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ORIGINAL ARTICLE

A novel design for sampling benthic zooplankton communities in disparate Gulf of Alaska habitats using an autonomous deep-water plankton pump

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Deep-water larval fish and zooplankton utilize structurally complex, cold-water coral and sponge (CWCS) habitats as refuges, nurseries and feeding grounds. Fine-scale sampling of these habitats for larval fish and zooplankton has proven difficult. This study implemented a newly designed, autonomous, noninvasive plankton pump sampler that collected large mesozooplankton within 1 m of the seafloor. It was successfully deployed in the western Gulf of Alaska between the Shumagin Islands (~158°W) and Samalga Pass (−170°W), and collected *in situ* zooplankton from diverse benthic communities (coral, sponge and bare substrates) at depths in excess of 100 m. Key design parameters of the plankton pump were its ability to be deployed from ships of opportunity, be untethered from the vessel during sampling and be deployed and retrieved in high-relief, rocky areas where CWCS are typically present. The plankton pump remains stationary while collecting from the water column, rests within 1 m of the seafloor and captures images of the surrounding habitat and substrate. This plankton pump design is a low-cost, highly portable solution for assessing the role of benthic habitat in the life cycle of mesozooplankton, a linkage that has been relatively underexplored due to the difficulty in obtaining near-bottom samples.

KEYWORDS: plankton pump; seafloor water sampling; deep-water habitat; benthic zooplankton; Alaska

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INTRODUCTION

Deep-water fishes and zooplankton utilize structurally complex, cold-water coral and sponge (CWCS) habitats as refuges, nurseries and feeding grounds (Brodeur, 2001; Auster et al., 2005; Busby *et al.*, 2005; Rooper *et al.*, 2007; Pirtle et al., 2012; Baillon *et al.*, 2012). Mobile bottom-fishing gear or deployed cameras are frequently used to summarize associations between benthic fishes and structure-forming invertebrate (SFI) assemblages such as corals and sponges (Yoklavich *et al.*, 2000; Tissot *et al.*, 2006; Beazley *et al.*, 2013; Laman *et al.*, 2015; Milligan et al., 2016; Schejter *et al.*, 2016, Schejter *et al.*, 2017). However, little attention has been given to the epibenthic planktonic community, particularly the zooplankton and ichthyoplankton communities associated with structure-forming habitats found within the bottom meter of the water column.

Traditionally, zooplanktons are sampled in the field using oblique or vertically stratified net tows. Obliquely towed plankton nets typically descend to within 5 or 10 m of the seafloor and collect samples through the water column integrated over distance and depth. Individual nets from vertically stratified tows (e.g. multiple opening/closing net and environmental sensing system, MOCNESS; Wiebe *et al.*, 1976) integrate over horizontal distances at discrete depths. Both methods are typically deployed to avoid contact with the seafloor and any high-relief habitat. Epibenthic sleds, which are designed to be towed along the seafloor, can also be used to collect zooplankton along the seafloor (Clark and Stewart, 2016), but sleds are typically restricted to soft bottom areas where deployment and retrieval without snagging on seafloor obstacles is possible. These methods all integrate samples over a wide range of distances and/or depths providing large-scale (100s to 1000s m^3) low-resolution zooplankton distribution and abundance patterns. CWCS habitats are not typically sampled during routine zooplankton and ichthyoplankton net surveys, as their proximity to the seafloor and presence in rough and rugose habitats create physical obstacles for traditional plankton net sampling.

The objective of this study was to develop an autonomous, noninvasive plankton pump that collected large (>300 μm) mesozooplankton and fish larvae within 1 m of the seafloor, and captured concurrent images of the surrounding seafloor habitat. Mesozooplankton were targeted in this study because of their importance as prey items for and larvae of commercially important fish species. Key design parameters of the plankton pump were its ability to be deployed from ships of opportunity, be untethered from the vessel during sampling and be deployed and retrieved in high-relief, rocky areas where CWCS are typically present.

