). If these tools are not available, cut 1 x 1 cm squares from the squid mantle.
- 2) **Secure squid to line** by threading a sewing needle with monofilament line. Pierce the bait with the needle, wrap the line around the bait, and tie a knot to secure.
- 3) **Attach to stake.** Cut line approximately 5 cm from squid bait. Wrap the free end of the line to the stake and attach it with tightly wrapped electrical tape. Leaving ~1 cm of line between the bait and the end of the stake (see Figure 2).
- 4) **Print out a field datasheet** for each site where you plan to deploy, preferably on waterproof paper, and take them with you to the field. If you are inexperienced with squidpops, print a copy of this protocol and bring it with you in the field



Figure 1. Squidpop baits are discs cut from mantle of dried squid using a cork borer or augur punch.

Note: Keep the squid dry until deployment otherwise the squid can become oily or slimy. We recommend keeping squidpops in the refrigerator until deployment.

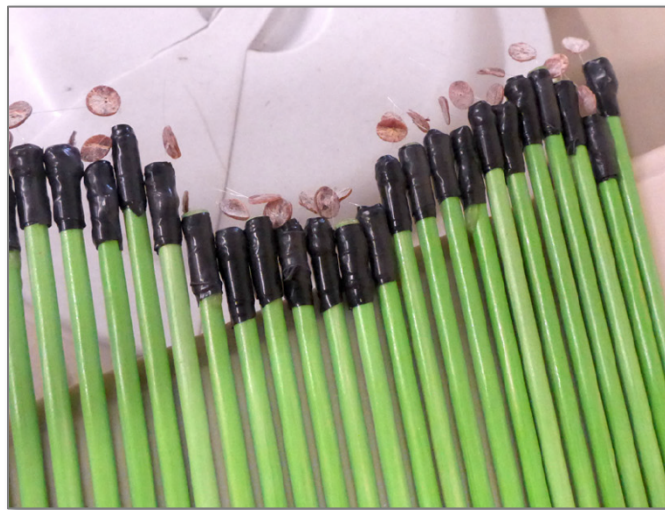


Figure 2. Squidpops ready for deployment

5. Field Work

- **Day 1: Deploying and scoring squidpops**

- 1) **Fill in the datasheet metadata:** site name, MarineGEO site code (if available), date (yyyy-mm-dd), habitat type, depth, and full names (first and last) of field team.
- 2) **Record site latitude and longitude** in decimal format using GPS and record on field datasheet. If GPS is not available, note carefully your position relative to land features that will help you find the squidpops later and identify your GPS coordinates on Google Earth.
- 3) **Deploy the squidpops** by pushing them firmly and deeply into the substrate to prevent dislodgement by waves, debris, or predators. Deploy all squidpops at roughly the same depth, in the same type of habitat, and separated from one another by ~2 m. Linear arrangement (space permitting) makes relocating stakes easier. Also, carefully noting terrain features and landmarks, and taking a compass heading can ease relocation. If desired, you can deploy a buoy or float near one end of the squidpop line, but be careful if this is likely to attract interference from people.
- 4) **Record the time** of deployment.
- 5) **After one hour:**
 - a. Examine each squidpop in situ. Record bait as present (1) or absent (0). Bait absent (0) means the *entire* bait is missing; partial baits are counted as present. Note any missing or dislodged squidpop stakes with an “M” on the datasheet.
 - b. *Leave all squidpops in place* whether or not bait is missing to prevent confusion when collecting after 24 hours. Squidpops will be collected on day 2.

- **Day 2: Scoring and retrieving squidpops**

- 1) **Roughly 24 hours after deployment:**
 - a. Examine each squidpop in situ and record bait as present or absent and any missing or dislodged squidpops. *Record bait missing from all squidpops, including those counted at 1 hour.*
 - b. Retrieve all stakes and any other materials (e.g., buoys) from the field.
 - c. Review field datasheets for completeness before departing the site. Make sure you have recorded GPS coordinates or described landmarks to help locate the site in Google Earth.

6. Entering the data

- 1) **Scan the completed field datasheets** as PDFs, name the PDF files with informative file names that include site and date, and store both paper and electronic copies in a secure folder designated for this purpose by your site PI. Then back them up.
- 2) **Enter the data**, along with all metadata, from the field datasheet into the MarineGEO squidpop data spreadsheet. Be sure to enter your full name and date of data entry, and fill in all columns as best you can.
- 3) **Save the file** with a unique and sensible suffix to distinguish it from the template. Save the data spreadsheet in the designated folder, and back it up.
- 4) **Email** the PDF copies of all field datasheets, the completed squidpop data spreadsheet, and any necessary notes or explanation to marinegeo-data@si.edu.
- 5) **You're done!**

7. “So, you’ve Squidpop-ed before”: A QuickStart guide

• Materials checklist (per deployment)

	Amount
<input type="checkbox"/> <i>MarineGEO squidpop field datasheet (waterproof paper)</i>	1
<input type="checkbox"/> <i>MarineGEO squidpop data entry template (electronic)</i>	1
<input type="checkbox"/> Pencils (bring a spare!)	2
<input type="checkbox"/> clipboard	1
<input type="checkbox"/> dried squid mantle baits (1 - 1.3 cm diameter)	25
<input type="checkbox"/> auger hole punch (1.3 cm diameter)	1
<input type="checkbox"/> green fiberglass garden stakes (~50cm)	25
<input type="checkbox"/> fine monofilament fishing line (2-10 lb. or similar)	~1 m
<input type="checkbox"/> electrical tape	1 roll
<input type="checkbox"/> sewing needle	1
<input type="checkbox"/> scissors	1
<input type="checkbox"/> GPS unit (optional)	1

• Key Steps

- ☐ Make 25 squidpops
- ☐ Print out field datasheet
- ☐ Fill out datasheet metadata (site name, code and lat./long., date (yyyy-mm-dd), habitat, depth, team member names)
- ☐ Deploy squidpops
- ☐ After one hour
 - Record bait as present (1) or absent (0) and any missing or dislodged squidpops (M)
 - Do not remove any stakes
- ☐ After 24 hrs
 - Record bait as present (1) or absent (0) and any missing or dislodged squidpops (M)
 - Remove stakes
 - Review datasheets for completeness
- ☐ Enter data
 - Scan datasheets as PDFs
 - Enter data into Data Entry Template spreadsheet
 - E-mail PDFs, spreadsheet, and anything else you’d like to share to marinegeo-data@si.edu