# Reviewer 1 Comments

**Comment 1.** Line 44: Perhaps note that NB and BC are in Canada; I would think many people have never heard of New Brunswick and may conflate it with New Brunswick, NJ in the USA.
**Response 1.** The end of this sentence has been edited to: "and the east and west coasts of Canada." (Line 89)

Comment 2. Lines 46-47: Is it possible that they were never identified because of sampling methods and a lack of awareness?
**Response 2:** Absolutely! The subsequent sentence was intended to suggest that the worm could have recently invaded, or been present all along but undetected (i.e. due to lack of awareness and sampling methods, in addition to relatively innocuous infestation rates). I have revised that sentence for clarity: The two sentences now read:

> "Despite previous observations of mud worms in nearby regions such as British Columbia (Bower et al. 1992) and California (Hartman 1961), shellfish growers have not historically identified shell-boring mud worms in Washington State. It is unclear whether the mud worms are recent invaders or have been present but were not previously detected due to low-level infestation, sampling methods or lack of awareness, nor is the state-wide infestation rate yet known." (Lines 92-97)

Comment 3. Lines 163-173: A focus is paced on salinity here, but there are other environmental (biotic and abiotic) conditions that can influence Polydora abundances. Indeed, some of these factors are mentioned later in the text. I think it would be beneficial to provide a review of environmental parameters and Polydora here. If this section gets large, perhaps an additional sub-section would be warranted.
**Response 3:** I added a paragraph that briefly describes the influence of environmental factors other than salinity on mud worm infestation. I still chose to focus most heavily on salinity, as I found several studies concluding that *Polydora* prevalence and infestation rate is influenced by salinity. There is less evidence that other environmental factors (T, DO, pH) influence infestation rates. Regarding siltation, tidal height, and host density, I present these factors briefly in the new paragraph, but then expand on them in the next section when I discuss farm management techniques. You'll also see that I include information on conditions in Washington State (this was suggested by another reviewer). And thank you for pointing out the Medcof (1964) paper, I had not seen that one!

> "Mud worm infestation may differ among locations due to environmental conditions, particularly salinity. Evidence from Nova Scotia, Canada indicates that mud worm infestation intensity in C. virginica and blister size are highest at sites with lowest salinity (Medcof 1946). A recent survey of wild C. virginica in two Gulf of Mexico estuaries found that P. websteri prevalence and abundance decrease with increasing salinity, with a marked drop in infestation at salinities exceeding 28 ppt (Hanley et al. 2019). High infestation rates were reported for C. gigas and C. virginica grown in low- and moderate-salinity locations across Virginia, but infestation rates were much lower in areas with high salinity (Calvo, Luckenbach & Burreson 1999). Mud worm infestation has also been associated with low-salinity environments in the Indian backwater oyster Crassostrea madrasensis (Stephen 1978). In Gulf of Mexico farms, P. websteri was

reportedly least abundant in C. virginica where salinity was most variable (Cole 2018). Whether salinity influences the current Polydora spp. distribution and abundance in Washington State is unknown. Salinity in Washington State estuaries typically ranges from 14–31 psu depending on sub-basin, season, weather, and proximity to river effluent (Babson, Kawase & MacCready 2006; Moore et al. 2008). In some parts of the Puget Sound estuary, for instance, salinity is relatively high and stable, such as in the Southern Puget Sound (26–28 ppt) and Main Puget Sound basins (28–30 ppt) (Babson, Kawase & MacCready 2006; Moore et al. 2008). Salinity is more variable near river mouths, such as in the Skagit River estuary where it typically ranges from 18–28 ppt, but can reach as low as 0.5 ppt (Moore et al. 2008). To understand whether salinity will influence mud worm distribution or prevalence in Washington State, it will be important to document the salinity range and variability on farms with and without mud worm infestations.