MATERIALS AND METHOD

Plankton pump system design

Our plankton pump design was largely based upon previous zooplankton sampling projects (Gori *et al.*, 2016; Madurell *et al.*, 2012), which collected samples from deep-water, epibenthic communities. The plankton pump was specifically designed to autonomously collect zooplankton and ichthyoplankton within 1 m of the seafloor. The pump consisted of five major structures: the sample collection tube and its components; two polyvinyl-chloride (PVC) waterproof housing units for electronics; a protective housing for the sample collection tube; the deployment, retrieval and landing gear and a camera with housing (Table I).

The sample collection tube consisted of a 1 m length, 10.2 cm diameter, clear PVC pipe that was split into three sections, joined together using PVC unions, allowing each component in the tube to be easily accessible. The contents of the collection tube included a General Oceanics mechanical flowmeter equipped with a one-way clutch (Model 2030RC), a 333 μm mesh plankton net with codend, and a Blue Robotics T200 thruster (Fig. 1). The flowmeter was suspended in the tube using a threaded bolt and rubber clamp to hold it 17 cm from the mouth of the tube and roughly 6 cm in front of the plankton net. The plankton net and codend were placed mid-tube, about 42 cm from the opening. The mesh size (333 μm) of

Table I: Plankton pump major structures and their components

Structures and components
Sample collection tube
———10.2 cm diameter clear PVC pipe
———10.2 cm diameter PVC unions
———General Oceanics flowmeter
———333 μm mesh plankton net w/codend
———Blue Robotics T200 thruster
———10.2 cm diameter PVC cap (w/8.9 cm diameter hole removed from center)
———Modified Van Dorn Lid w/neodymium magnet
PVC waterproof housing units
———5 cm diameter gray PVC pipe
———9 V NiMH batteries
———Arduino microcontroller
———Magnet retractor mechanism
•Motorized threaded rod
•Gear motor
•Neodymium magnet
Protective housing for sample collection tube
———25.4 cm diameter PVC protective pipe
Deployment and landing gear
———Welded steel landing base
———15.9 mm floating line
———_Buoys
Digital camera and housing

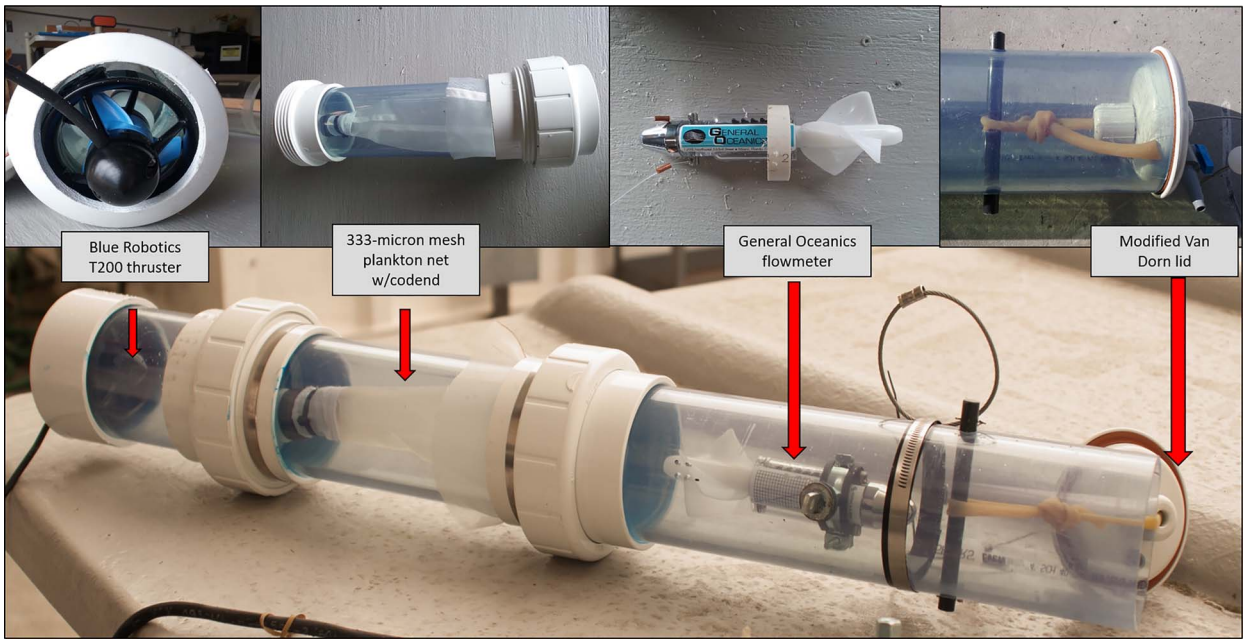

Fig. 1. Plankton pump sample collection tube and its components.

