Protocol: Squidpop predation assay

V20180817

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1. Summary

This protocol provides a simple, quick, standardized assay of fish predation intensity. When coupled with camera deployments, it can also be part of a Baited Remote Underwater Video (BRUV) sampling of the fish community. The latest version of this and other MarineGEO protocols, along with field data sheets, data templates, instructional videos, and standardized analysis scripts, are hosted and updated at the MarineGEO GitHub site[1].

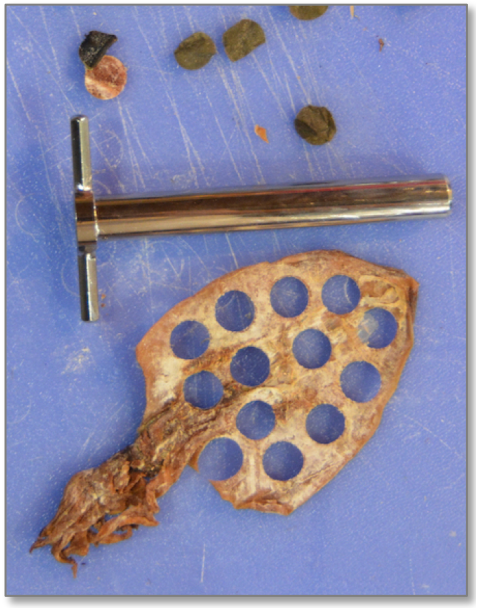

Figure 1. Squidpop baits are discs cut from mantle of dried squid using a cork borer or augur punch.

2. Rationale

Fish predation is an important process structuring communities and a conduit of production up the food web in nearly all marine ecosystems. The goal of this protocol is to quantify the relative intensity of feeding by generalist predators using a standard bait and method that is simple and comparable across a wide range of sites and conditions. We use dried squid as bait because most marine predators are generalists who readily eat it, it is widely available (e.g., in Asian grocery stores), and the dried bait can be shipped and stored without refrigeration. It is important to get the type of squid in which the mantle (main part of body) remains whole, resembling a sheet (see appendix for a source). The protocol has been used to address a variety of questions (see 'Selected Literature', below).

3. Requirements

Personnel: 2

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

Frequency: Variable (at least annually)

Assay design: The assay measures the rate of fish predation as loss of standard bait deployed for 24 hours in the field. The dried squid (Figure 1) is cut into pieces of standard size (~1.3 cm diameter), attached to stakes (squidpops) with monofilament fishing line, and deployed in the

[1] MarineGEO GitHub: https://marinegeo.github.io/

field in groups of 25. The number of baits remaining is scored after 1 hour, then scored again and retrieved after 24 hours. Baits are scored as present or absent.

4. Measured Parameters

- Bait loss after 1 hour
- Bait loss after 24 hours

5. Preparations prior to field work

Goal: Construct squidpops and gather field materials

See instructional video at: https://marinegeo.github.io/modules/squidpops

1) Use auger punch or cork borer to cut 25 pieces, 1.3 cm diameter, from the dried squid mantle (Figure 1). If these tools are not available, cut 1 x 1 cm squares from the squid mantle.
2) Thread a sewing needle with thin monofilament line (2-10 lb. test). Pierce the bait with the needle, wrap the line around the bait, and tie a knot to the lead line to secure squid to the line.
3) Cut the line approximately 5 cm from the 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 data sheet for each site where you plan to deploy (available at https://marinegeo.github.io/modules/squidpops), 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

Note: Keep the squid dry until deployment. The squid can become oily or slimy when immersed and may foul the water. We recommend keeping squidpops in the refrigerator until deployment.

6. In the field, day 1: Deployment and scoring of squidpops

Goal: Deploy squidpops and measure predation rate after 1 hour

1) Fill in metadata on the field data sheet: site name, MarineGEO site code (if available), date (yyyy-mm-dd), habitat type, depth, and full names (first and last) of field team.
2) Use GPS to measure and record site latitude and longitude, preferably in decimal format, and record on field data sheet. 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 thrusting them firmly and deeply into the substratum to prevent dislodgement by waves, debris, or energetic predators. Deploy all squidpops at roughly the same depth in the same type of habitat, separated from one another by 1-2 m. They may be

placed in linear or other arrangement depending on site conditions. Take care to deploy squidpops in such a way that they can be easily relocated after 24 hours—note carefully the terrain and landmarks. 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.

Figure 2. Squidpops ready for deployment

4) Record the time of deployment.
5) One hour after deployment, examine each squidpop in situ and record how many stakes are *missing* bait.
6) Make sure all squidpop stakes are still in place and record how many stakes are *remaining*. Avoid disturbing or removing the stakes, which will be collected the following day. Bait is recorded as present (1) or absent (0), that is, absent means the entire bait is missing; partially remaining baits should be counted as present.
7) *Do not retrieve stakes with missing bait at this time*. Leave all stakes in place, whether or not bait is missing. This prevents confusion when collecting after 24 hours.