Other environmental factors can influence mud worm infestation rates. Higher infestation is associated with higher siltation levels (Clements et al. 2017a; Nell 2007), more densely grown shellfish (Smith 1981), and lower tidal height (Handley & Bergquist 1997; Medcof 1946). Several of these environmental factors, such as tidal height and shellfish density, can be manipulated by Washington State farmers to manage mud worm infestation (described further in the next section). Other factors may influence mud worm prevalence and intensity naturally. For instance, P. websteri infestation is significantly lower in oyster shells exposed to severe acidification (pH 7.0) compared to more alkaline conditions (pH 8.0) (Clements et al. 2017b). Estuaries in Washington and the broader Pacific Northwest region experience periods of low pH due to natural estuarine processes and coastal upwelling, but which are being amplified by acidifying oceans (Feely et al. 2008; Feely et al. 2012). It is possible that carbonate conditions in some parts of Washington State could naturally limit the spread of P. websteri and other mud worm species, although this hypothesis remains to be tested." (Lines 837-886)

Comment 4. Line 192: Just as a heads-up, I suspect there will be a publication coming out soon documenting some interesting Polydora species in Maine using molecular tools that may be applicable for Washington.

**Response 4:** Very good to know! I look forward to seeing that.

Comment 5. Lines 197-201: I'm curious as to whether or not the outbreak has been sustained in recent years? The outbreak we saw in eastern Canada back in 2016 only lasted a single year, and we have not had any reported issues since (we were lucky!). Has this been the case in WA, or has the outbreak remained across multiple years?

**Response 5:** After mud worms were first identified in Washington State (Martinelli et al. 2020), a larger study was funded to survey shellfish farms in Alaska, Washington, Oregon, and California. The first round of sampling occurred in Autumn/Winter 2019, and preliminary results do suggest that infestations are persistent in Washington State (albeit prevalence differs depending on the farm/subbasin). The farms are being re-sampled this spring, and again in autumn 2020 and spring 2021. The preliminary data is not in a place to confidently posit whether worms are still localized around introduction sites and therefore eradication is possible, or if they are more widespread (but the latter is more likely). In its current state, we do not feel confident enough to reference the unpublished data in the current manuscript.

Comment 6. Lines 202-208: This paragraph feels a bit out of place here. Perhaps it would be better suited near the beginning of this section?
**Response 6:** Yes, I agree. This paragraph has moved to the beginning of the section.

Comment 7. Lines 252-253: Off-bottom methods do not always slow oyster growth rates. In fact, depending on localized conditions, they can actually increase growth rates as they do in Atlantic Canada.
**Response 7**: Great insight! The sentence has been revised to the following: “These off-bottom methods have proven effective for avoiding high rates of infestation, but can slow oyster growth rates in some regions … ” (Lines 966-967)

Comment 8. Lines 260-262: And perhaps other potential foulers such as tunicates or hydroids at sites where these pests are problematic
**Response 8:** The sentence has been augmented as follows:

> “Frequent cleaning can also reduce impacts of non-boring spionids, such as P. nuchalis and P. cornuta, and other taxa such as tunicates and hydroids, which foul culture equipment with large masses of organisms, sediment, and tubes (Bailey-Brock 1990; Fitridge et al. 2012).” (Lines 973-976)

Comment 9. Lines 323-324: This is an excellent suggestion! I agree.
**Response 9**: Thank you!

Comment 10. Lines 357 onward: I very much like this progression from global to regional with respect to mitigation - nice job! I wonder if it might be better (from an organizational standpoint) to include the sections from here onward (or perhaps the next 3 sections) as subsections in a larger, overarching "Mitigation" section.
**Response 10:** Definitely, and I received this comment from another reviewer as well. There is now a broader section entitled “Status of Polydora monitoring and regulations”, which has a short introductory paragraph (below) and which contains three subsections: 1) Examples of mitigation strategies globally, 2) Polydora status in the United States, and 3) Live shellfish regulations in Washington State. Section intro:

> “Few countries formally regulate mud worm translocation or monitor outbreaks to mitigate infestations in regions with naturalized populations. The following is a brief discussion of regulatory approaches (or lack thereof) that this review identified at the global and national scales, followed by a more comprehensive survey of existing regulations in Washington State that could be leveraged to control mud worm distribution within the state.” (Lines 1266-1287)