the plankton net was selected to collect large zooplankton that serve as food for fish species, but any mesh size could be used in the design. Finally, the Blue Robotics thruster was placed at the end opposite of the mouth, where it pulled (or pushed) water through the collection tube. The inner diameter of the sampling tube (10.2 cm diameter) was selected to specifically fit the outer diameter of the thruster unit. A 10.2 cm diameter PVC cap with an 8.9 cm hole was glued over the end of the sampling tube to ensure the thruster stayed in place and water flowed unobstructed.

The PVC waterproof housing units, which contained the electronics, consisted of 39 cm length, 5 cm diameter gray PVC pipe with threaded waterproof caps and wire access points (Fig. 2). One housing contained the power provided by 12 V NiMH 9 Ah battery packs. The second housing contained an Arduino microcontroller and a magnet gear motor used to activate the closure of the sample collection tube. The Arduino microcontroller was programmed to control the timing of the Blue Robotics thruster activation, speed and direction, as well as the activation of the magnet gear motor. A modified, one-sided Van Dorn lid was used as a "door" to seal the mouth of the sample collection tube upon completion of sampling (Fig. 1). The lid was held open during deployment and sample collection with a coated high-powered neodymium magnet (the exterior magnet) that adhered to the outside of the PVC waterproof housing unit containing a second high power magnet (the anchor magnet). Initial Arduino programing set the door opening mechanism by initiating the forward rotation of the gear motor to push the anchor magnet close to the inside wall of the PVC housing. This rotation continued until the magnet seated itself against a simple switch, which instructed the gear motor to cease turning. The exterior magnet attached to the plankton pump door was then strongly attracted to the outside of the PVC housing unit due to the anchor magnet. After sample collection was complete, a motorized threaded rod slowly pulled the anchor magnet away from the exterior magnet, causing the exterior magnet to release and snap the Van Dorn lid closed. The threaded rod system was composed of commercially available hardware (threaded rod and stainless-steel nuts), a 12 V brushed DC gear motor and the 3D printed frame for the pulley system. The gear motor was controlled by a DC motor driver carrier, which was powered by the NiMH batteries.

The protective, deployment/retrieval and landing gear consisted of a plastic PVC pipe, a welded steel base, line and floats (Fig. 2). The majority of the sample collection tube was enclosed in a PVC pipe (25.4 cm diameter) to protect it from potential damage during deployment and retrieval. Metal bolts at one end of this protective pipe prevented the collection tube from exiting out the back, while quick-release clips and cable ties were used to secure the sampling tube at the front of the protective pipe. The electronic waterproof housing units described above were attached to the top of the protective pipe using threaded bolts and rubber clamps. The welded steel base weighed ~27 kg in air and provided a stable platform

