Invertebrate settlement and diversity on a glass sponge reef

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Abstract
Glass sponge reefs are an ecosystem unique to the continental shelf of the northeast Pacific Ocean. Due to their vulnerability and limited distribution, several sponge reef protection initiatives exist within Canadian waters with the common goal of conserving biodiversity. To date, the biodiversity associated with sponge reefs has largely been assessed using remote video methods that allow us to describe large fauna associated with the reefs. However, small organisms are typically missed, resulting in an underestimate of reef-associated biodiversity. In this study we aimed to further describe invertebrate biodiversity associated with sponge reefs. Sponge reefs recently discovered in Howe Sound, British Columbia are within safe recreational SCUBA diving limits allowing us to examine macrofaunal settlement timing and community structure using diver-deployed settlement plates. We examined the effect of settlement plate material and elevation above the benthos within the reef on invertebrate community structure. A total of 70 taxa settled on the plates representing 10 phyla, including two phyla not previously described on sponge reefs: Nemertea (ribbon worms) and Platyhelminthes (flatworms). There were no significant differences in taxa richness, diversity, or community structure associated with settlement plate material or height above the benthos. Ours is the first report of invertebrate settlement on a sponge reef in the Salish Sea and the first description of larval settlement timing for nine invertebrate species in the northeast Pacific.

Key words: Glass sponge reefs; invertebrates; Porifera; community structure; juvenile settlement

Introduction
Glass sponge reefs, an ecosystem unique to the continental shelf of the northeast Pacific Ocean, are found from the Salish Sea in the south to Portland Canal, on the Canada-Alaska border in the north (Conway et al. 1991; Stone et al. 2014; Dunham et al. 2018). The sponge reefs are large biogenic structures that play an important role in carbon, nitrogen, and silica cycling and support more diverse and abundant faunal communities than the surrounding sea floor (Chu and Leys 2010; Chu et al. 2011; Kahn et al. 2015, 2018; Maldonado et al. 2016; Dunham et al. 2018). The reefs are formed by three species of glass sponges: Cloud Sponge (Aphrocallistes vastus), Farrea occa (no common name and absent on reefs in the Salish Sea), and Goblet Sponge (Heterochone calyx). These sponges occur throughout the world but have only been reported to form reefs in the north-east Pacific. They can form reefs because they possess skeletons of fused silica spicules which maintain their three-dimensional structure after the sponges’ death. The erect dead sponge skeletons serve as settlement substrate for juvenile sponges and are infilled with baffled sediment, a process that results in reef formation after multiple generations of sponges. While the sponge skeletons are rigid, they are fragile. Consequently, the sponges and reefs are vulnerable to damage from human activities that contact the bottom, such as trawl and trap fishing, and cable laying (Dunham et al. 2015; Kahn et al. 2016). Because of their vulnerability to human impacts and their limited global distribution the sponge reefs are a high conservation priority in Canada. As a result, one marine protected area (MPA) and 17 bottom contact fishing closures have been created to protect sponge reefs and the biodiversity they support (DFO 2015, 2019).

Despite the high priority of protecting biodiversity associated with sponge reefs, we have a limited under
standing of the life histories of many organisms residing on the reefs. In particular, we do not know when many of the organisms reproduce or when larvae settle onto the reefs. For example, the exact timing of spawning and larval settlement for reef-forming glass sponges is not known, although evidence points to at least one spawning period in the winter months for sponges in the Salish Sea (Kahn et al. 2016) and potentially multiple settlement periods in Hecate Strait (Guillas et al. 2019). Knowing when reef-associated fauna (including reef-forming sponges) recruit to the sponge reefs can help guide monitoring, management, and potential restoration activities in the future.

In addition to a lack of knowledge of the life history of reef-associated fauna, it is likely that our understanding of reef-associated biodiversity is incomplete. To date the biodiversity associated with sponge reefs has largely been assessed using remote video methods because most reefs occur outside safe recreational SCUBA diving limits (i.e., deeper than 40 m; but see Krautter et al. 2001; Cook 2005; Guillas et al. 2019). While remote video surveys allow us to describe large fauna (≥24 cm) associated with the reefs, smaller organisms (hereinafter referred to as macroinvertebrate fauna), such as polychaetes and bryozoans, are typically missed. Several of the reef complexes recently discovered in Howe Sound (Clayton and Dennison 2017; Dunham et al. 2018) are significantly shallower than other known reefs (22–127 m compared to 90–300 m), occurring within safe recreational SCUBA diving limits. The discovery of these shallow reefs allowed us, for the first time, to examine macrofaunal community structure and settlement timing on a glass sponge reef in the Salish Sea using diver-deployed settlement plates.