## Reviewer 2 Comments

**Comment 1.**
It is not immediately clear why the authors have focused only on Polydora species, and not also on related species such as Boccardia that have similar life histories and impacts. Either the authors should expand to include shell-boring polydorid species in general, since these are mentioned in Table 1, or they must specify clearly why they are focusing only on Polydora – after all, there is always a chance that Boccardia species or species from other genera may soon be introduced to the area, or may already be there, but at undetectable levels.
**Response 1.**
This is a valid and much appreciated critique. The majority of mud worms identified in Washington State in 2017 by Martinelli et al. 2020 were *P. websteri* and other *Polydora* spp. We therefore felt that focusing on Polydora spp. would provide the most pertinent information to stakeholders. However, farmers do not necessarily care about the species of mud worm infesting their farms, but rather the impact. We therefore broaden our discussion and literature review to include all shell-boring spionids. You'll see that Table 1 has been expanded to include a more comprehensive list of *Dipolydora* and *Boccardia* spp reports in cultured shellfish, and we incorporate additional references where pertinent (for instance in the translocation section we include a discussion of *Boccardia proboscidea* in South Africa). We do still focus slightly on P. websteri, as that was the species positively identified in 2020, and have added the following clarifying statement to the introductory section:

> "We provide information relevant to all boring spionids that infest cultured shellfish, which includes ten species of Polydora, eight Boccardia spp., and three Dipolydora spp. (Table 1). Where pertinent, we focus more heavily on the cosmopolitan invader P. websteri, due to its confirmed presence in the 2017 Puget Sound oyster survey (Martinelli et al. 2020) (Table 1), and its global status as a pest to oyster aquaculture (Radashevsky, Lana & Nalesso 2006)." (Lines 238-242)

**Comment 2.**
Additionally, the authors identify the main Polydora species in Washington as P. websteri, but only intermittently seem to single out this species in their discussions in the different subdivisions. For example, the authors switch between general discussion of larval developmental mode in Polydora species in general and P. websteri in particular, but don't provide any information on what is known on the species in Washington State. This occurs intermittently throughout the manuscript, and I suggest that the authors provide more coherent information regarding the species.
**Response 2**. I hope that you'll find the updated manuscript to more cohesively present information on the generic mud worm. However, where there was ample literature or where I felt it was particularly useful, I still opted to dive deeper into *P. websteri* because the species was detected in Washington State 2017 (confirmed using molecular markers).

**Comment 3.**
It would be useful if the order in which the subdivisions are discussed is re-organised to minimise repetition.

a. For example, the authors discuss the identification of the species AFTER they provide quite a bit of information on the biology. This should, I think, come earlier, to avoid repetition and to better contextualise the specific focus on P. websteri. And on the topic of identification – I am not sure what the point is in repeating the phylogenetic trees from an already published paper; they don't add anything to the paper.

b. Similarly, having a specific subsection on the impacts also repeats quite a bit that is said early on in the manuscript. It should probably be combined with Host Pathology.
c. The monitoring and regulation section can include a brief overview, and subdivisions considering global, USA and Washington examples.

**Response 3.**

A. To provide more context before launching into *Polydora* biology, the section entitled "Recent Polydora identification in Washington State" has been moved up, immediately following the introduction. The trees have been removed from the manuscript. Now that Martinelli et al. (2020) has been published (it was previously under review), I agree that the readers can reference that publication should they be interested in the identified species.
B. The information contained within sections entitled "Impacts to aquaculture production" and "host pathology" have been integrated into one section. This combined section has also been moved earlier in the manuscript, immediately after the identification section, such that it will grab the attention of shellfish aquaculturists, who may be less interested (or distracted) by the worm biology.
C. I received this comment from another reviewer as well. There is now a broader section entitled "Status of Polydora monitoring and regulations", which has a short introductory paragraph (below), followed by three subsections: 1) Examples of mitigation strategies globally, 2) Polydora status in the United States, and 3) Live shellfish regulations in Washington State.