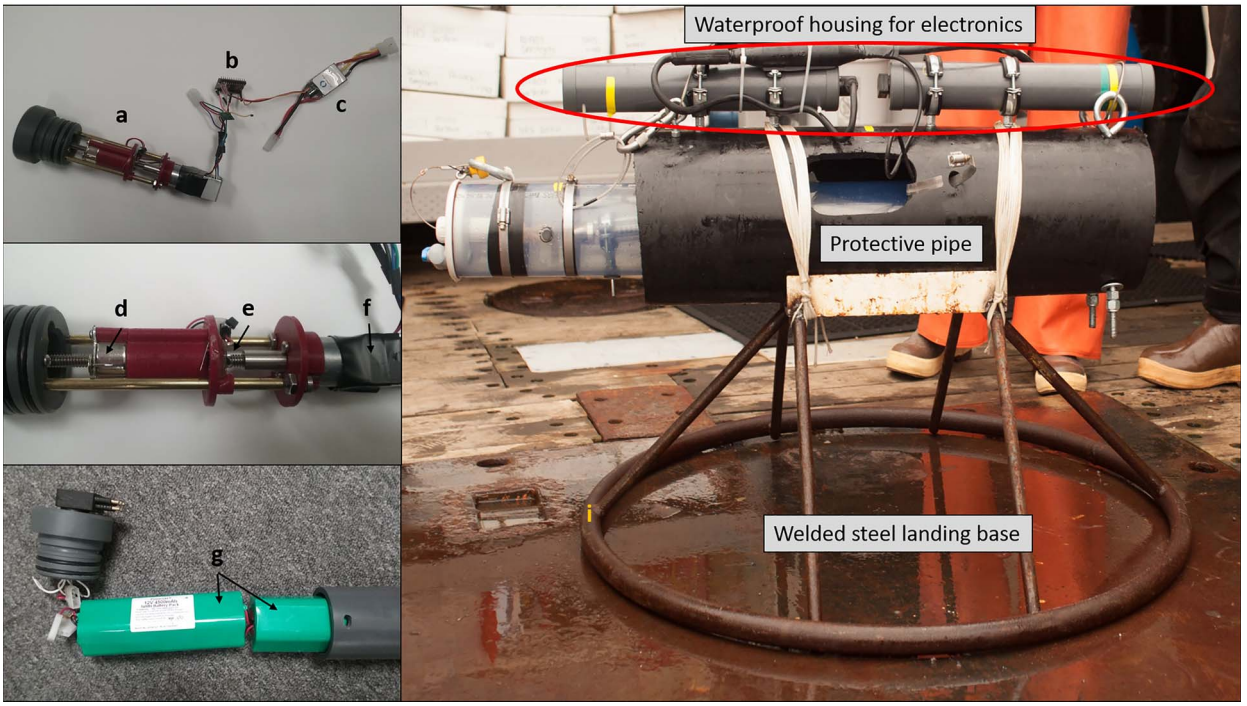

Fig. 2. Autonomous plankton pump system assembled. (**a**) Magnet retractor mechanism, (**b**) Arduino microcontroller, (**c**) Blue Robotics thruster control, (**d**) neodymium magnet, (**e**) motorized threaded rod, (**f**) Gear motor and (**g**) 9V NiMH batteries.

for anchoring the unit near the seafloor. Deployment and retrieval line consisted of 150 m of 15.9 mm floating polyline with two buoys attached at the top that were used to locate and retrieve the unit.

A digital camera (Canon Powershot 300 HS point and shoot camera) in a DELRIN plastic underwater housing was attached to the side of the plankton pump protective housing. This camera was not integrated into the electronic programming of the plankton pump system. The camera was controlled by installing an alternative firmware [Canon Hackers Development Kit (CHDK)], an open source software project. The CHDK firmware allowed control scripts to set delay intervals between image captures.

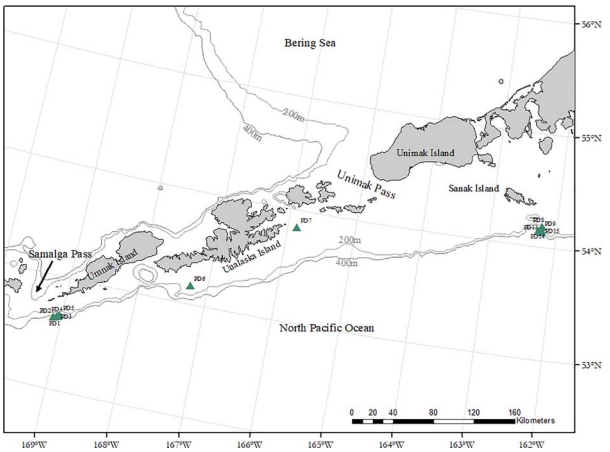

Fig. 3. Sampling stations ($n = 15$) from the North Pacific side of the eastern Aleutian Islands from Samalga Pass to Sanak Island.

