A multi-scale analysis of habitat-mediated megafaunal invertebrate distribution at two locations in the Monterey Bay National Marine Sanctuary

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Abstract:

Effective marine policy depends on the timely dissemination of research results, informed management agencies, and a knowledgeable public community. However, resource managers frequently lack important information on the locations, resources, and ecological processes in the areas they manage. The inherent patchiness of marine systems impacts the distribution of these resources, requiring detailed research results to be provided to managers on the distribution of taxa and habitats. The reality of scientific analysis often prolongs the time between data collection and dissemination. Using video records, the analysis of data at multiple scales can be conducted to determine if data collected “on-the-fly” adequately records taxa abundance and distribution. This study analyzed towed camera sled video collected at two study sites within the Monterey Bay National Marine Sanctuary (MBNMS) at two sampling scales to determine the utility of data collected at a coarser sampling scale in characterizing the distribution and habitat associations of megafaunal invertebrates. The two approaches to the collection of data from that videographic imagery were a frame-by-frame approach for fine-scale research questions and data collected in real-time at one-minute intervals provided to management agencies. Taxa-habitat associations were compared at these multiple sampling schemes. The one-minute sampling scale was able to record similar taxa-habitat associations as the frame-by-frame approach, but does not adequately record taxon presence within individual transects or differences in taxa-habitat associations between study sites. The frame-by-frame approach collects fine-scale data on taxa abundance and taxa-habitat associations, but is time consuming to analyze. Sampling scale may not be a factor in recording taxa-habitat associations; however, a fine-scale sampling scale is required to determine taxa abundance and overall distribution. On-the-fly sampling techniques are adequate to estimate taxa-habitat associations and allow results to be disseminated to management agencies much more rapidly. This study demonstrates that sampling scale in videographic analysis is important and the extent to which it represents the species within the Sanctuary needs to be understood.

Introduction:

Effective marine policy depends on the timely dissemination of robust research results, informed management agencies, and a knowledgeable, supportive public community (Robison 1993; Brody 1998; Morin 2001; Chandler and Gillelan 2005; Auster et al. 2008; MBNMS 2008). However, resource managers frequently lack important information on many locations, resources, and ecological processes in the areas they manage, even where a great deal of science and monitoring has been conducted (MBNMS 2008). This makes it important for scientists to provide managers with adequate and accurate information on these areas as quickly as possible. Conversely, the reality of scientific data processing and analysis often prolongs the time between data collection and dissemination, which in turn inhibits the rate at which scientific results become available to management agencies. The implications of delayed reporting of scientific data are particularly acute where management agencies and policy-makers are anticipating the arrival of information to support environmental decision-making (Stafford et al. 1994). Such implications make it important to have detailed results available to resource managers virtually immediately post-cruise (Stafford et al. 1994).

Populations and sizes of commercial fisheries species, such as rockfish (*Sebastes* spp., Scorpaenidae), have been declining for years along the central coast of California (Mason 1998; Starr et al. 2002). The decline in groundfish populations along the west coast has resulted in research and management actions focusing on habitat-based strategies (Miller et al. 2008; PFMC 2008; Tissot et al. 2008). With ecosystem-based management gaining momentum it is important to clearly define habitats supporting existing groundfish populations in order to provide proper management for the recovery of commercial fish stocks (Rosenberg et al. 2000; Pirtle 2005). Part of the focus is on megafaunal invertebrates as living components of habitat and their ecological relationships with groundfish (Pirtle 2005).

Survivorship of commercially important species has been linked to habitat complexity (Auster et al. 1996, Auster 1998, Lindholm et al. 1999, 2001). Large, complex, or densely aggregated invertebrates augment the three dimensional relief of substrates, providing shelter, feeding, and spawning locations for fishes (Carlson and Straty 1981; Auster 1997; Lindholm et al. 2001; Auster et al. 2003; Pirtle 2005; Tissot 2006; Tissot et al. 2007). These megafaunal invertebrates are important habitat features for many commercial fish species with the additional habitat they provide acting as nursery grounds for juveniles or as refuge from predators (Carlson and Straty 1981; Auster 1997; Lindholm et al. 2001; Auster et al. 2003; Pirtle 2005; Tissot et al. 2007). Furthermore, many megafaunal invertebrate species tend to be long-lived and ill-adapted to frequent disturbances such as those often caused by bottom-contact fishing practices (Auster et al. 1996, Watling and Norse 1998; Dayton et al. 2000; Pirtle 2005). Many megafaunal invertebrates' specific habitat requirements and functional role as habitat for fish species are not well understood, making it important to clearly define their distribution (Langton et al. 1995; Pirtle 2005).

Deep-sea communities are characterized by the patchiness of the benthos (Cosson et al. 1996). Additionally, many spatial scales are not known before sampling is conducted which can result in a high variation within sites (Morrisey et al. 1992). Morrisey et al. (1992) found that the abundance of infauna on soft sediments varied spatially with sampling size, which can have important consequences on habitat studies of organisms. Ellingsen (2001) found that pattern and variability are likely to change with scale and the measurement of marine biodiversity may therefore be dependent on spatial scale. The Monterey Bay contains a variety of habitat types with these diverse substrates supporting diverse species assemblages (Stein et al. 1992; Love and Yoklavich 2006). The inherent patchiness found in the marine environment makes it important to test multiple scales for sampling species and habitats when extrapolating results into a larger context and when providing the results to management agencies. By determining if sampling at a larger spatial scale adequately captures species distributions and habitat associations, less time would be required on analysis allowing results to be disseminated quickly to resource managers.

One methodology for assessing marine habitats is using video platforms such as towed camera sleds, remotely operated vehicles (ROVs) and human occupied submersibles (Carlson and Straty 1981; Pirtle 2005; Tissot et al. 2007; Tissot 2008; Laidig et al. 2009). Despite time needed for analysis, videographic imagery is ideal for studies on distribution and habitat associations, because video records allow researchers to view fish and invertebrate species in their natural habitats as well as preserve a permanent record of the study site (Spencer et al. 2005; Tissot 2008). By sampling areas “on-the-fly,” collecting *in situ* video observations, researchers can gain an overview of the area and associated resources without requiring further analysis time. However, the inherent patchiness of marine ecosystems can pose problems, possibly skewing the data since samples are generally taken at larger spatial scales than species occur.

The overall goal of this study was to review two difference sampling schemes and determine the utility of a coarser sampling scale at recording taxa distributions at two study areas in the Monterey Bay National Marine Sanctuary (MBNMS). The objectives of this study were to: 1) characterize the distribution and habitat associations of sessile, megafaunal invertebrates at two locations in the MBNMS using a frame-by-frame analysis; 2) quantify any differences in megafaunal invertebrate distribution and habitat associations between study sites; and 3) analyze the data collected at one-minute intervals in the field to evaluate the utility of real-time field data collection for quantifying megafaunal invertebrate distribution and habitat associations. By determining if data collected "on-the-fly" record similar taxa distributions to the finer-scale sampling collected in the lab, my results will inform the on-going discussion about the utility of collecting such data and their appropriateness for management. It is important to understand the impact of different sampling scales in recording taxa distributions, especially when extrapolating the results to habitat-based management approaches. This type of information will benefit ecosystem-based management approaches by incorporating habitat requirements of biophysical structures utilized by commercially important species and by determining the utility of data collected in real-time in recording these resources.

Methods:

Study Sites

Research cruises were conducted in 2006, 2007 and 2008 aboard the NOAA Research Vessel *Fulmar* off of Point Lobos and adjacent to Point Sur along the central California coast (Figure 1). These study areas, both of which are within the MBNMS, are relatively close geographically and share similar habitat types (Yoklavich and Starr 2008). Both study areas also include newly implemented marine protected areas (MPAs); the Point Lobos State Marine Conservation Area, Point Lobos State Marine

Reserve, Point Sur State Marine Conservation Area and Point Sur State Marine Reserve. Twelve predetermined transect lines were used for analysis; six from Point Sur and six from Point Lobos. Transects were selected randomly based on their depth range (60-150 meters) to encompass the depth range of all sessile invertebrates included in analysis.