> "Few countries formally regulate mud worm translocation or monitor outbreaks to mitigate infestations in regions with naturalized populations. The following is a brief discussion of regulatory approaches (or lack thereof) that this review identified at the global and national scales, followed by a more comprehensive survey of existing regulations in Washington State that could be leveraged to control mud worm distribution within the state." (Lines 1266-1287)

**Comment 4.**

I also think that it would be better if after each topic is reviewed, the information is contextualised with respect to their occurrence in Washington (and or P. websteri) – for example, the authors summarise the annual cycles in Polydora larvae abundance (seasonal, when high phytoplankton is available), but there are no predictions as to when this may occur in Washington. Check all subsections and recontextualise the information accordingly.

**Response 4.** This is an excellent suggestion. I have included the following additional information that contextualizes the various sections for Washington State:

> "If, instead, Polydora spp. have been present in Washington State for a long period of time but at low levels that until recently escaped detection, the high infestation intensity reported by Martinelli et al. (2020) may be the result of a recent uptick in abundance, caused by factors such as genetic changes, relaxation of biotic pressures (e.g., predators), or environmental changes (e.g., ocean warming, siltation) (Clements et al. 2017a; Crooks 2005). The recent marine heat waves, for instance, that resulted in anomalously elevated ocean temperatures in Washington State from 2014-2016

(Gentemann, Fewings & Garcia-Reyes 2017) may have enabled mud worm outbreaks directly, such as by increasing reproductive output (Blake & Arnofsky 1999; Dorsett 1961), or indirectly due to shifts in trophic ecology (e.g., altered phytoplankton community composition or phenology) (Peterson et al. 2017)." (Lines 321-331)

"Since half-shell oysters are the most lucrative product option for oyster farmers, and mud worm-infested oysters are often not salable on the half-shell market, infestation substantially depreciates oyster products. As Washington State oysters are increasingly prized and marketed for their half-shell presentation (Washington Sea Grant 2015), the state's oyster industry is particularly vulnerable to impacts of widespread mud worm infestations." (Lines 398-402)

"Growth rate in the larval stage depends on ambient water temperature; thus, the time spent in the water column differs among species and across environmental conditions, and may last as long as 85 days (Blake & Arnofsky 1999; Blake & Woodwick 1971). This potential for a long pelagic larval duration, particularly in cooler climates such as Washington State where spring temperatures typically average from 8–14°C, may allow for long dispersal distances (Graham & Bollens 2010; Moore et al. 2008; Simon & Sato-Okoshi 2015)" (Lines 594-600)

"In Washington State, phytoplankton blooms peak in late winter or spring (Horner et al. 2005), but smaller, successive blooms occur throughout the summer and into fall (Nakata & Newton 2000; Winter, Banse & Anderson 1975). It is therefore likely that mud worm larvae will be most abundant in Washington State in the spring but remain present through fall. Studies are needed to identify the seasons of greatest transmission risk and the drivers of high mud worm larval abundance in Washington State. These studies should be prioritized in South Puget Sound where Poydora spp. have already been observed and the majority of oyster aquaculture operations are established." (Lines 749-771)

"Whether salinity influences the current Polydora spp. distribution and abundance in Washington State is unknown. Salinity in Washington State estuaries typically ranges from 14–31 psu depending on sub-basin, season, weather, and proximity to river effluent (Babson, Kawase & MacCready 2006; Moore et al. 2008). In some parts of the Puget Sound estuary, for instance, salinity is relatively high and stable, such as in the Southern Puget Sound (26–28 ppt) and Main Puget Sound basins (28–30 ppt) (Babson, Kawase & MacCready 2006; Moore et al. 2008). Salinity is more variable near river mouths, such as in the Skagit River estuary where it typically ranges from 18–28 ppt, but can reach as low as 0.5 ppt (Moore et al. 2008). To understand whether salinity will influence mud worm distribution or prevalence in Washington State, it will be important to document the salinity range and variability on farms with and without mud worm infestations." (Lines 848-873)