Deployment and retrieval

The plankton pump was autonomous and could operate completely separate from the research vessel. It was deployed during daylight hours only in the western Gulf of Alaska (GOA) between the Shumagin Islands (~158°W) and Samalga Pass (−170°W). This region has a relatively narrow and shallow (<200 m) continental shelf that extends ~20 to ~125 km from the Alaska mainland. The dominant current along the shelf is the Alaska Stream, which flows from east to west (Stabeno *et al.*, 2004, 2005). Tidal currents are important features, especially in the passes of the eastern Aleutian Islands where velocities can be in excess of 4.0×10^6 m^3 s^{-1} (Stabeno *et al.*, 2005). During this study, 15 locations were sampled from 28 May to 8 June 2017 (Fig. 3). These locations were identified from previous studies (NOAA, 2015; Rooper *et al.*, 2007) as locations likely to have deep-sea and surface current

Table II: Station environmental data

Sampling Stations	Depth (m)	Latitude	Longitude	Surface current speed (kts)
Station 2	123	52 38.6	169 07.4	1.2
Station 4	120	52 39.8	169 03.8	1.7
Station 7	88	53 51.7	165 52.5	0.0
Station 8	80	54 11.8	162 13.6	0.5
Station 9	98	54 09.5	162 14.0	0.5
Station 10	92	54 09.3	162 16.3	0.8
Station 11	94	54 09.8	162 14.2	0.5
Station 12	105	54 08.7	162 13.4	0.5
Station 13	105	54 08.7	162 13.3	0.5
Station 14	93	54 09.7	162 13.8	0.5
Station 15	90	54 09.7	162 13.8	0.5

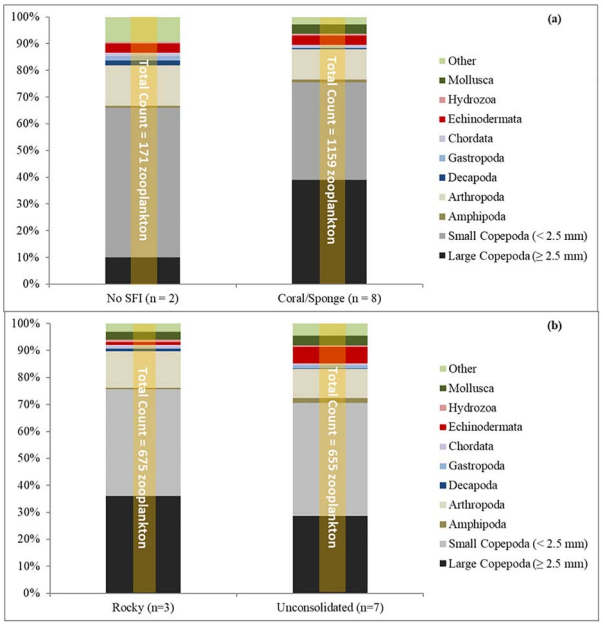

Fig. 4. Species composition among (**a**) habitat types (no SFI and coral/sponge) and by (**b**) substrate types (rocky and unconsolidated).

speed derived from ship logs (Table II). Initial deployments encountered some equipment and deployment failures that were addressed, resulting in a final sample size of eight successful deployments. Pump speed was also adjusted throughout the sampling process to increase sample collection volumes.