Juvenile settlement is driven by several factors, including water flow and the surface texture and chemistry of available substrate (Rodriguez et al. 1993). Because little is known regarding the settlement requirements for glass sponges and other reef-associated fauna we used two settlement plate materials (frosted glass and ceramic clay) in order to capture a wider range of organisms. We hypothesized that frosted glass plates would attract a more diverse and abundant community as this surface approximates the bottom and middle portions of the stem (Figure 2a, b). Determining whether elevation above the benthos impacts the diversity of benthic invertebrates settling on the reef has implications for reef conservation as well. When human activities damage sponge reefs they destroy the three-dimensional structure of the reef, often crushing or toppling both live and dead sponges. If a more diverse invertebrate community settles on plates higher above the benthos, this destruction of the three-dimensional structure may have larger impacts on reef-associated biodiversity than previously reported. We hypothesized that plates located higher above the benthos would attract a more diverse and abundant settler community as water flow around these plates should be higher. Overall, the goal for this study was to describe the biodiversity of small benthic invertebrates associated with sponge reefs and determine if settler diversity is affected by plate material and/or height above the benthos. Additionally, we report the first description of larval settlement timing for several invertebrate species in the northeast Pacific.

**Study Area**

Our study occurred at the Halkett Point glass sponge reef in Howe Sound, Salish Sea on the southwest coast of British Columbia (BC), within the Halkett Bay Provincial Marine Park northwest of the city of Vancouver (Figure 1a). The glass sponge reef was first discovered by G. Dennison in 1996 and first formally described by Clayton and Dennison (2017). The reef was federally protected from all bottom-contact fishing activities in 2019, but at the time of our study, in 2017, fishing restrictions were not yet in place (DFO 2019). The main sponge reef occurs between 22–90 m on a pinnacle that is irregular in shape with a relatively flat top and gently sloping sides (Figure 1b; Clayton and Dennison 2017; DFO 2018). Glass sponge reefs are naturally patchy with areas of live, erect dead, and buried dead sponge present throughout the reef (Dunham et al. 2018). At Halkett Point sponge reef live reef-building sponges cover 4% of the reef and 40% of the reef was classified as live reef habitat (i.e., live sponges dominate the benthos; Dunham et al. 2018); 31 associated species have been documented on the reef (DFO 2018; Dunham et al. 2018). Some rocky outcroppings are found on the western side of the pinnacle.

**Methods**

**Tree design, deployment, monitoring, and retrieval**

Two trees with eight settlement plates each were constructed using a 1.5 m length of 20 mm PVC pipe (sold as ¾ inch PVC pipe) for the stem with two sets of 0.30 m length PVC branches extending from the top and middle portions of the stem (Figure 2a, b). Each branch was further divided into two additional branches, each supporting a 0.11 m × 0.11 m × 0.03 m settlement plate made of either ceramic clay or frosted glass. Plates were fastened to the PVC branch with a
Figure 1. a. The location of the Halkett Point glass sponge reef as marked by the black dot, and b. a detailed bathymetric map of the reef area. Bathymetry contours were derived from a $3 \times 3$ m resolution survey of the area using downwards sonar conducted by G. Dennison. The location of the settlement trees is marked on b with a black dot.
plastic bracket (Figure 2a). Each tree was designed to hold two ceramic and two glass settlement plates on each of the upper and lower branches for a total of four of each plate type; the upper and lower branches were 24 cm apart. The brackets holding the ceramic and glass plates were designed in SOLIDWORKS® 3D mechanical CAD software Rev 2016 (Dassault Systèmes SolidWorks Corporation, Waltham, Massachusetts, USA) and produced with a Stratasys Mojo 3D printer (Computer Aided Technology, Buffalo Grove, Illinois, USA). The bracket was designed to allow for easy removal and installation of the plates. Each set of glass and ceramic settling plates was identified with a unique tag secured to the corresponding branch of the tree.

The trees were deployed 11.8 m apart on 4 March 2017. Trees were installed with settlement plates attached by penetrating the stem of the tree into the soft surface layer of the reef (e.g., Figure 2b). The trees were deployed in an area next to low-density sponge (31.7 m depth, hereinafter tree 1) with the lower branches ~0.5 m above the benthos and in an area next to high density sponge (at 33.5 m depth, hereinafter tree 2) with the lower branches ~0.65 m above the benthos.

ATidBit® v2 (Onset Computer Corporation, Bourne, Massachusetts, USA) temperature logger was attached to the lower branch of each tree to record temperature every 15 min over the course of the study. Data from both temperature loggers were collected from 12 March to 16 August 2017; the temperature logger attached to tree 1 failed to record from 16 August 2017 to 21 April 2018 and thus only data from tree 2 were available for this time period.