"Other environmental factors can influence mud worm infestation rates. Higher infestation is associated with higher siltation levels (Clements et al. 2017a; Nell 2007), more densely grown shellfish (Smith 1981), and lower tidal height (Handley & Bergquist 1997; Medcof 1946). Several of these environmental factors, such as tidal height and shellfish density, can be manipulated by Washington State farmers to manage mud worm infestation (described further in the next section). Other factors may influence mud worm prevalence and intensity naturally. For instance, P. websteri infestation is significantly lower in oyster shells exposed to severe acidification (pH 7.0) compared to more alkaline conditions (pH 8.0) (Clements et al. 2017b). Estuaries in Washington and the broader Pacific Northwest region experience periods of low pH due to natural estuarine processes and coastal upwelling, but which are being amplified by acidifying oceans (Feely et al. 2008; Feely et al. 2012). It is possible that carbonate conditions in some parts of Washington State could naturally limit the spread of P. websteri and other mud worm species, although this hypothesis remains to be tested." (Lines 874-886)

Comment 5.

Some topics are addressed very superficially.

a. For example, on p10, lines 195-197: But if eradication of P. websteri is not possible, it could still be contained to a few Puget Sound basins through education, mitigation, and regulation (Çinar 2013; Paladini et al. 2017) – expand on the kinds of education, mitigation and regulation programmes you have in mind. Even by referring to a different part of the manuscript.
b. lines 197 – 200, you say: If P. websteri has been present but dormant, the high infestation intensity reported by Martinelli et al. (2019) may be the result of a recent outbreak, caused by factors such as genetic changes, relaxation of biotic pressures (e.g. predators), or environmental changes (e.g., ocean warming, siltation). But you don't expand on this – is there any evidence that predation pressure has relaxed, or that the area has experienced ocean warming and siltation?
c. When you discuss control measures used elsewhere, you discuss quite a few, but only discuss how one (using hypersaline work) may be applied in Washington. I suggest you consider all the options and their applicability – why would exposure during low tide not work in Washington? Why are any of the others applicable or not.

**Response 5.**

a. This section has been refined to more clearly introduce containment strategies (education, management, regulation), then specify that we discuss these in more detail throughout the paper:

    "It is unknown whether Polydora spp. were historically present in Washington State at low abundance or recently introduced. If the species were recently introduced, eradication might be possible (see Williams & Grosholz, 2008 for examples of successful eradication programs), or they could still be contained to a few Puget Sound basins through stakeholder awareness education, farm management, and state-wide regulation, which we discuss in more detail throughout this review (Çinar 2013; Paladini et al. 2017)." (Lines 316-321)

b. I expanded these thought by adding details of a recent environmental perturbation, the NE Pacific Ocean marine heat wave that impacted temperatures throughout the state, and is one candidate trigger for increased mudworm abundance, as follows:

    "The recent marine heat waves, for instance, that resulted in anomalously elevated ocean temperatures in Washington State from 2014-2016 (Gentemann, Fewings & Garcia-Reyes 2017) may have enabled mud worm outbreaks directly, such as by increasing reproductive output (Blake & Arnofsky 1999; Dorsett 1961), or indirectly due to shifts in trophic ecology (e.g., altered phytoplankton community composition or phenology) (Peterson et al. 2017)." (Lines 326-331)

c. This section has been re-organized to focus less on the hypersaline treatment as the best option for Washington farmers. Instead, it presents all treatments and suggests some caveats for farmers (feasibility, applicability for locally grown species and conditions).

Comment 6.
Please check your citations – in some places a lot of information is provided without any citations. At least one citation (Williams and Grosholz, 2008) does not appear in the reference list and in text citations are listed inconsistently – are they meant to be in chronological or alphabetical order?
**Response 6.** In-line citations have all been updated for alphabetical order, and citations have been added (see responses to line-specific comments).