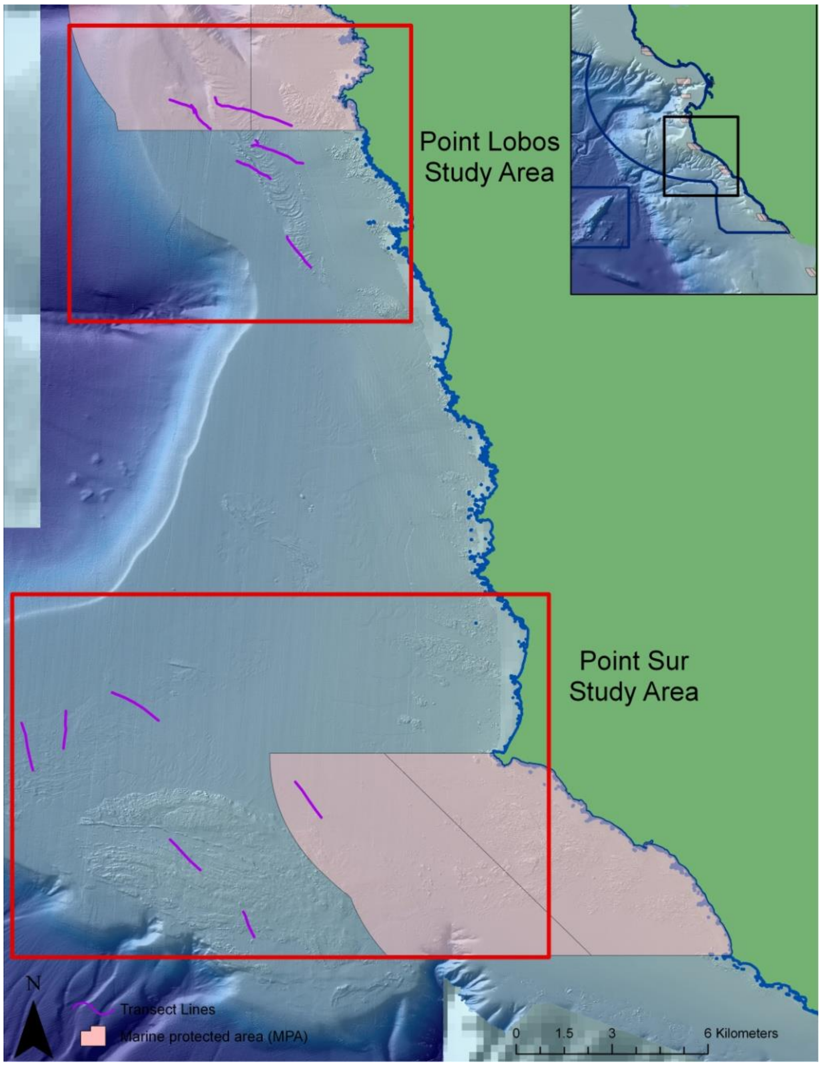


Figure 1: Map of the transect lines collected in the Point Lobos and Point Sur study areas.

Towed Camera Sled

Videographic imagery was collected with a towed camera sled (Figure 2). The camera sled is comprised of a single forward-facing high resolution video camera mounted at an angle, paired sizing lasers spaced at 10 cm (for organism sizing), two high-powered quartz halogen lights, and a computer that determines the camera depth and altitude above the seafloor. These components are protected by a sturdy aluminum frame and powered by a 250 meter umbilical tether attached to the R/V *Fulmar* that streamed live video to the scientists in the dry lab on board. The camera sled was flown at a mean altitude of 1 meter above the seafloor at a speed of approximately 1 knot (0.514 meters per second). Altitude and speed were adjusted based on conditions. The inconsistent altitude and speed of the camera sled limited the data collection to coarse quantitative sampling at-sea, where scientists assessed the presence of macrofauna (Anglin and de Marignac 2008,).

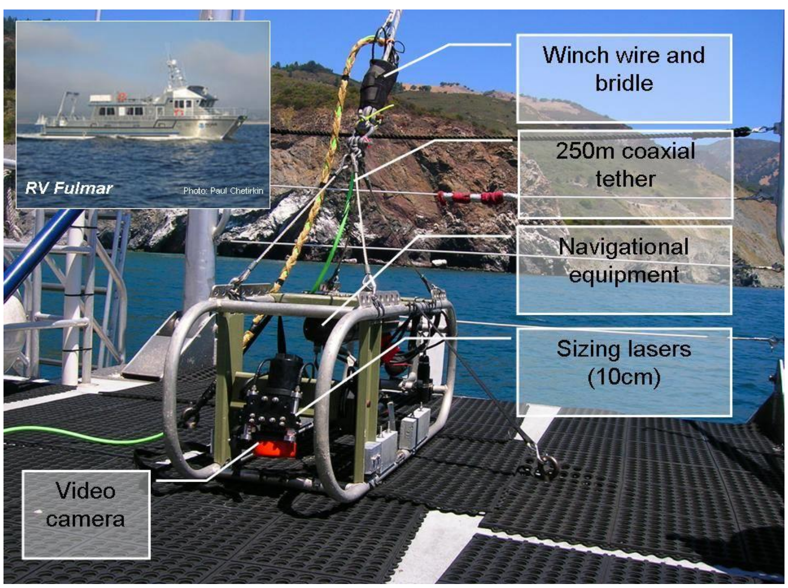


Figure 2: Image of the towed camera sled aboard the R/V *Fulmar* (Photo By: Ashley Knight).

"Real-Time" Data Collection

Anglin and de Marignac (2008) developed the protocols for the real-time data collection in the field. By using the X-Keys TM programmable keyboard (PI Engineering, Williamston, MI) at-sea, a coarse set of data were collected "on-the-fly" in real-time at one-minute intervals to provide summary data virtually immediately post-cruise (Figure 3). The X- Keys ^{TM} system is a keyboard where, instead of letters, the keys are programmed for species and habitats observed. This allows species and habitats to be recorded rapidly, without requiring the operator to type out each individual species observed. This keyboard was used in the field for data collection of observed species and habitat types. In the field, a scientific observer observed the live streaming video in the dry lab via the deck monitor. Data were collected in a presence/absence format and allowed for rapid real-time collection of observed features, taxa presence, and habitat types during seafloor video transects. Samples were collected during the first twenty seconds of every minute while an observer called out the presence of each taxa observed, the primary and secondary habitat type, and associated primary and secondary habitat relief to the X-Keys^{TM} operator. The X-Keys^{TM} operator entered the taxa and habitat observed by the scientific observer using the X-Keys^{TM} keyboard, which included a total of 129 keys encompassing fish, invertebrates and seafloor habitats and associated relief.

Figure 3: The X-Keys^{TM} system used for data collection at-sea programmed with fish and invertebrate species and habitat categories and associated relief.

There were seven different categories of substratum used to characterize habitat by estimated grain size (Table 1 & Figure 4) (Greene 1999). Hard substrates have relief codes based on the vertical relief of the physical substrate off the seafloor including: low (<1 meter), moderate (1-5 meters), high (>5 meters) and rock wall (a vertical wall of outcropping bedrock). Soft sediment habitats have separate relief codes including: flat (0-5cm), ripple (10-60cm), wave (>60cm) and bioturbated (sediments that have >50% surface area of biological disturbances). Primary habitat consists of the habitat type that was observed >50% of the observation period. The secondary habitat is the habitat observed >20% of the observation period. This process for classifying habitats is commonly used for habitat characterization and is based on the procedure described in Hixon et al. (1991), Stein et al. (1992) and Greene et al. (1999) (Tissot 2008).

Table 1: Habitat categories used to characterize habitat by decreasing particle size.