Monitoring of settlement on the plates was done with macrophotography. Divers visited the site on 9 July 2017, 16 August 2017, 2 December 2017, and 8 April 2018 to photograph the settlement plates. Photos were taken in a sequence that included an initial photo of the unique identifier tag followed by photos of the frosted glass and ceramic clay settlement plate associated with each tag; photos of the entire settlement plate and of each plate’s surface were taken at a greater magnification. These photos were not used in our analysis as many of the taxa that settled on the plates are difficult, if not impossible, to identify even to phylum at this level of resolution. However, for slightly larger taxa (≥ ~2 cm) these photos serve as a permanent record of plate colonization and are available for future study (e.g., Figure 2c).

Settlement plates were retrieved on 8 and 21 April 2018 after ~13 months of immersion. Two plates were recovered on 8 April to test the protocol for plate recovery; the remainder were recovered in the next

![Figure 2.](image-url)
Community sampling

Once at the surface, the 16 containers with the individual plates were processed sequentially. Each container was placed inside a larger aluminum pan to capture any spillage of seawater as the lid was removed. Approximately three-quarters of the seawater in the container was poured through a 0.25 mm sieve to capture any macrofauna that had been dislodged during transport from depth to surface. These dislodged or ‘mobile’ macrofauna are herein referred to as flocculent. Container spillage in the aluminum catch-pan was also poured through the sieve. Photographs were taken of the top and bottom sides of the plate in a preprocessing state to assist with taxa documentation and identification (e.g., Figure 2d). The flocculent residue on the 0.25 mm sieve was returned to the original LOCK&LOCK container using pre-filtered saltwater (0.25 mm sieve) along with a waterproof label identifying each plate. The container was then topped up with a 30% solution of 95% ethanol and pre-filtered saltwater to anesthetize macrofauna on the plate and in the flocculent. Several hours later, the anesthetizing fluid was decanted through a 0.25 mm sieve, the flocculent residue on the sieve returned to the container, and the container topped up with 95% ethanol. The lid of the container was securely relocked and placed into a cooler for transport upright. Four settlement plates were resting directly on a clump of sponge while the other four plates on the tree were not in contact with the sponge.

Statistical analysis

Univariate measures of biodiversity (taxa richness and Shannon’s diversity) on the plates were compared between the relative height above the benthos (lower branch versus upper branch, \( n = 4 \) plates per tree for a total of eight plates) and plate material (ceramic clay or frosted glass, \( n = 4 \) plates per tree for a total of eight plates) using a linear mixed effects model with tree as a random factor to account for any effects due to tree placement and account for the nested structure of our study design. We did not examine the interaction between plate material and the relative height off the bottom, as this was not relevant to our hypotheses. The significance of all variables (fixed and random) was assessed using the lmerTest package in R version 3.6.1 (Kuznetsova et al. 2017; R Core Team 2019). All taxa associated with a single plate were pooled separately for solitary and colonial organisms due to differences in how their abundance was recorded. Diversity was analyzed to differences in how their abundance was recorded. Diversity was analyzed to differences in how their abundance was recorded. Diversity was analyzed to differences in how their abundance was recorded. Diversity was analyzed to differences in how their abundance was recorded.

For all RDA analyses the community data were transformed with a Hellinger transformation to meet the assumptions of the analysis. All RDAs included height above benthos, plate material, and tree as predictor variables. Overall RDA significance and the significance of individual axes were evaluated using analysis of variance. Taxa were con-
considered associated with an axis if their goodness-of-fit score was 0.5 or higher. All analyses were conducted in R (version 3.6.1; R Core Team 2019).

Life history information and settlement timing

We assumed that juvenile taxa had settled on the plates relatively recently, within the previous two months. A list was generated of all taxa that were both identified to at least the genus level and had juveniles present on the plates. For these species we searched the literature for information regarding when spawning and/or larval settlement are known to occur. We also searched for information regarding larval and juvenile duration. The search was first restricted to reports from the northeast Pacific and then expanded to a global search. For species where information was found, we compared the published information with our findings.

Results

Water temperature differed significantly between the settlement trees during the period from March to August 2017 (paired t-test, $t_{110} = 153.91, P < 0.0001$): tree 1 was in slightly warmer conditions (mean 9.15°C, range 8.25–10.96°C) compared to tree 2 (mean 9.06°C, range 8.17–10.76°C). Overall temperature range recorded in this study was 8.02–10.96°C.