Comment 7.
You need to check the correct identifications of the species cited. You refer to P. ciliata as a borer in multiple places – undoubtedly this is how the original authors of the papers identified them, but you need to acknowledge that these identifications (form various places around the world) are probably incorrect as P. ciliata is not a shell-borer (see Blake and Kudenov 1978 who suggested that at least all records of this species as shell-borer in Australia may actually be of P. websteri. This is also discussed in Simon and Sato-Okoshi 2015). Additionally, P. uncinata was synonymised with P. hoplura (Sato-Okoshi et al. 2017 and Radashevsky et al, 2017).
**Response 7.** Mud worm species taxonomy is indeed a complicated landscape. Your notes were very helpful, and I have incorporated them into Table 1, and to the introduction as follows:

> “It is important to note that mud worm identification is difficult, and there are ongoing debates regarding spionid taxonomic classification. For instance, because P. ciliata is not a shell-boring species, mud worms reported from shellfish and classified as P. ciliata are instead likely to be P. websteri (Blake & Kudenov 1978; see Simon & Sato-Okoshi 2015 for a discussion of commonly mis-identified species). For the purposes of this review, we will refer to the species names as they were reported by the authors.” (Lines 242-248)

Comment 8.
Although the summary includes a brief overview on future research, etc., I feel that the manuscript will benefit from a more structured ‘plan of attack’ for future research, and modified management strategies and regulations. It may help to include a flow chart to show how different information may be collated to this end.
**Response 8.** We had originally included more detailed action items for Washington shellfish stakeholders to control further mud worm spread. However, after mud worms were first identified in Washington State (Martinelli et al. 2020), a larger study was funded to survey shellfish farms in Alaska, Washington, Oregon, and California. The first round of sampling occurred in Autumn/Winter 2019, with additional sampling occurring this spring, autumn 2020 and spring 2021. The preliminary data do suggest that infestations are more widespread across Washington State that previously though, however it is not in a place to confidently posit whether worms are still localized at farms and therefore eradication is possible, or if they are more widespread (but the latter is more likely). Because the situation is uncertain, we do not feel

confident enough to present a roadmap for control or containment at this time. Instead, we hope this manuscript will alert farmers, tribal growers, and managers of the impacts of mud worms.

Comment 9. Line 19: Include: 'and related genera' since it's not only Polydora spp that can have such impacts.
**Response 9**. "And related genera" has been added. (Line 86)

Comment 10. Line 33: Include the authority
**Response 10**. The authority (Hartman) has been added. (Line 79)

**Comment 11**. Line 67: what is the difference between tunnel and burrow? Be consistent. Suggest you rather use burrow as that is the term used more widely.
**Response 11**. All instances of "tunnel" have been replaced with "burrow".

**Comment 12**. Line 87 (re: P. ciliata): Probably incorrect identification
**Response 12**. See Response 7.

Comment 13. Line 91: Is is also possible that it may be related to age? I.e., those that are more infested are actually older?
**Response 13**. Thank you for calling my attention to this reference, and giving me a chance to review it more closely. Upon inspection, I am not confident in the experimental design of Schleyer 1991 to draw conclusions regarding fecundity ~ infestation in S. margaritacea. The more infected / fecund oysters were grown at a different site from the less infected / fecund oysters. While the authors posit that food availability was not likely a factor (based on data from a nearby site, not reported), they do not present other environmental data (exposure time, temperature) from the two locations which may have influenced fecundity. I therefore removed Schleyer 1991 from this section.

Comment 14. Line 103: 'Excavate' is a more appropriate verb.
**Response 14**. "Building" has been replaced with "excavate".

Comment 15. Line 104-106: This doesn't really make sense - boring into the centre (without turning back toward the margin) implies a blind-ending burrow. But infestation by Polydora spp and relatives is usually recognised by the presence of two openings next to each other - and 'entrance' and 'exit'.
**Response 15**. This sentence now reads:

> "After a planktonic larval stage, a burrowing spionid worm settles onto the prospective host's shell margin, and begins to excavate a burrow. Mud worms in the genus Polydora create a characteristic U-shaped burrow, such that two adjacent openings are created at the margin (an "entrance" and an "exit") (Figure 2)." (Lines 530-533)

**Comment 16**. Line 110: not sure how this figure supports or illustrates this point
**Response 16**. This figure reference has been removed. I now reference Figure 3 when discussing the 5th setiger.

**Comment 17**. Line 111: there is at least one later publication by Blake that should also be cited, https://doi.org/10.1201/9781482280159

**Response 17**. The book chapter by Blake 2006 has been included as a reference:

> Blake, J. A., 2006. Spionida. In B. G. M. Jamieson, F. Pleijel, & F. Pleijel (Eds.), Reproductive Biology and Phylogeny of Annelida (pp. 565–638). Taylor & Francis Group.