Habitat type	**Grain size**
Bedrock	>3.0 m
Boulder	0.25-3.0 m
Cobble	64-256 mm
Pebble	2-64 mm
Gravel	24 mm
Sand	0.06-2 mm
Mud	< 0.06 mm

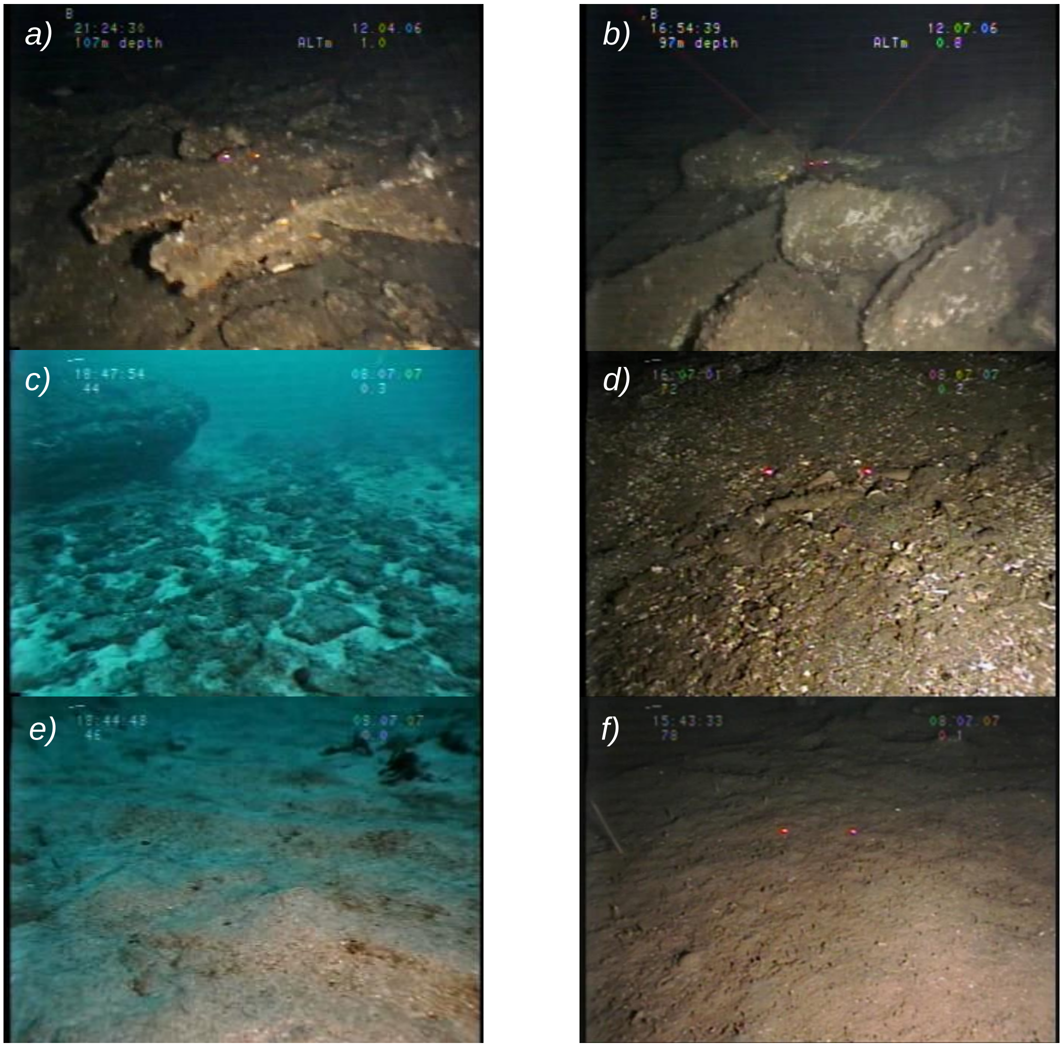


Figure 4: Representative habitats taken by the towed camera sled by decreasing particle size: a) rock; b) boulder; c) cobble; d) pebble/gravel; e) sand; and f) mud.

Frame-by-Frame Data Collection

Videographic data taken from the same transects were analyzed post-cruise on a frame-by-frame basis noting sessile invertebrate taxa and habitat type during the twelve selected transects. Data were entered using the X-Keys ™ programmable keyboard in a format similar to the real-time data collection. However, the frame-by-frame sampling scheme treated the video as a series of non-overlapping quadrats (Figure 5). Each frame was determined by the paired 10cm sizing lasers to ensure that each frame was the same size. Within each frame all sessile invertebrates were counted, except for brachiopods (which were recorded in a presence/absence format due to high aggregations), and entered into a spreadsheet using the programmable keyboard.

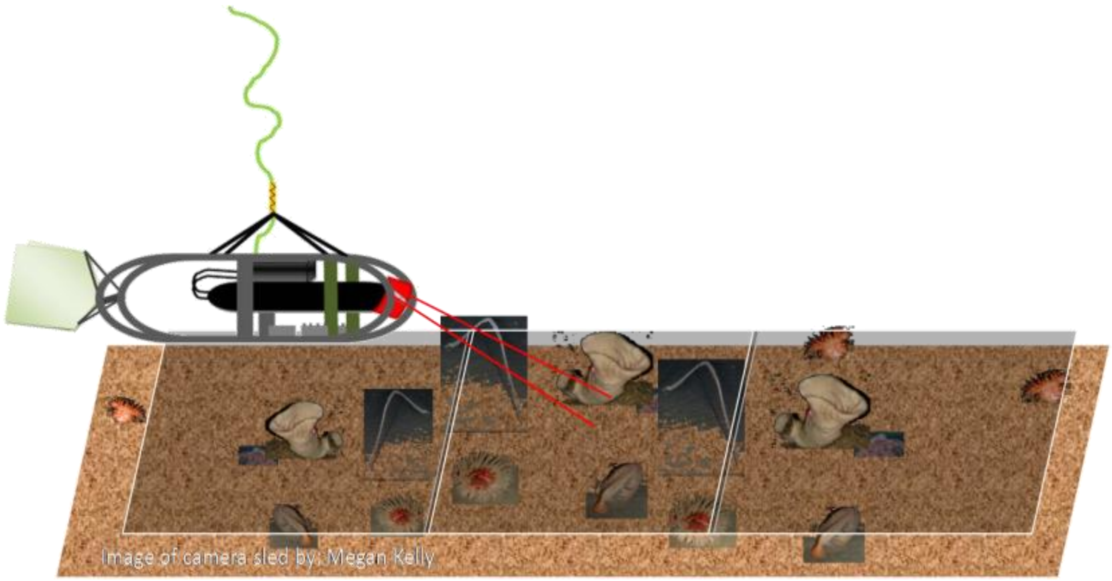


Figure 5: Animation of collecting data at a frame-by-frame scale using the 10cm sizing lasers as frame size reference.

Sessile invertebrates included in this analysis were; Phylum Porifera (sponges (not encrusting/extending beyond the substrate)), Class Ascidiacea (tunicates), Class Anthozoa (solitary anemones), *Metridium spp.* (white-plumed anemones), *Pachycerianthus fimbriatus* (tube anemone) *Lophogorgia chilensis* (red gorgonian), Order Gorgonacea (seawhips), Order Pennatulacea (sea pens), and Phylum Brachiopoda (brachiopods) (Figure 6). Some groups were only identified at the phylum or subphylum level (Poriferans and Brachiopods), Anthozoan cnidarians were identified at the subclass level or below, and gorgonians were identified to order or species.