Qualitative description of the invertebrate community

Overall, 70 taxa from 10 phyla were observed on the settlement plates ($n = 16$, including the top, bottom, and flocculent material for all plates). Most taxa (58, or 82.9%) were found on fewer than 50% of the plates and 28 species (40%) were found on a single plate. Three species were found on all 16 plates: Alcyonidium cf. polyoom (no common name, Bryozoa), Irregular Calcareous Tubeworm (Crucigerica irregularis, Annelida), and Western Calcareous Tubeworm (Pseudochitinopoma occidentalis, Annelida). A full list of taxa identified on the plates can be found in Table S1. Annelids were by far the most taxa-rich phylum on our plates, with 23 taxa observed, then by Cnidaria with 12 taxa observed, followed by Platyhelminthes, Mollusca, and Nemertea. Despite having a single taxon representative, both Nemertea and Porifera were found on multiple plates (two and seven plates, respectively). Fewer taxa were observed on the tops of plates (33) than either in the flocculent material (47) or on the bottom side (49) of the plates. When only considering taxa that were observed more than once (i.e., multiple individuals or distinct colonies were seen) several taxa were found to occur on a single side of the plate. There were four taxa, each from a different phylum, that occurred only on the bottom sides of plates. These were Stomatopora sp. (Bryozoa), Tiny White Tunicate (Bathypera feminalba, Chordata [Tunicata]), Epiactis sp. (Cnidaria) and Doridacea indet. (Mollusca). A single taxon from Arthropoda, Balanomorpha indet., was found to only occur on the tops of the plates. Eleven taxa from four phyla (Annelida, Cnidaria, Mollusca, and Nemertea) were found only in the flocculent material (marked with a star in Table S1). All taxa found only in the flocculent material are mobile species. Regardless of the taxonomic level of organization examined, the bottom sides of plates were the most taxa-rich (Figure S1). Contrary to the flocculent material was consistently more diverse when considering solitary organisms (Figure S2) while there was no difference between plate sides in the diversity of colonial organisms (Figure S3; no colonial organisms were recorded in the flocculent material).

Effects of substrate and relative height above the benthos on invertebrate community composition and structure

Taxa richness on individual plates ranged from 11 to 25 (17.69 ± 4.00, mean ± SD). There was no difference in species richness attributable to the relative height off the benthos ($F_{1,12} = 0.16, P = 0.70$), the plate material ($F_{1,12} = 0.07, P = 0.79$), or the tree the plate was attached to ($\chi^2_1 = 0.54, P = 0.46$). Similarly, the diversity of both solitary and colonial organisms were not influenced by relative height off the benthos (solitary: $F_{1,12} = 0.52, P = 0.48$; colonial: $F_{1,12} = 0.12, P = 0.73$), plate material (solitary: $F_{1,12} = 1.79, P = 0.21$; colonial: $F_{1,12} = 3.62, P = 0.08$), or the tree to which the plate was attached (solitary: $\chi^2_1 = 2.19, P = 0.14$; colonial: $\chi^2_1 = 2.43, P = 0.12$). When the entire community was examined as a whole using presence-absence data and relative height off the benthos, plate material, and the tree the plate was attached to as explanatory variables, the RDA had an $R^2_{adj}$ of 0.14 and one significant axis ($F_{1,12} = 3.41, P = 0.001$). The first and only significant axis separated the communities occurring on the two trees (Figure 3a). The barnacle Balanomorpha indet., the nudibranch Aeolidioidea indet., and the hydrozoan Clytia hemisphaerica were strongly associated with tree 2. When solitary taxa were analyzed separately, the same predictor variables resulted in an $R^2_{adj}$ of 0.09 and one significant axis ($F_{1,12} = 2.53, P = 0.02$). The significant axis again corresponded with the tree to which the plates were attached (Figure 3b) and the barnacle Balanomorpha indet. and the nudibranch Aeolidioidea indet. were associated with tree 2. For colonial organisms, the RDA had an $R^2_{adj}$ of 0.39 and a single significant axis ($F_{1,12} = 11.25, P = 0.002$), which again corresponded with the tree to which the plates were attached (Figure 3c). The hydrozoan C. hemisphaerica was associated with
tree 2 while the bryozoan *Amathia gracilis* was associated with tree 1.