**Comment 18**. Line 113: fertilised

**Response 18**. “Fertilized” has been added.

**Comment 19**. Line 126: has it been determined whether P websteri in Washington are exclusively planktotrophic, or are they also peocilogenous?

**Response 19**. Haigler (1969) observed two modes of larval development in *P. websteri* when conducting tests in the lab: 1) fertilized eggs develop mostly in sync, and are released as planktivorous larvae. 2) eggs develop at different rates, and hatched larvae remain in the burrow to feed on undeveloped eggs until they are released at a much later larval stage. There have been no studies examining mudworm larval development in Washington state (as they were just recently observed in the state, see [Martinelli et al. 2020](#)). However *P. websteri* was the species examined by Haigler, and has been positively identified in the state. We present therefore present the multiple larval development modes to inform WA growers of the possible larval sources, and have adjusted the language as follows:

> “Additionally, in some spionid species, including P. websteri, early hatched larvae can feed on underdeveloped eggs (“nurse eggs”) and remain in the burrow for a portion of their larval phase (Haigler 1969; Simon & Sato-Okoshi 2015). This can result in mud worm larvae being released at a much later stage. As mud worms colonize hosts during the larval phase, multiple modes of development and stages at release make it possible for larvae to be both locally sourced (e.g., autoinfection or from the same farm) or carried from distant wild or farmed shellfish.” (Lines 600-730)

**Comment 20**. Line 162: But this is not a problem for oysters grown in the water column. Can pollutants affect non-burrowers in a way that will make them more susceptible to infestation?

**Response 20**. To complete this thought and connect possible interactions between pollutatns/stressors and oyster infestation rates, the following has been added to the end of the paragraph:

> “In oysters, exposure to pollutants and other environmental stressors can reduce calcification rates and shell integrity (Frazier 1976; Gazeau et al. 2007; Gifford et al. 2006), which could render them more susceptible to mud worm infestation (Calvo, Luckenbach & Burreson 1999), although this mechanism has yet to be tested.” (Lines 832-836)

**Comment 21**. Line 195: not in reference list

**Response 21.** Williams & Grosholz 2008 has been added to the reference list:

> Williams, S. L., & Grosholz, E. D., 2008. The Invasive Species Challenge in Estuarine and Coastal Environments: Marrying Management and Science. Estuaries and Coasts 31(1): 3–20.

**Comment 22**. Line 197: provide more practical ways by which such measures may be implemented.
**Response 22**. This section has been refined to more clearly introduce containment strategies (education, management, regulation), and specify that we discuss these in more detail throughout the paper:

> "It is unknown whether Polydora spp. were historically present in Washington State at low abundance or recently introduced. If the species were recently introduced, eradication might be possible (see Williams & Grosholz, 2008 for examples of successful eradication programs), or they could still be contained to a few Puget Sound basins through stakeholder awareness education, farm management, and state-wide regulation, which we discuss in more detail throughout this review (Çinar 2013; Paladini et al. 2017)." (Lines 316-321)

Comment 23. Line 200: is there evidence of such changes occurring within the study region?
**Response 23**. I added details of a recent environmental perturbation, the marine heat wave that impacted temperatures throughout the state and is one *possible* impetus for increased mudworm abundance, as follows:

> "The recent marine heat waves, for instance, that resulted in anomalously elevated ocean temperatures in Washington State from 2014-2016 (Gentemann, Fewings & Garcia-Reyes 2017) may have enabled mud worm outbreaks directly, such as by increasing reproductive output (Blake & Arnofsky 1999; Dorsett 1961), or indirectly due to shifts in trophic ecology (e.g., altered phytoplankton community composition or phenology) (Peterson et al. 2017). " (Lines 326-331)

Comment 24. Line 212: please check Simon & Sato-Okoshi 2015 regarding this species in aquaculture
**Response 24**. This sentence has been removed from the manuscript. Also, see response to Comment 7.