7. In the field, day 2: Scoring and retrieval of squidpops

Goal: Measure predation rate after 24 hours

1) Roughly 24 hours after deployment, examine each squidpop again in situ and record how many stakes are *missing* bait.
2) Be sure to record bait missing from *all* squidpops, including those you already counted at 1 hour, to capture cumulative bait loss. For example, if 5 baits were missing at 1 hour, and an additional 10 were missing at 24 hours, record 15 baits missing after 24 hours. Be sure to record the number of baits *missing*, not the number remaining.
3) Count the squidpop stakes and record how many stakes *remain* in place.
4) Retrieve all stakes and any other materials (e.g., buoy) from the field.
5) Review field data sheets for completeness before departing the site. Make sure you have recorded GPS coordinates or described landmarks to help locate the site in Google Earth.

8. In the lab, day 2: Curating the data

Goal: Secure the data and submit to MarineGEO

1) Scan the completed field data sheets to PDF, 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 data sheet into the MarineGEO squidpop data spreadsheet (available at https://marinegeo.github.io/modules/squidpops). 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 data sheets, the completed squidpop data spreadsheet, and any necessary notes or explanation to marinegeo-data@si.edu.
5) You're done!

9. Selected literature

Duffy, J. E., S. L. Ziegler, J. E. Campbell, P. M. Bippus, and J. S. Lefcheck. 2015. Squidpops: A Simple Tool to Crowdsource a Global Map of Marine Predation Intensity. PLoS ONE 10:e0142994. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0142994

Gauff, R. P. M., S. Bejarano, H. H. Madduppa, B. Subhan, E. M. A. Dugény, Y. A. Perdana, and S. C. A. Ferse. 2018. Influence of predation risk on the sheltering behaviour of the coral-dwelling damselfish, *Pomacentrus moluccensis*. Environmental Biology of Fishes 101:639–651. https://link.springer.com/content/pdf/10.1007%2Fs10641-018-0725-3.pdf

Gross, C., C. Donoghue, C. Pruitt, A. C. Trimble, and J. L. Ruesink. 2017. Taxonomic and functional assessment of mesopredator diversity across an estuarine habitat mosaic. Ecosphere 8:e01792. http://doi.wiley.com/10.1002/ecs2.1792

Gusmao, J. B., M. R. Lee, I. MacDonald, N. C. Ory, J. Sellanes, Les Watling, and M. Thiel. 2018. No reef-associated gradient in the infaunal communities of Rapa Nui (Easter Island) – Are oceanic waves more important than reef predators? Estuarine Coastal and Shelf Science 210:123–131. https://doi.org/10.1016/j.ecss.2018.06.019

Lefcheck, J. S., S. J. Brandl, P. L. Reynolds, A. R. Smyth, and S. T. Meyer. 2016. Extending Rapid Ecosystem Function Assessments to Marine Ecosystems: A Reply to Meyer. Trends in ecology & evolution 31:251–253. http://dx.doi.org/10.1016/j.tree.2016.02.002

Rodemann, J. R., and S. J. Brandl. 2017. Consumption pressure in coastal marine environments decreases with latitude and in artificial vs. natural habitats. Marine ecology progress series. Oldendorf 574:167–179. http://www.int-res.com/articles/meps2017/574/m574p167.pdf

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10. Materials checklist (per deployment)

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

[2] Available at: https://marinegeo.github.io/

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11. Sources of materials

Squid: http://www.amazon.com/Hang-Marine-Products-Dried-Squid/dp/B00HN18KA6/ref=sr_1_14?ie=UTF8&qid=1439399988&sr=8-14&keywords=dried+squid

Fishing line: http://www.amazon.com/Stren-Original-330-Yard-Spool-Pound/dp/B001H32I7M/ref=sr_1_1?ie=UTF8&qid=1422284845&sr=8-

Electrical Tape: http://www.amazon.com/EL7566-AW-Synthetic-Rubber-Premium-Electrical/dp/B000TPEHMS/ref=sr_1_1?s=electronics&ie=UTF8&qid=1422453815&sr=1-1&keywords=electric+tape

Cork Borer: http://www.amazon.com/American-Educational-Piece-Nickel-Borer/dp/B005QDWU18/ref=sr_1_1?ie=UTF8&qid=1404146522&sr=8-1&keywords=cork+borers

Fiberglass Stakes: http://www.amazon.com/EcoStake-Stakes-Garden-Tomato-Training/dp/B00YNU1BDS/ref=sr_1_3?ie=UTF8&qid=1454247202&sr=8-3&keywords=fiberglass+plant+stake

12. About the Smithsonian MarineGEO program

The **Marine G**lobal **E**arth **O**bservatory (MarineGEO) is a community of practice, led by the Smithsonian Institution, with the mission of understanding how coastal ecosystems work, and how to keep them working. We use standardized, comparative methods to do so, focusing on nearshore seabed communities because this is where marine biodiversity is concentrated and where humans interact with it most. Our mission is realized through two main components: (1) repeated measurements of key environmental and biological components to produce long-term time series; and (2) experiments leveraging these observations across sites to discover ecological mechanisms in a comparative framework. To place these continuing studies in context and maximize their effectiveness we are assembling a rich database of existing knowledge and new biodiversity collections at each partner site.