**Settlement timing**

Juveniles of 17 taxa identifiable at the species or genus level were found on the plates at the time of collection (April 2018; Table 1). Of these, we were able to find information regarding spawn timing or larval settlement periods in the literature (for either the species we observed or members of the same genus) for 15 taxa. In most cases the information regarding reproductive timing was not based on studies from the northeastern Pacific. Regardless, the reported spawning or larval settlement timing generally agreed with our findings (Table 1). In four cases, *Paleanotus bellis*, *Pododesmus* sp., *Prionospio (Minuspio) multibranchiata*, and *Prionospio (Minuspio)* sp., our observation of juveniles on the plates in April does not correspond to spawning seasons reported in the literature. In all cases we observed juveniles of these species earlier than reported spawning periods. For *P. bellis* we found reports of spawning occurring between May and November with juvenile settlement occurring approximately three weeks later (Rasmussen 1956; Bhaud et al. 1987; Table 1), but we recorded juveniles in April. Similarly, for *Pododesmus* sp. our findings suggest spawning occurred earlier than has been previously reported, as Drozdov et al. (2009) reported spawning occurring in the Sea of Japan in May and then again from August to October. We did not find any reports on larval duration for this genus. In Atlantic Canada members of the *Prionospio* genus were found spawning between May and August (Lacalli 1981). It should be noted that *Prionospio patagonica* larvae were found in October, November, and March in Chile (Radashevsky et al. 2006), timing that corresponds seasonally to the appearance of juveniles on our plates in April, because Chile is in the southern hemisphere. In two cases, *Lanassa venusta venusta* and *Proclea* sp., we were unable to find any reports of spawn timing in the literature for species more closely related than the family level (i.e., in a different genus within the same family). For these two species there are reports of reproductive timing.

**Figure 3.** Biplot of the three redundancy analyses conducted using a. presence-absence data from the entire community, b. abundance data for solitary, and c. colonial organisms on the settlement plates. Species displayed were strongly associated with one or both of the axes displayed (goodness-of-fit of at least 0.50). There are significant differences between the communities found on the two trees. Plate material did not significantly influence community structure. The taxa names are abbreviated on the figure panels so that A = Aeolidioidea indet. (nudibranch), AG = *Amathia gracilis* (Bryozoan), B = Balanomorpha indet. (barnacle), and CH = *Clytia hemisphaerica* (hydrozoan).
| Phylum   | Class      | Order     | Family     | Genus & species | Spawn timing | Larval settlement | Larval Duration | Location(s) of study | Comments | Citation(s) |
|----------|------------|-----------|------------|-----------------|--------------|------------------|----------------|----------------------|----------|-------------|
|          | Annelida   |           |            |                 |              |                  |                |                      |          |             |
|          | Polychaeta |           |            |                 |              |                  |                |                      |          |             |
|          | Phyllodocida |          |            |                 |              |                  |                |                      |          |             |
|          | Chrysopetalidae |      |            |                 |              |                  |                |                      |          |             |
|          | *Paleanotus bellis* | |            |                 | May–Nov      | North Sea       | >3 wk          | NE Atlantic         |          | Rasmussen (1956) |
|          | Polynoidae |           |            |                 |              |                  |                |                      |          |             |
|          | *Harmothoe sp.* |        |            |                 | Dec–May       | NE Atlantic     | All months      | NE Pacific           |          | Daly (1972) |
|          | Syllidae   |           |            |                 |              |                  |                |                      |          |             |
|          | *Proceraea sp.* |      |            |                 | Dec–Aug       | North Sea       |                | NW Pacific           |          | Rasmussen (1973) |
|          | Sabellida  |           |            |                 |              |                  |                |                      |          |             |
|          | Serpulidae |           |            |                 |              |                  |                |                      |          |             |
|          | *Crucigera irregularis* | |            |                 | Mar–May       | NE Pacific     |                |                      |          | Strathmann (1987) |
|          | *Pseudochitinopoma occidentalis* | |            |                 | All months    | NE Pacific     |                |                      |          |             |
|          | Spionidae  |           |            |                 |              |                  |                |                      |          |             |
|          | Spionidae  |           |            |                 |              |                  |                |                      |          |             |
|          | *Prionospio (Minuspio) multibranchiata* | |            |                 | May–Aug       | NW Atlantic     |                |                      |          | Lacalli (1981) |
|          |            |           |            |                 | Oct, Nov,      | SE Pacific      |                |                      |          |             |
|          |            |           |            |                 | Mar*          |                  |                |                      |          | Radashevsky *et al.* (2006) |
### Table 1. Continued.