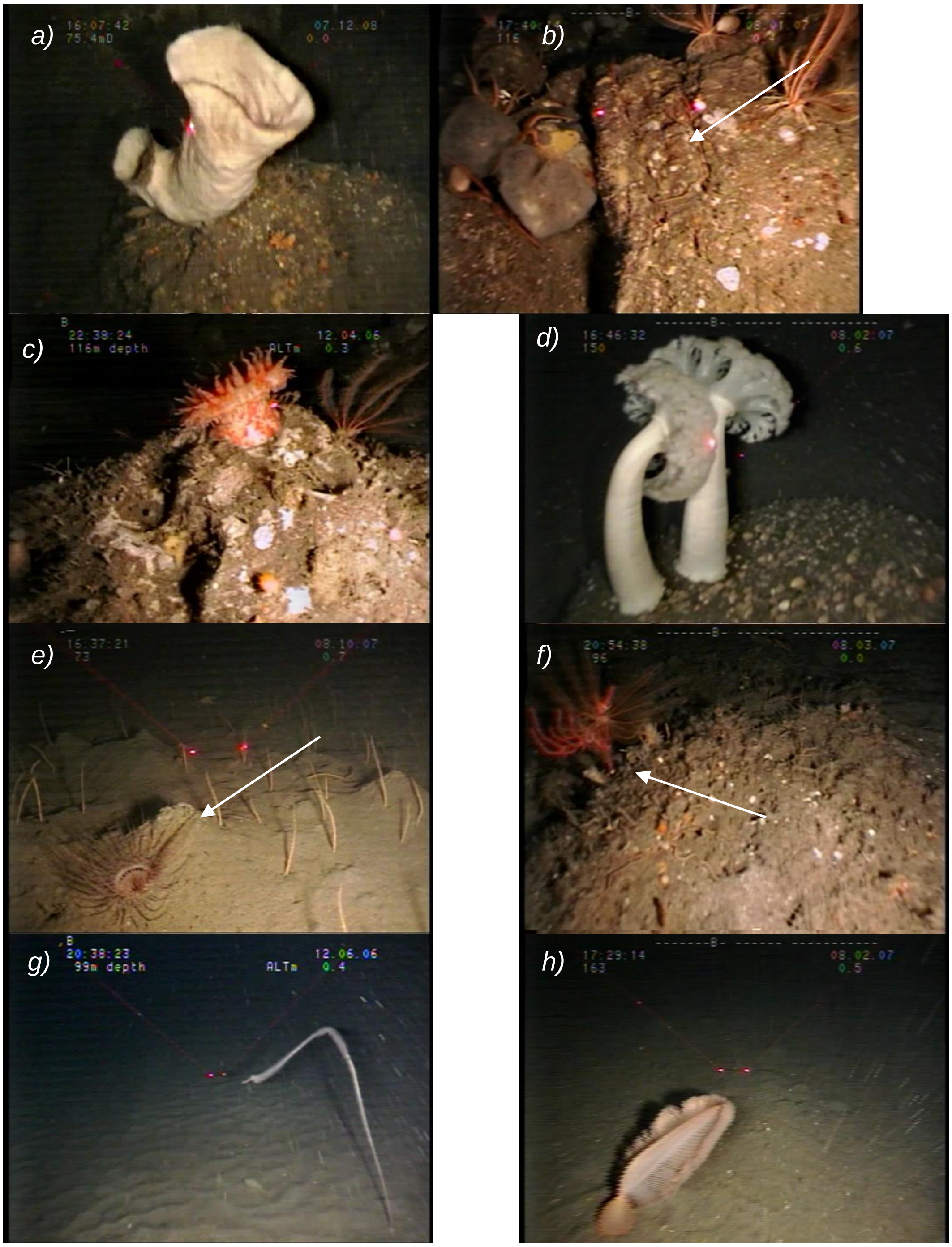

a)
b)
c)
d)
e)
f)
g)
h)

Figure 6: Representative structure-forming invertebrates in Monterey Bay, CA: a) sponge; b) tunicate; c)anemone; d) *Metridium*; e) tube anemone; f) gorgonian; g) seawhip; h) sea pen; and i) brachiopods.

After concluding the frame-by-frame analysis for each selected transect, an accurate count of all sessile invertebrate taxa observed as well as habitat type was available. Thus allowing for the taxa distribution and habitat associations to be analyzed and compared to the data collected in the field at one-minute intervals as well as to know what taxa were within each transect, how many, and where they were located.

Data Analyses

The crux of this study was to analyze two sampling scales in recording taxa distribution, abundance and habitat associations. First, was to address the differences between the two sampling scales at recording taxa-habitat associations . Second, was to analyze if there was a difference in the ability of the coarser sampling scale in recording differences in taxa abundance and habitat associations between the two study sites. Lastly, was to determine the ability of the coarser sampling scale to record similar taxa accumulation within individual transects sampled.

Differences in taxa-habitat associations between the frame-by-frame and one-minute sampling scales:

Taxa-habitat associations were determined by using data sampled at a frame-by-frame scale. A Chi-square goodness of fit test was used to determine if there was any association with habitat for each taxon. If a taxon was neither attracted to nor repelled by a habitat, then the expected proportion of those invertebrates on that habitat would be proportional to the amount of that habitat available in the survey (Love and Yoklavich 2008). The efficacy of the real-time data collection was tested by taking the data collected at-sea at one-minute intervals and conducting a Chi-square goodness of fit test to determine the habitat associations recorded by a coarser sampling scale. The results of the Chi-square test were compared to the habitat associations calculated using the frame-by-frame analysis above to determine the ability of data collected in real-time in capturing the distribution of sessile invertebrates.

Differences in megafaunal invertebrate distribution and habitat associations between study sites at a frame-by-frame and one-minute sampling scale:

The frame-by-frame data was used to determine if there were any differences in distribution of habitats between study sites (Point Sur and Point Lobos) by conducting a one-way ANOVA and graphing the abundance of each habitat type. A one-way ANOVA was also conducted to determine differences in taxa abundance between study sites. A Chi-square goodness of fit test was conducted to determine spatial differences in taxa-habitat associations of sessile invertebrates.

Using the data collected in real-time, a one-way ANOVA was conducted to determine differences in abundance of taxa between study sites. Differences in taxa abundance between sites were compared to those recorded at the frame-by-frame scale to determine if the real-time data recorded the same differences in taxa abundance. A Chi-square goodness of fit was conducted to test differences in habitat associations of each taxon between study sites. The results of the Chi-square test were compared to the habitat associations recorded at a frame-by-frame scale to determine if the one-minute sampling scale recorded the same habitat associations between sites.

Statistically comparing the two sampling scales was not possible due to the fact that the frame-by-frame sampling scale collected total abundance of taxa while the one-minute sampling scale recorded presence. These transects also do not meet the independence assumption. When conducting the one-way ANOVA, if assumptions such as equal variances or normality weren't met, a Kruskal-Wallis was conducted instead. By determining the distribution and habitat associations of the taxa sampled at the separate scales the results could be compared and contrasted to determine similarities in the data recorded between the two scales.

Differences in taxa accumulation between the two sampling scales:

Patterns in taxa accumulation curves were analyzed to determine differences between sampling scales at recording taxa presence and rate of accumulation within individual transects used in analysis. Differences in rate of accumulation and number of taxa recorded were the patterns observed between the two sampling scales.

Results:

Differences in taxa-habitat associations between the frame-by-frame and one-minute sampling scales:

Sponges, brachiopods, red gorgonians and *Metridium spp.* were the most abundant taxa observed occurring primarily over hard substrates (Table 1). Sea pens, seawhips and tube anemones had a low frequency of occurrence and occurred only over soft sediments. Other anemones and tunicates occurred across all habitat types in lower frequencies.

Table 1: Total number of macro-invertebrates in each habitat category observed sampled at a frame-by-frame scale.

	Brachiopod	**Sea pen**	**Seawhip**	**Red gorgonian**	**Tube anemone**	***Metridium***	**Other Anemone**	**Tunicate**	**Sponge 3D**

ROCK	606	0	0	896	0	173	16	14	2451
BOULDER	558	0	0	348	0	7	41	1	1433
COBBLE	545	1	0	41	0	9	27	11	475
GRAVEL	1	0	0	1	0	0	0	3	15
SAND	111	60	86	54	6	5	92	5	238
MUD	261	56	24	2	1	11	25	0	3

Sand was the dominant habitat type in this study, followed by mud and rock habitats (Figure 7). These habitats can be separated into three categories: hard, mixed and soft habitats. Hard substrate habitats supported the highest abundance of taxa; sponges, red gorgonians, tunicates, *Metridium spp.* and brachiopods. Mixed habitats are those that are composed of both hard and soft habitats and are able to support a high diversity of taxa that are associated with both hard and soft habitat types that occur on the interface of habitat patches such as; anemones, sponges, tunicates, *Metridium spp.* and brachiopods. Soft sediment habitats were the most abundant habitat type with half of the total frames observed consisting of soft sediment habitats. Associated taxa were tube anemones, seawhips and sea pens that had very patchy distributions and low abundances.

