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We are still developing and optimizing this protocol

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A. verticillata sampling in San Diego, CA

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ABSTRACT

This is the sampling protocol.

Amathia verticillata collection

1. Three replicate colonies (of low, medium, and high biogenic material) of *A. verticillata* were collected from the side of the dock by surrounding the colony (depending on its size) with a 100 µm, 18 x 42 cm or 10.5 x 20 cm mesh bag

2. Place the sample into a gallon-sized Ziplock bag, and storing it in a cooler with ice to prevent degradation during transport to the lab for further processing.

Amathia verticillata sorting

3. Each *A. verticillata* colony was removed from the collection bags and placed in a 5-gallon bucket filled with seawater

4. The *A. verticillata* colony was shaken 10 times by hand to remove all associated marine organisms, and the colony was returned to the Ziplock bag for processing after sample sieving.

5. The bucket water was filtered through nested 100, 200, and 400 µm mesh sieves to collect the organisms associated with *A. verticillata.*

6. Repeated three times to ensure that most of the organisms had been successfully removed from each *A. verticillata* colony

7. Placed organisms in containers by sieve size and preserved in 100% ethanol until they could be counted and identified.

8. Each *A. verticillata* colony was removed from its Ziplock bag and five random kenoozoids per colony were selected to measure widths using a Meiji Techno stereo microscope with a RZ PLAN 1x lens and an ocular micrometer

9 Once measured, the colony was placed in a drying oven at 50 °C for 24 h to obtain the *A. verticillata* dry weight of each sample.

Associated Invertebrate Sorting

10 The preserved invertebrate samples were divided using a Folsom plankton splitter if they were dense, then sorted under a Meiji Techno stereo microscope with a RZ PLAN 1x lens.

11 Organisms were separated into broad taxonomic groups: amphipods, isopods, tanaids, polychaetes, copepods, unknowns, and other organisms that could be identified but were less abundant, such as gastropods, bivalves, and nematodes (called “others”). In addition, invertebrates were categorized into four main life history stages: immature organisms, adults (males and females without eggs), females with eggs, and unknowns.

12 The organisms collected in the 100 µm sieve were primarily pelagic copepods which were assumed to be swimming in and around *A. verticillata* colonies and not necessarily using the bryozoan as a benthic habitat; thus, they were not identified to species and were excluded from further analysis. The 200 and 400 µm sieved amphipods, isopods, tanaids, and polychaetes were identified to the lowest taxonomic level possible using published resources (SCAMIT 2004, 2023) and by working with taxonomists (Dean Pasko and Tony Phillips, personal communication).

13 We classified the introduction status for the 12 peracarid crustaceans and polychaetes that we could identify to species as native, NIS, likely NIS, and cryptogenic using published records (Menzies 1952; Light 2007; Maloney 2007b, a; Fofonoff et al. 2018) and taxonomic experts (Dean Pasko and Tony Phillips, personal communication).