| Phylum | Class | Order | Family | Genus & species | Spawn timing | Larval settlement | Larval Duration | Location(s) of study | Comments | Citation(s) |
|--------|-------|-------|--------|----------------|--------------|------------------|-----------------|----------------------|----------|-------------|
|        |        |       |        | **Prionospio (Minuspio) sp.** | May–Aug | NW Atlantic | Report from a member of the same genus from Atlantic Canada | Lacalli (1981) |          |
|        |        |       |        |                | Oct, Nov, Mar* | SE Pacific | Planktonic larvae of *Prionospio patagonica* reported in October, November, and March in Chile | Radashevsky *et al.* (2006) |          |
|        | Terebellida | Terebellidae |        | **Lanassa venusta venusta** | May–July | NE Pacific | No reports of spawn timing in the literature | McHugh (1993) |          |
|        |        |        |        | **Neoamphitrite sp.** | May–July | NE Pacific | No reports of spawn timing in the literature | McHugh (1993) |          |
|        |        |        |        | **Proclea sp.** | No reports of spawn timing in the literature |          |          |          |          |            |
| Chordata (Tunicata) | Ascidiae | Ascidiacea | Corellidae | **Corella willmeriana** | All months | All months | NE Pacific | Lambert (1968) |          |
|        | Stolidobranchia | Pyuridae | **Bathypera feminalba** | Feb–Apr | NE Pacific | Species described from Saanich Inlet, BC | Young and Vazquez (1995) |          |
| Cnidaria | Anthozoa | Actiniaria | Actiniidae | **Epiactis sp.** | Jun, Oct | NE Pacific | Observations of spawning in California | Dunn (1975) |          |
|        |        |        |        | Summer months* | NE Pacific | Weddell Sea | inferred from samples of gonads in Weddell sea | Rodriguez *et al.* (2013) |          |
### Table 1. Continued.

| Phylum   | Class    | Order     | Family      | Genus & species | Spawn timing | Larval settlement | Larval Duration | Location(s) of study | Comments                                                                 | Citation(s)                  |
|----------|----------|-----------|-------------|-----------------|--------------|-------------------|-----------------|----------------------|--------------------------------------------------------------------------|-------------------------------|
| Mollusca | Bivalvia | Adapedonta| Hiattellidae|                |              |                   |                 |                      |                                                                          |                               |
|          |          |           |             |                 | Dec–Feb, May*|                   |                 | SW Pacific          | Also references Kuafman (1977) as a report of spawn timing. We could not confirm this report as it is only available in Russian | Booth (1983)                  |
|          |          |           |             |                 | Jun–Sep      | Jun–Nov           |                 | Barents Sea         |                                                                          | Flyachinskaya and Lesin (2006) |
|          |          |           |             |                 | All months    |                   |                 | NE Atlantic          | In a population at 12 fathoms (~22 m) females with mature ova were found throughout the year | Hunter (1949)                 |
|          |          |           |             |                 | All months    |                   |                 | NE Atlantic          |                                                                          |                               |
| Mytilida | Mytilida | Mytilidae | Mytilidae    | Mytilus sp.    | All months    |                   |                 | NE Pacific           | Recruitment lowest in Jan-March                                         | Connolly et al. (2001)        |
| Pectinida| Anomiidae|           |             |                 |              |                   |                 |                      |                                                                          |                               |
|          |          |           |             |                 | May,          | Aug–Oct           |                 | NW Pacific           | Report from Sea of Japan                                               | Drozdov et al. (2009)         |
| Gastropoda| Nudibranchia|   | Dendronotidae| Dendronotus sp.| Mar–Oct      |                   |                 | Several              | Several species from the Arctic, Barents Sea, and the NW Pacific        | Ekmova et al. (2015)          |
|          |          |           |             |                 | Mar–Oct      | Short term        |                 |                      | No clear definition of short-term but appears to be ~50 days from egg-larval development | Todd and Doyle (1981)         |
for closely related genera. However, our search was restricted to members of the same genus and therefore we did not include this information in Table 1. For the 15 species where we could find reports of the timing of reproduction and/or larval settlement, we were unable to find reports from the northeast Pacific for seven species. Therefore, our contribution represents the first clues to larval settlement timing in the northeast Pacific for nine species.

Non-indigenous species

None of the organisms identified to species in our study are known to be non-indigenous in BC. We recorded taxonomic relatives of non-indigenous species in BC with similar life histories (e.g., *Eusyllis blomstrandii* and *Eusyllis habeii*) are members of the same genus as *Eusyllis japonica*, a non-indigenous species in BC; *Lu et al.* (2007). It should be noted that *E. blomstrandii* may be part of a cryptic species complex in the northeastern Pacific (*Kudenov et al.* 1995).

Discussion

An important step in the processes of ensuring effective ecosystem-level conservation is building a thorough understanding of the systems we are attempting to protect. Sponge reefs are a high conservation priority because of their global rarity, slow growth and low recovery potential, and the biodiversity they support (*Cook et al.* 2008; *Chu and Leys* 2010; *Kahn et al.* 2015, 2016; *Dunham et al.* 2018). Previous work using remote video surveys has provided a thorough description of the larger fauna occurring on the reefs, yet a gap remained in our understanding of small taxa not detectable using remote methods. Here we present the first report of invertebrate settlement on a sponge reef in the Salish Sea and increase our understanding of biodiversity on sponge reefs.