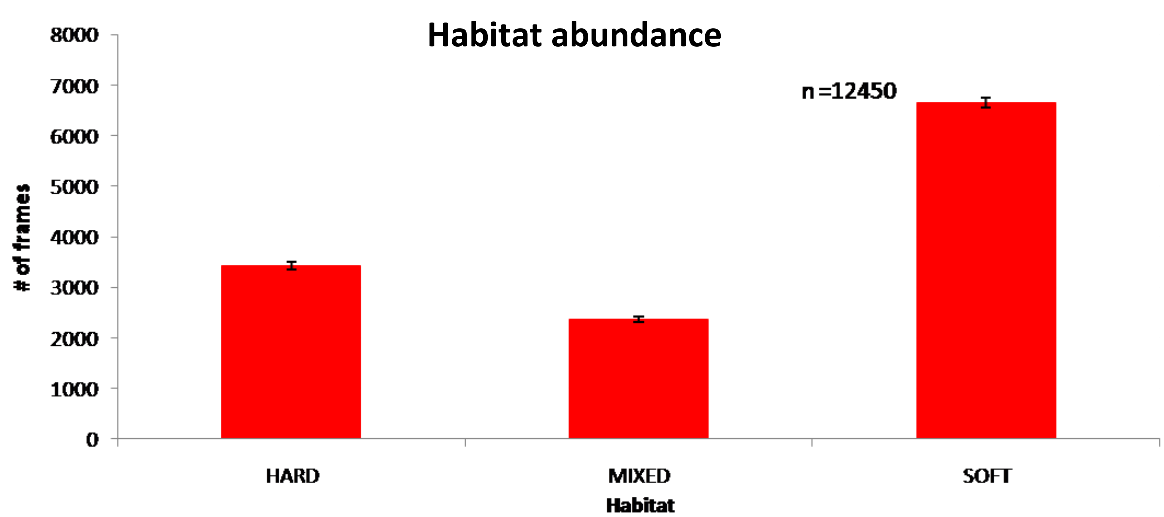


Figure 7: Physical characteristics of the study sites in Monterey Bay by pooled substrate type with standard error bars. Seafloor types are combined by decreasing particle size, where hard substrate in composed of combinations of rock, boulder and cobble; mixed habitats are composed of both hard and soft sediments, and soft sediments are gravel, sand and mud habitats. Total sample size was n = 12450 frames.

There were clear habitat associations observed by almost every megafaunal invertebrate sampled at a frame-by-frame scale (Table 2). Sponges and red gorgonians were associated with rock and boulder habitats and were negatively associated with cobble, gravel, sand and mud habitats. Tunicates were associated with rock and cobble habitats and negatively associated with boulder, gravel and sand habitats. Other anemones and seawhips were associated with sand habitats and negatively associated with rock, cobble and mud habitats. *Metridium spp.* were associated with rock habitats and negatively associated with boulder, cobble, sand and mud habitats. Brachiopods were associated with rock, boulder and cobble habitats and negatively associated with gravel, sand and mud habitats. Sea pens were associated with sand and mud habitats and negatively associated with cobble habitats. Lastly, tube anemones occurred primarily over sand habitats, but were the only taxa that did not have a significant habitat association (χ^2 =3.571, p = 0.059).

Table 2: Habitat associations observed by each megafaunal invertebrate taxa at a frame-by-frame scale (* < 0.05, ** < 0.01, *** < 0.001).

Taxa	Habitat associations
Brachiopod	Rock, boulder, cobble***
Sea pen	Sand, mud***
Seawhip	Sand***
Red gorgonian	Rock, boulder ***
Tube Anemone	N/A
Metridium	Rock***
Anemone	Boulder, sand***
Tunicate	Rock, cobble***
Sponge	Rock, boulder***

The one-minute sampling scale captured the same habitat associations for seawhips, anemones and sponges and similar habitat associations for brachiopods, *Metridium spp.* and red gorgonians. Brachiopods were associated with rock, boulder and mud habitats. Seawhips were associated with sand habitats. Red gorgonians were associated with rock habitats. *Metridium spp.* were associated with rock and sand habitats. Anemones were associated with boulder and sand habitats. Sponges were associated with rock and boulder habitats. Sea pens (χ^2 =1.636, p = 0.201) and tube anemones (χ^2 =3.200, p = 0.202) were not associated with any habitat type. There weren't enough tunicates observed to run the Chi-square goodness of fit test. The habitat associations recorded at one-minute intervals were similar to those recorded at a frame-by-frame sampling scale (Table 4).

Table 4: Taxa-habitat associations at the two sampling scales ((* < 0.05, ** < 0.01, *** < 0.001). (Note: purple denotes that the results of taxa-habitat associations were the same between the two sampling scale).

Taxa	Frame-by-frame	One-minute
Brachiopod	Rock, boulder, cobble***	Rock, boulder, mud***
Sea pen	Sand, mud***	N/A
Seawhip	Sand ***	Sand***
Red gorgonian	Rock, boulder***	Rock***
Tube Anemone	N/A	N/A
Metridium	Rock***	Rock, mud**
Anemone	Boulder, sand***	Boulder, sand***
Tunicate	Rock, cobble***	N/A
Sponge	Rock, boulder***	Rock, boulder***

Differences in megafaunal invertebrate distribution and habitat associations between study sites at a frame-by-frame and one-minute sampling scale:

There were significantly more hard substrate habitats in Point Sur than in Point Lobos ($F_{(1,10)}$ = 6.503, p = 0.029) (Figure 8). There were no significant differences in mixed substrate ($F_{(1,10)}$ = 0.167, p = 0.691) and soft sediment ($F_{(1,10)}$ = 1.591, p = 0.236) habitats between the two sites.