At deployment, the plankton pump system was lowered to the seafloor (with the lid open) using a polyline with surface buoys and a two-point bridle system at the attachment point on the plankton pump. The plankton pump was lowered at an angle by using different-sized bridle lengths at the attachment points so that the sample collection tube opening was angled toward the surface and water allowed to pass through from back to front. During deployment, the plankton pump thruster ran in reverse, expelling water through the front of the collection tube for 15 min during descent and after first settlement on the seafloor. Expelling water out the front of the sample collection tube eliminated any plankton collection during descent. The deployment process (lowering the plankton pump system to the seafloor and releasing the buoys) took <10 min from the time the unit was initially powered to the time the plankton pump was resting untethered from the surface vessel on the seafloor. To further evacuate any contents of the sampling tube, the thruster ran at high speed in reverse for 1 min once settled on the seafloor. The pump system then rested for 1 min allowing surrounding fauna to settle, at which point the thruster began filtering water through the plankton net, collecting a sample for 15 min. At 12 min after the sample collection was initiated, the magnet gear motor began rotating the threaded rod and retracting the anchor magnet to initiate the lid release. The gear motor retracted the anchor magnet for a set number of rotations (equaling ~6 cm). This ensured that the magnet released and the door closure occurred just prior to the thruster shutting off. The timing of the lid closure eliminated any potential contamination during ascent and prevented any captured organisms from escaping. The plankton pump Arduino program halted all activity at this point. The code for the full program can be found online (https://github.com/rooperc4/Plankton-Pump). Once the lid was sealed and the plankton sampling was completed, the camera began collecting images at a rate of one photo every 7–15 s for 10 min. The pump system was then retrieved by capturing the surface buoy and winding in the polyline using a hydraulic crab block of the research vessel. The retrieval time using this method was typically <5 min once the buoy was captured, meaning the entire deployment and retrieval cycle could be completed in about an hour (our target sampling time). The flowmeter revolutions during deployment were recorded and samples were extracted and preserved in plastic containers with a 95% ethanol/glycerol solution.

Fig. 5. Images from camera from left to right. Primnoid corals and small demosponge, *Hippoglossus stenolepis* (Pacific halibut), *Sebastes* sp. (rockfish), juvenile *S. ruberrimus* (yelloweye rockfish) next to boulder and *Bathymaster signatus* (searcher).

Sample and image processing

The collected samples were processed using a dissecting microscope to identify preserved zooplankton and ichthyoplankton. Zooplanktons were identified to family level. Copepods were separated by size into large (>2.5 mm) and small (≤2.5 mm) categories. Fish larvae were identified to the lowest taxonomic level possible and then genetically verified (I. Spies, Alaska Fisheries Science Center, National Marine Fisheries Service, unpublished data). The number of individuals in taxonomic group was counted for each individual sample. The density of each taxon was standardized among samples using the volume of water filtered during the deployment.

$$\text{Density} = \frac{n}{(3.14159 \times (\text{Net Mouth Radius})^2) \times \text{Flowmeter Distance}}$$

The camera images were reviewed for the presence of SFIs (i.e. coral and/or sponge). The presence of a single sponge or coral in the field of view of the camera resulted in classification as SFI present, and absence of sponge or coral from the field of view of the camera resulted in a classification of SFI absent. The substrate type at each location was classified as either rocky or unconsolidated from the images. Rocky substrates were those containing exposed bedrock or boulders (estimated at diameter > 25.5 cm; Wentworth, 1922). Unconsolidated substrates were those containing only sandy, muddy or cobble substrates (estimated at diameter < 25.5 cm; Wentworth, 1922). Fishes in the images were counted and identified to species. A summary of fish and substrate types with example images can be found in the supplementary information.

RESULTS

Stations and habitats sampled

As this was a pilot study using novel equipment and deployment techniques, there were some adjustments made in the field. So, of the 15 stations sampled, the initial seven were used to fine-tune the plankton pump settings. The changes made over these test deployments included changing the angle of the bridle to point the unit upwards 30° during retrieval. This was done so that the water pressure from ascending through the water column assisted in keeping the lid tight to the collection tube, thus eliminating the potential for animals to escape during retrieval. In addition, we modified the speed of the thruster in the field. Initially, the speed of the thruster was tested in a closed system tank at the Alaska Fisheries Science Center to determine flow rates that would be appropriate for catching larval fish (e.g. faster rates than larval fish were expected to swim or > ~2 cm s^{-1}); however, we found that in the open waters of the GOA, *in situ* benthic currents required a much faster thruster speed to overcome bottom current strength. In the field, the thruster performed well at slightly less than full speed, and extremely well at full speed (~60 cm s^{-1}).