There have been three previous studies on small macroinvertebrate fauna on sponge reefs in BC. However, all of these studies have occurred on the reefs in Hecate Strait (over 600 km to the northwest) and have studied animals found in cores or settled on sponges collected from the reef. *Krautter et al.* (2001) collected cores from the reefs in Hecate Strait and described a macroinvertebrate fauna dominated by polychaetes and bryozoans, consistent with what we found in Howe Sound. *Cook* (2005) described the polychaete community on sponge reefs from cores taken within and around the Hecate Strait sponge reefs. They found 105 species of polychaetes from 27 families. We identified 22 polychaetes from nine families (Table S1). Interestingly we did not find any species reported in *Cook* (2005) and only two genera and four families were found in both studies. The Hecate Strait reefs and the reef in our study are separated by over 600 km and there is over a 100 m difference in the depth, those in Howe Sound being shallower. Therefore, it may be unsurprising that the taxa found in the two studies overlapped so little. *Cook* (2005) found a statistically significant difference in the polychaete communities on sponge reefs when compared to non-reef habitat immediately adjacent to the reefs. In the future it may be interesting to repeat a study similar to ours but with settlement plate trees located at similar depth in the off-reef habitat.

In a recent study, *Guillas et al.* (2019) looked at the macrofauna settled on *F. occa* individuals collected from a Hecate Strait reef. Despite the fact that their study and ours had only one species in common (Arctic *Hiatella*, *Hiatella arctica*) the overall community structure was similar with a community composed largely of sponges, bryozoans, molluscs, and polychaetes. However, our study documented two new phyla occurring on sponge reefs (*Nemertea* and *Platyhelminthes*) and expanded our knowledge of the settlement timing of invertebrates in this system. While our study failed to document any reef-building sponge settlement, *Guillas et al.* (2019), who collected reef-building sponges with both live and dead portions, found many small reef-building sponges potentially representing many settlement pulses over the past year. This is consistent with observations made by *Kahn et al.* (2016), who observed evidence of multiple reproductive events within a year on the Galiano Ridge sponge reef in the Salish Sea. While it is still unclear what environmental cues lead to spawning in reef-building glass sponges, it is likely, given other studies that report evidence of multiple spawning events within a calendar year, that reef-building sponges spawned while our settlement plates were deployed, but larvae did not recruit to the plates.

We did not find any difference in taxa richness, diversity, or community structure associated with the relative height above the benthos. In other words, height above benthos did not appear to structure the community on our settlement plates. This may have been for several reasons. First, the two trees were pushed into the substrate to different depths so that the branches were at 0.5 (tree 1), 0.65 (tree 2), 0.74 (tree 1), and 0.89 m (tree 2) above the benthos. If the data are reanalyzed so that actual height above benthos, rather than relative height above benthos, is included as a predictor variable the conclusions are still the same. A second reason is that a 0.24 m difference in height between the two branches is not large, particularly considering that sponges on BC’s sponge reefs can often grow to be over 1 m tall (*Conway et al.* 2005). However, in other systems, such as seagrass beds, similar height differences have been found to influence survival, and therefore community structure,
of invertebrates (Pohle et al. 1991). Plate material similarly had no impact on taxa richness, diversity, or community structure. It is possible that both plate materials presented too similar a surface to influence community structure.

Although we did not test for it statistically, we did find that the side of the settlement plate was an important factor influencing taxa richness and diversity. Many marine invertebrates have a pelagic portion of their larval life history during which they are photosensitive, meaning exposure to light influences their behaviour (Thorson 1964). The vast majority of photosensitive larvae become photonegative, or move away from light, as they begin to settle out of the water column and into the benthic realm (Thorson 1964). This behaviour often results in larvae settling onto the undersides of available surfaces as these are the surfaces with the lowest light levels (Thorson 1964), which is consistent with our findings. However, light levels are consistently low at our study site and further research would be necessary to determine if this behaviour is the cause of the patterns we observed. The high richness and diversity of taxa found in the flocculent material is also not surprising; these taxa were overwhelmingly motile. Other settlement plate studies, with sampling protocols that allowed for the capture and enumeration of motile taxa, have similarly reported high diversity in this group (Gartner 2010). Perhaps more surprising was the consistent difference in community between the two trees. One of the trees (tree 2) was toppled over at some point in the last three months of our study, it is possible that this led to the differences we observed as there was a significant difference in the total abundance of solitary taxa between the two trees ($t_{12.51} = -2.71$, $P = 0.02$). Additionally, we did observe a small, but statistically significant difference in the water temperatures recorded at each tree in the beginning of the study. While it is unlikely that this small difference in temperature would drive the differences in diversity, it may be indicative of an environmental gradient present within the sponge reef that relates to water movement and sedimentation and thus, likely, larval dispersal within the sponge reef. It is also possible that the difference in surrounding reef-building sponge abundance affected local fine-scale currents and influenced the species settling on each tree. Further study is warranted to investigate environmental gradients and the importance of local fine-scale current patterns within sponge reefs and how these relate to larval settlement and biodiversity patterns.