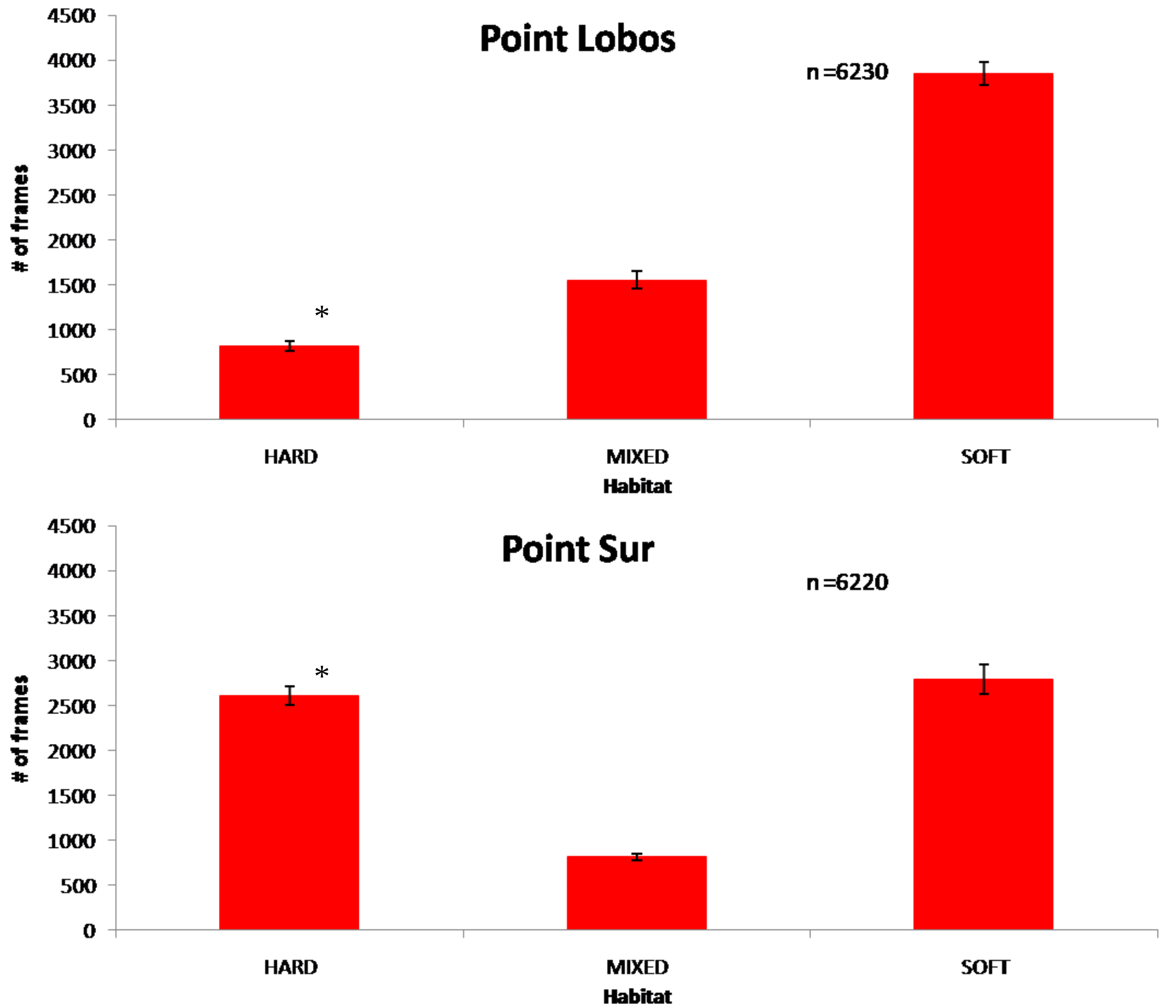


Figure 8: Physical characteristics of each study site, Point Sur and Point Lobos, by substrate type with standard error bars. Seafloor types are combined by decreasing particle size. Total sample size was *n* = 6220 frames in Point Sur and *n* = 6230 in Point Lobos (* < 0.05, ** < 0.01, *** < 0.001).

Taxa abundance and distribution differed between study sites for some taxa at a frame-by-frame scale (Table 5). Sea pens were more abundant in Point Lobos than in Point Sur. *Metridium spp.* were also more abundant in Point Lobos than in Point Sur. There was no difference in abundance of the remaining taxa between the two study sites: brachiopods (Levine's test = 7.503, p = 0.021; χ^2 =0.104, p = 0.747); seawhips

(Levine's test = 5.130, p = 0.047; χ^2 =1.103, p = 0.294); gorgonians (Levine's test = 6.700, p = 0.027; χ^2 =.006, p = 0.936); tube anemones (Levine's test = 5.568, p = 0.040; χ^2 =0.815, p = 0.367); anemones ($F_{(1,10)}$ = 4.444, p = 0.061); tunicates (Levine's test = 7.967, p = 0.018; χ^2 =0.264, p =0.607); and sponges ($F_{(1,10)}$ = 4.497, p = 0.060).

Table 5: Differences in abundance of megafaunal invertebrates between study sites sampled (* < 0.05, ** < 0.01, *** < 0.001).

Taxa	Difference in abundance between sites
Brachiopod	No difference between sites
Sea pen	More in Point Lobos than Point Sur*
Seawhip	No difference between sites
Red gorgonian	No difference between sites
Tube Anemone	No difference between sites
Metridium	More in Point Lobos than Point Sur**
Anemone	No difference between sites
Tunicate	No difference between sites
Sponge	No difference between sites

The one-minute sampling scale recorded the same differences in abundance for almost every taxon between the two sites that the frame-by-frame scale recorded. There were more *Metridium spp*. and anemones in Point Lobos than in Point Sur. There were no differences in brachiopod ($F_{(1,10)}$ = 0.001, p = 0.976), sea pen ($F_{(1,10)}$ = 3.025, p = 0.113), seawhip ($F_{(1,10)}$ = 3.055, p = 0.111), red gorgonian (Levine's test = 6.272, p = 0.031; χ^2 = 0.027, p = 0.870), tube anemone ($F_{(1,10)}$ = 0.192, p = 0.670), tunicate (Levine's test = 6.250, p = 0.031; χ^2 = 1.000, p = 0.317) or sponge ($F_{(1,10)}$ = 2.410, p = 0.152) abundances between sites.

There was strong similarity between the frame-by-frame and one-minute sampling scales when testing differences in taxa abundance between sites with only sea pens and anemones differing between the two sampling scales (Table 6). Brachiopods, seawhips, red gorgonians, tube anemones, *Metridium spp.*, tunicates and sponges were the same between the two sampling scales. Almost all of the taxa, except two, exhibited the same differences in abundance between sites at the different sampling scales.

Table 6: Differences in taxa abundance between sites (Point Lobos and Point Sur) (* < 0.05, ** < 0.01, *** < 0.001). (Note: purple denotes that the results of taxa-habitat associations were the same between the two sampling scales).

Taxa	Frame-by-frame	One-minute
Brachiopod	No difference between sites	No difference between sites
Sea pen	More in Point Lobos than Point Sur*	No difference between sites
Seawhip	No difference between sites	No difference between sites
Red gorgonian	No difference between sites	No difference between sites
Tube Anemone	No difference between sites	No difference between sites
Metridium	More in Point Lobos than Point Sur**	More in Point Lobos than Point Sur**
Anemone	No difference between sites	More in Point Lobos than Point Sur*
Tunicate	No difference between sites	No difference between sites
Sponge	No difference between sites	No difference between sites

Taxa-habitat associations differed between study sites at a frame-by-frame scale (Table 7). Sponges and red gorgonians were associated with rock, sand and cobble habitats in Point Lobos and in Point Sur were associated with rock and boulder habitats. Tunicates did not have significant habitat associations at either Point Lobos ($\chi^2 = 7.143$, $p = 0.067$) or Point Sur ($\chi^2 = 3.000$, $p = 0.223$). Anemones were associated with sand habitats in Point Lobos and boulder and sand habitats in Point Sur. Metridium were associated with rock habitats in Point Lobos and had no significant associations in Point Sur ($\chi^2 = 0.000$, $p = 1.000$). Tube anemones had no significant habitat associations at Point Lobos ($\chi^2 = 1.800$, $p = 0.180$) and habitat associations were not able to be analyzed at Point Sur. Seawhips were associated with sand habitats in Point Lobos and habitat associations were not able to be analyzed for Point Sur. Sea pens were associated with mud and sand habitats in Point Lobos and with sand habitats in Point

Sur. Lastly, brachiopods were associated with cobble and mud habitats in Point Lobos and in Point Sur were associated with rock and boulder habitats.

Table 7: Taxa-habitat associations between study sites sampled (* < 0.05, ** < 0.01, *** < 0.001).