Data from 15 stations were collected at depths of 80 to 105 m; however, some collection modifications were necessary *in situ* and in three cases, there was an issue with camera image collection. Of the 15 sampled stations, 10 were able to be image analyzed for benthic habitat, substrate type and associated adult fishes (Supplementary Table A1). Two stations contained no identifiable SFI, while eight samples were collected in and among corals and/or sponges. Six stations had sponges present and two sites had corals present. The

Table III: List of zooplankton organisms identified from 11 sampling stations

	Sample stations											
	S2	S4	S7	S8	S9	S10	S11	S12	S13	S14	S15	Total
Amphipods												
Gammaridea	1	0	1	0	1	0	0	0	1	1	0	5
Hyperiidea	0	0	0	0	1	0	0	9	1	2	0	13
Arthropoda												
Barnacle Cyprid	1	1	24	0	49	14	2	15	7	4	10	127
Cumacean	0	0	0	0	0	0	0	14	2	3	0	19
Mysid	0	0	0	0	0	0	0	0	0	0	1	1
Euphausiid	0	1	0	0	0	0	0	0	0	1	0	2
Calyptopis												
Euphausiid	0	0	0	1	0	2	1	0	0	57	2	63
Furcilla												
Chordata												
Larvacean	0	0	2	0	2	0	2	1	0	2	4	13
Sebastes sp.	0	0	0	0	0	0	0	0	0	1	0	1
Copepoda												
Copepods (<2.5 mm)	3	15	81	1	26	25	7	34	66	179	130	567
Copepods (≥2.5 mm)	0	14	3	0	37	14	8	20	44	232	96	468
Decapoda												
Crab Zoea	0	0	1	0	0	0	0	0	0	0	0	1
Shrimp	0	0	2	0	0	0	0	1	0	3	0	6
Echinodermata												
Echinoderm Juvenile	1	1	4	1	0	1	1	21	3	1	3	37
Echinoderm Pluteus	1	0	1	0	0	1	0	1	0	1	6	11
Gastropoda												
Gastropods	3	1	2	0	0	1	0	0	0	0	2	9
Hydrozoa												
Hydroid Polyp	0	0	0	0	0	0	0	1	1	0	0	2
Hydrozoan Jelly	0	0	1	0	0	1	0	1	0	5	0	8
Mollusca												
Pteropod	0	0	0	0	0	0	0	20	1	19	3	43
Other												
Chaetognath	0	0	1	0	1	1	0	2	0	2	4	11
Bryozoan Colony	0	0	2	0	0	0	0	0	2	1	1	6
Bryozoan	0	0	0	0	0	0	0	1	0	2	0	3
Cyphonaut												
Polychaete	0	2	0	0	2	4	1	9	0	1	1	20
Unidentified Invertebrate	0	0	11	0	0	0	0	0	1	0	1	13

remaining two sites had no SFI present. In addition, the 10 stations were almost evenly divided between substrate types of rocky ($n = 4$) and unconsolidated ($n = 6$) based on sediment grain size. Eight disparate species of fish were identified in view of the camera, but rockfish (*Sebastes* spp.) and particularly northern rockfish (*Sebastes polyspinis*) were the most commonly identified fish (Supplementary Table A1).

Zooplankton sample collection

A summary list of the zooplankton collected at 11 of the stations is available in Table III. The mean abundance for all invertebrates was 133.1 ± 45.2 individuals per station, with a total of 1528 individuals collected. A single larval northern rockfish (*S. polyspinis*) measuring 3.38 mm total length and weighing 0.20 g was collected in rocky, coral habitat. For the four stations where flowmeter measurements were available, the total volume of water filtered ranged from 4.35 to 9.71 m^3 (Table IV). Total zooplankton densities at these stations ranged from ~31 to 53 individuals m^{-3} (Table IV).

Copepods were the dominant organisms, accounting for 71% of all identified zooplankton in comprising the bulk of all habitat and substrate types, but had the largest numbers in coral/sponge and/or rocky habitats (Fig. 4). Euphausiids were almost 8 times more abundant in coral/sponge habitats, although they were completely absent from habitat with no SFI. Conversely, barnacles were ~2 times more abundant in habitat with no SFI. Unfortunately, sample sizes were not adequate to apply statistical tests determining significant differences.