This is the first published settlement plate study at these depths in BC (31.7–33.5 m). Consequently, the species found on our plates largely differed from those in other, shallower, settlement plate studies in BC, but the overall community structure observed was similar with polychaetes, hydroids, and bryozoans being prevalent (Greene and Schoener 1982; Greene et al. 1983; Gartner 2010). One species we observed, L. v. venusta (phylum Annelida), is reported to be commensal with pagurids (hermit crabs; Hoberg et al. 1982), which have been observed on sponge reefs in the Salish Sea (Dunham et al. 2018). Interestingly, we did not observe any plume anemone (Metridium) species settling on the plates despite the fact that they are a common member of settlement plate communities in BC (Greene and Schoener 1982; Greene et al. 1983; Gartner 2010) and are commonly observed around sponge reefs in the area (Dunham et al. 2018). The presence of predators can significantly influence the development of fouling communities (Nydam and Stachowicz 2007). While the majority of the species we observed were filter-feeders, we did collect several predatory species including a number of polychaetes, a nudibranch, and some parasitic species. For example, members of the genus Proceraea (phylum Annelida) have been reported as predators and parasites of bryozoans, sponges, ascidians, echinoderms, cnidarians, and decapods (Martin and Britayev 1998). Consequently, the presence of predatory polychaetes and nudibranchs on our plates may have prevented us from detecting the settlement of some common members of the invertebrate community. However, it should be noted that there were records of unidentified Actiniaria and Anthozoa juveniles from four plates. It is possible that these may have been Metridium juveniles. Future work is needed to fully unravel processes determining macroinvertebrate community development within sponge reefs.

We found juveniles identifiable to at least the genus level from four phyla present on our settlement plates (Annelida, Chordata [Tunicata], Cnidaria, and Mollusca; Table 1). Of the 17 unique taxa that we observed as juveniles we could find records of the timing of spawning and/or larval settlement for 15. Of these 15 taxa, eight had either spawn or larval settlement times reported in studies in the northeast Pacific and only one species had been studied in BC. Our observations of juveniles corresponded with the published timing of spawning or larval settlement for all taxa in studies conducted in the northeast Pacific. To the best of our knowledge our study is the first to elucidate potential spawn timing in L. v. venusta and a Proclea species. Members of this family are reported to spawn anytime throughout the year and display a wide range of life history strategies (McHugh 1993). Despite the wide variation in reproductive strategies in this family, all species seem to have a relatively short planktonic larval duration period (0–7.5 days; McHugh 1993 and references therein). Consequently, we can infer that spawning likely occurred in these
species in the weeks leading up to plate collection. Overall, over 51% of the individual organisms we observed, across all plates and taxa, were juveniles. The high number of juveniles on our settlement plates at the time of collection corresponds well with the hypothesis that many invertebrates in coastal BC waters spawn in the spring (Gartner 2010).

Although we did not have any reef-building sponges recruit to our settlement plates, continued settlement plate deployments focussed on testing different settlement materials and placement within the reefs could help fill important knowledge gaps on the factors influencing reef-building sponge recruitment. Additionally, continued settlement plate deployment could serve as an important monitoring tool for early detection of aquatic invasive species. Overall, our study helps to improve our understanding of biodiversity on sponge reefs.

Author Contributions
Conceptualization: S.K.A., S.B., A.D., G.D., and L.M.; Methodology: S.K.A., S.B., A.D., G.D., and L.M.; Investigation: S.B., G.D., and L.M.; Formal Analysis: S.K.A.; Writing – Original Draft: S.K.A.; Writing – Review & Editing: S.K.A., S.B., A.D., G.D., and L.M.; Funding Acquisition: A.D.

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Supplementary Materials:

Table S1. All taxa recorded on the settlement plates.

Figure S1. Mean taxa richness observed on a plate by taxonomic level and the side of the settlement plate (n = 16 per bar).

Figure S2. Mean taxa diversity for solitary organisms observed on a plate by taxonomic level and the side of the settlement plate (n = 16 per bar).

Figure S3. Mean taxa diversity for colonial organisms observed on a plate by taxonomic level and the side of the settlement plate (n = 16 per bar).