	Associated habitat	
Taxa	**Point Lobos**	**Point Sur**
Brachiopod	Cobble, mud***	Rock, boulder***
Sea pen	Mud, sand***	Sand*
Seawhip	Sand*	not significant
Red gorgonian	Rock, sand, cobble***	Rock, boulder***
Tube anemone	not significant	N/A
Metridium	Rock ***	not significant
Anemone	Sand***	Boulder, sand***
Tunicate	not significant	not significant
Sponge	Rock, sand, cobble***	Rock, boulder***

Taxa-habitat associations recorded at the one-minute sampling scale also differed between sites (Table 8). Sponges were associated with boulder, cobble, rock and sand habitats in Point Lobos and rock habitats in Point Sur. Tunicates habitat associations were not able to be calculated in any year sampled. Anemones were associated with sand and boulder habitats in Point Lobos and did not have significant habitat associations in Point Sur (χ^2 =0.200, p = 0.905). *Metridium spp.* were associated with rock and mud habitats in Point Lobos but had no significant habitat associations in Point Sur (χ^2 =0.000, p = 0.100). Tube anemones did not have a significant habitat association in Point Lobos (χ^2 =0.000, p = 1.000) and habitat associations were not able to be calculated for Point Sur. Red gorgonians did not have a significant habitat association at Point Lobos (χ^2 =6.500, p = 0.165), but a Point Sur were associated with rock habitats. Seawhips were associated with sand habitats in Point Lobos, but habitat associations weren’t able to be calculated for Point Sur. Sea pens were associated with mud habitats in Point Lobos, but habitat associations weren't able to be calculated for Point Sur. Brachiopods were associated with mud and boulder habitats in Point Lobos and with rock and boulder habitats in Point Sur. The one-minute sampling scale did a decent job of recording differences in taxa-habitat associations between the two study sites. Over a quarter of the taxa-habitat associations between the two study sites were exactly the same as the frame-by-frame results, with an overall 61% similarity.

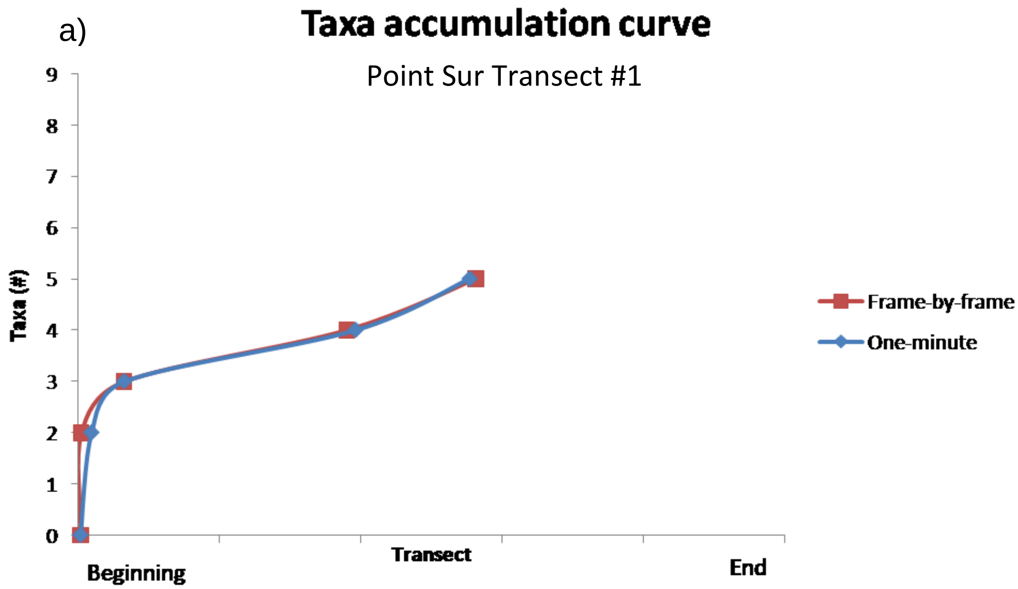
Table 8: Differences in taxa-habitat associations across years when sampled at a one-minute sampling scale (* < 0.05, ** < 0.01, *** < 0.001). (Note: purple denotes that the results of taxa-habitat associations were the same between the two sampling scales).

	Associated habitat	
Taxa	**Point Lobos**	**Point Sur**
Brachiopod	Mud, boulder***	Rock, boulder***
Sea pen	Mud**	N/A
Seawhip	Sand**	N/A
Red gorgonian	not significant	Rock***
Tube anemone	not significant	N/A
Metridium	Rock, mud**	not significant
Anemone	Sand, boulder***	not significant
Tunicate	N/A	N/A
Sponge	Boulder, cobble, rock, sand***	Rock***

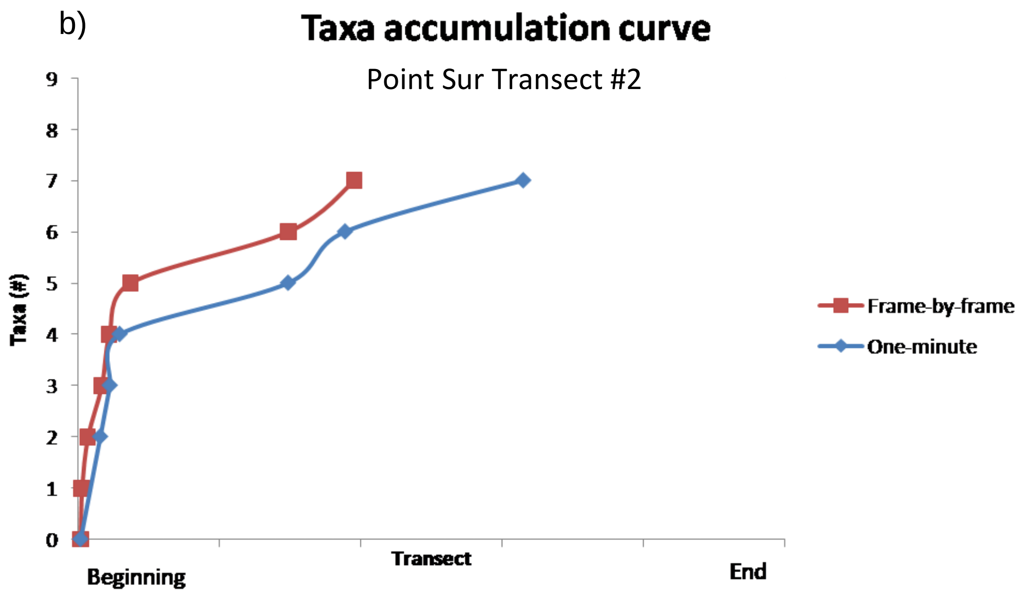
Differences in taxa accumulation between the two sampling scales:

There were differences in the accumulation of taxa between sampling scales (Figure 9). There were fewer taxa recorded at a one-minute sampling scale as opposed to the frame-by-frame scale. The coarser sampling scale was able to record the same rate of taxa accumulation as the finer-scale frame-by-frame. However, there were also instances where the coarser sampling scale was not able to record the same rate of accumulation nor the presence of all of the taxa along the transect. In some instances the coarser sampling scale recorded the same number of taxa observed within a transect, but the rate of accumulation was much lower. Additionally, in some transects the coarser sampling scale did not record the presence of some taxa that were captured at a finer sampling scale.

a)



b)



c)

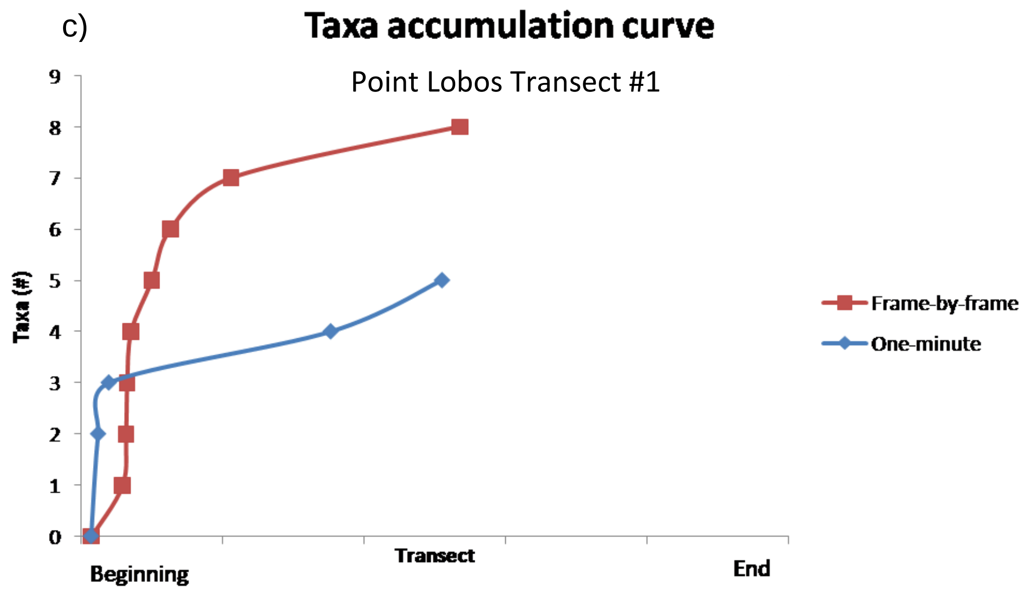


Figure 9: Taxa accumulation curves for three separate, representative transects analyzed at a frame-by-frame and one-minute sampling scale: a) coarser sampling scale recorded the same rate of taxa accumulation as the frame-by-frame scale; b) both scales recorded the same number of taxa, but rate of accumulation is different; and c) the coarser sampling scale did not record the same rate of accumulation nor the presence of all of the taxa along the transect.