Table IV: Summary of flowmeter outputs, zooplankton counts and densities for 11 sampling stations; flowmeter volume is based on 15 min of filtering seawater

Sampling stations	Flowmeter speed (cm s^{-1})	Flowmeter volume (m^3)	Zooplankton counts	Ind./m^3
Station 2	–	–	10	–
Station 4	–	–	35	–
Station 7	–	–	136	–
Station 8	–	–	3	–
Station 9	–	–	119	–
Station 10	–	–	64	–
Station 11	–	–	22	–
Station 12	63.46	4.67	150	33.40
Station 13	59.11	4.35	129	30.11
Station 14	131.97	9.71	517	53.35
Station 15	89.02	6.55	264	40.61

DISCUSSION

Isolating sample collections to within 1 m of the seafloor or an SFI (i.e. sponge or coral) can be difficult, particularly in deep-sea environments, yet planktonic communities associated with the seafloor are likely different from mid- and surface-water communities. In fact, Mullineaux *et al.* (2005, 2013) found greater larval abundances within 5 m of the seafloor than 50 m above the seafloor. We tried to design a plankton pump to overcome some of these hurdles in order to sample near-bottom habitats in a non-invasive way. For example, the plankton pump described here remains stationary, rests within 1 m of the seafloor and captures images of the surrounding habitat and substrate. The current design is highly portable, lightweight, easily deployable from a wide array of platforms and capable of filtering water volumes of ∼100 m^3 h^{-1} (in lab tests with no conflicting water currents). To put that into perspective, Beaulieu *et al.* (2009) filtered water volumes of ∼1.7 m^3 h^{-1} using a 3 cm diameter sampler, while the autonomous underwater vehicle sampler used in Billings *et al.*, 2017 was capable of sampling water volumes of 6000 m^3 h^{-1} at 5 m off the seafloor. No zooplankton count data were provided in Billings *et al.* (2017), but average zooplankton sample sizes ($n = 1104$ h^{-1}) collected during this study were almost 3 times larger than those collected by the Beaulieu *et al.* (2009) plankton sampler ($n = 392$ h^{-1}). Neither of these units was able to collect samples within 1 m of the seafloor.

Commercially available plankton pumps have been applied to plankton research in rugged and remote locations such as under sea-ice in Antarctica (Winslow *et al.*, 2014) or the Chukchi Sea in the Arctic (Lalande *et al.*, 2007) or deep ocean seeps (Billings *et al.* (2017). All these methodologies were highly effective at collecting samples, but each was costly to make, purchase and/or implement (∼$20 000–$100 000). In addition, these sampling strategies collected samples well above the height of most benthic structures, or were not stationary. Our autonomous plankton pump design successfully collected *in situ* zooplankton from near-bottom communities in the western GOA, and it performed well at depths in excess of 100 m. It is unique in its self-contained ability to collect data without surface input or attachment. The plankton pump design and costs were relatively inexpensive (∼$1500 per unit) when compared to what is currently commercially available. The low cost of construction makes this design highly replicable and encourages multiple plankton pump deployments simultaneously in a variety of locations. In addition, the benthic habitat was reliably categorized from the camera images, and all associated images were successfully processed for habitat, substrate and fish identification Fig. 5.

Specimen quality was excellent with ∼99% zooplankton collected undamaged. Species collected represented a wide array of mobile capabilities, including highly mobile larval fish and chaetognaths, indicating that the pump strength was sufficient for collecting a representative sample of the benthic community within 1 m of the seafloor. There have been no published studies of the near-bottom zooplankton communities in the northwestern GOA, but our results were similar to other surface and mid-water tow studies in the central and western GOA, which indicated a dominance of copepods in the region (Pinchuk and Coyle, 2003, 2005; Wang, 2007; Sousa *et al.*, 2016; Kimmel *et al.*, 2017; D. Kimmel, Alaska Fisheries Science Center, Unpublished results). Future improvements in design and a minor increase in costs would increase the likelihood of greater deployments of this plankton pump design and greatly improve site-specific understanding of benthic zooplankton and ichthyoplankton communities.

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