Discussion:

Megafaunal invertebrates at two sites in the MBNMS occurred in association with distinct physical habitats which varied spatially, with some taxa being more abundant between sites sampled. Data collected in real-time at a coarser sampling scale captured taxa-habitat associations and spatial differences in abundance when compared to the finer-scale frame-by-frame analysis. However, the coarser sampling scale was not always effective in recording taxa presence within individual transects or differences in taxa-habitat associations between study sites.

Megafaunal invertebrates provide additional structure and complexity to the physical habitats in the two areas sampled and have distinct habitat associations. These results are consistent with previous studies elsewhere along the west coast. Tissot et al.

(2004) found similar habitat associations of megafaunal invertebrates on Heceta Bank, Oregon. They found rocky ridge habitats to have high abundances of gorgonians and *Metridium spp.*, sponges occurred on boulder-cobble habitats and sea pens on mud habitats. Graiff (2008) found similar habitat associations of taxa in the Monterey Bay at three sites; Portuguese Ledge, Point Sur and Big Creek. Graiff found sponges and gorgonians were more abundant on high relief hard rock and mixed substrate habitats and sea pens were more abundant on low-relief soft sediments. Pirtle (2005) observed similar habitat associations of invertebrates in Cordell Bank. Sea pens were associated with sand and mud habitats, gorgonians and anemones on hard substrates in mixed habitat communities and sponges and *Metridium spp.* were associated with high-relief rock ridges. Pirtle also found distinct associations between many fish taxa with various megafaunal invertebrates, showing that these structure-forming invertebrates are important aspects of many commercially important taxa life-stages.

A range of depths and locations were covered by towed camera sled transects providing a wide representation of the physical habitats off Point Lobos and Point Sur that included six categories of substrate and combinations of each to document variation in community composition. Variation observed within physical habitat types occurred in hard substrate habitats between the two study areas with higher abundances of hard substrate habitats occurring in Point Sur. These findings are consistent with those of Yoklavich and Starr (2008).Transects collected in Point Sur were comprised of primarily hard substrate with an equal distribution of high-relief and low-relief habitats with areas of soft sediment and cobble patches. Transects collected in Point Lobos were comprised primarily of soft sediment with distinct hard substrate patches and cobble fields.

There was a difference in the abundance of hard substrate between the two study sites. However, the only taxa that differed in abundance between the two sites were *Metridium spp.* and sea pens. In both cases there was a higher abundance in the Point Lobos study area than in Point Sur. There was more soft sediment sampled at Point Lobos, though not statistically different from Point Sur, which can explain the difference in sea pen abundance between the two sites. Sea pens had a very patchy distribution, occurring few and far between, so the more soft sediment available there more sea pens can be supported. However, with *Metridium spp.* being associated with rock habitats one would expect to find a higher abundance in the Point Sur study area since it supported more high-relief rock habitats. All other taxa displayed no differences in abundance between study sites.

Data collected “on-the-fly” was successful in recording taxa-habitat associations. The coarser sampling scale recorded the same habitat associations for seawhips, anemones and sponges when compared to the frame-by-frame results. It also recorded similar habitat associations for brachiopods, red gorgonians and *Metridium spp.* with one associated habitat being different from the frame-by-frame results. For example, the frame-by-frame scale recorded that brachiopods are associated with rock, boulder and cobble habitats while the one-minute scale recorded brachiopods as being associated with rock, boulder and mud habitats. The one-minute sampling scale is a good estimate for sessile taxa-habitat associations within the MBNMS and allows researchers to know what taxa occur where post-cruise, without requiring additional analysis time.

Overall, the one-minute sampling scale successfully recorded taxa-habitat associations for all transects sampled, but did not always record all of the taxa present within individual transects that the frame-by-frame scale recorded. Morrisey et al. (1992)

and Ellingsen (2001) found that spatial scale is important when recording infaunal invertebrate distribution patterns in soft sediment habitats. Both studies were sampled in soft sediments using grabs to collect samples. Similar studies on the effects of sampling scale on recording taxa distributions in hard substrates are limited. The current study shows that spatial scale is important when recording epifaunal invertebrates across all habitat types.

Future studies should analyze differences in mobile species-habitat associations at these sampling scales to determine the effect spatial scale has when recording mobile species. Taxa analyzed in this study were sessile, allowing us to understand the capabilities of the one-minute sampling scale at recording similar habitat associations to a finer-scale approach. However, the results may differ when looking at mobile invertebrates and fish species that can easily be missed when sampling "on-the-fly." Future work should also look at specific relationships between fish species and these megafaunal invertebrates to determine if megafaunal invertebrates are essential aspects of fish habitats at these sites in the MBNMS.

Future studies can also analyze depth and the flow of oceanographic currents and upwelling impact the distribution of these species. This study only analyzed habitat as a factor for taxa distributions, but Pirtle (2005) found that currents in Cordell Bank affected the distribution of cnidarian-dominated encrusting taxa due to flow requirements favorable for feeding and growth. Tissot et al. (2004) found that habitats were distributed based on depth in Heceta Bank, Oregon, which in turn determines the distribution of associated species.

All data collected at a frame-by-frame scale were collected by me. This limited the amount of human error in data collection. This is not the case for the data collected at one-minute intervals. There were multiple people that collected the data at at-sea; in many cases less experienced people were entering observations in the X-Keys TM. This could have impacted the results due to the potential for human error. It would be beneficial to conduct a study determining the accuracy of the data entered in the X-KeysTM at-sea to what was actually observed. This can be easily conducted by listening to the audio recorded on each transect of the observer and recording the taxa and habitat called using the same X-Keys TM keyboard used at-sea and comparing the results to those data actually collected at-sea. This will allow us to know any errors occurring when data are collected.

In conclusion, the one-minute sampling scale is a good predictor for habitat associations and spatial differences in abundance of sessile invertebrates. However, the coarser sampling scale did not effectively record spatial differences in taxa-habitat associations. This shows that, depending on the goals of the study and the target audience, that a sampling scale may not matter when it comes to recording taxa-habitat associations, but a finer-scale is required to record taxa abundance and overall distribution and, in some cases, taxa presence. By sampling areas “on-the-fly” management agencies can gain understand the distribution of taxa without requiring additional analysis time post-cruise, but a coarser sampling scale does not always capture the patchiness of these systems and thus is not able to record all taxa present within individual transects. This is important to document, especially when providing data to management agencies and extrapolating to results to a larger context. Data collected “on-the-fly” were able to record taxa distribution and habitat associations when there was a large sample size of transects included in analyses. The frame-by-frame approach collects fine-scale data in taxa abundance and taxa-habitat associations and is the most reliable in regards to analysis, but is far more time consuming to analyze. “On-the-fly” sampling techniques are adequate to estimate taxa-habitat associations and allow research results to be disseminated to management agencies much more rapidly. By reducing the time required to disseminate results to management agencies more informed decisions can be made regarding habitat studies of managed resources, such as commercial fish species. This study demonstrates that sampling scale in marine environments and in videographic analysis is important to determine and to understand the extent to which these scales represent the taxa within the Sanctuary.

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