Comment 25. Line 214: there is some repetition of the impacts, which have already been mentioned previously
**Response 25**. Indeed. The information contained within sections entitled "Impacts to aquaculture production" and "host pathology" have been integrated into one section and redundancies have been addressed.

Comment 26. Line 218: please check the order of references - should they be chronological or alphabetical?
**Response 26**. In-line citations have all been updated for alphabetical order, and citations have been added (see responses to line-specific comments).

Comment 27. Line 235: References?
**Response 27**. The following citations were added to provide evidence of culturing practices that growers use to control mudworm:

- Morse, D. L., P. D. Rawson & J. N. Kraeuter. 2015. Mud blister worms and oyster aquaculture. Maine Sea Grant Publications. 46. Available at: https://digitalcommons.library.umaine.edu/seagrant_pub/46/
- Nell, J. 2007. Controlling mudworm in oysters. New South Wales Department of Primary Industry Primefact 590. Available at: https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0010/637633/Controlling-mudworm-in-oysters.pdf

Comment 28. Line 261: Can you provide insight into how easily it would be to apply these measures to oyster culture in Washington?
**Response 28**. It is difficult to characterize oyster culture in Washington State, as farms differ considerably in their acreage, remoteness, staff size, and grow methods. I therefore hesitate to tell growers what they can/cannot feasibly do to manage outbreaks. Additionally, so little is known about mud worm physiology and distribution in Washington's environmental conditions, and how oysters grown in Washington will respond to treatments used in other regions, that it is difficult to predict which treatments will be effective in Washington. I therefore present methods that have worked in other regions to provide options that WA growers and extension specialists can test based on intimate knowledge of their farms.

Comment 29. Line 265: references?
**Response 29**. The following references have been included in-line (which I also reference in subsequent sentences, where I provide treatment details):

- Bishop, M. J. & P. J. Hooper. 2005. Flow, stocking density and treatment against Polydora spp.: Influences on nursery growth and mortality of the oysters Crassostrea virginica and C. ariakensis. Aquaculture 246:251–261.
- Brown, Shannon W. 2012. Salinity tolerance of the oyster mudworm Polydora websteri. Honors thesis. Honors college, University of Maine, Orono, Maine. Available at: https://digitalcommons.library.umaine.edu/honors/41
- Cox, B., P. Kosmeyer, W. O'Connor, M. Dove & K. Johnstone. 2012. Oyster over-catch: cold shock treatment. The Seafood CRC Company Ltd, the Fisheries Research and Development Corporation, Port Stephens Fisheries Institute, Industry & Investment NSW and Tasmanian Oyster Research Council Ltd. Project 734. Available at: http://www.frdc.com.au/Archived-Reports/FRDC%20Projects/2010-734-DLD.pdf
- Dunphy, B. J., R. M. G. Wells & A. G. Jeffs. 2005. Polydorid infestation in the flat oyster, Tiostrea chilensis: hyposaline treatment for an aquaculture candidate. Aquac. Int. 13:351–358.
- Gallo-García, M. C., M. G. Ulloa-Gómez & D. E. Godínez-Siordia. 2004. Evaluation of two treatments in polychaete worm intensity associated with Crassostrea gigas (Thunberg, 1873) oyster valves. Cienc. Mar. 30:455–464.

Comment 30. Line 288-290: don't think this is necessary.
**Response 30**. This sentence has been removed.

**Comment 31**. Line 290: such as? Technically speaking, salt is a chemical, so a hypersaline treatment may also be considered a chemical treatment.
**Response 31**. These sentences now read:

"In other regions, non-saline chemical treatments such as calcium hydroxide (lime) and mebendazone have effectively controlled mud worm infestations (Bilboa et al. 2011; Gallo-García, Ulloa-Gómez & Godínez-Siordia 2004). However, environmental, health, and safety regulations will probably preclude chemicals other than salt from being used in Washington State (Morse, Rawson & Kraeuter 2015)." (Lies 1039-1044)

Comment 32. Line 295: provide more detail.

**Response 32**. This sentence now reads:

"Growers incur expenses associated with handling and specialized equipment, such as increasing staff hours to perform treatments, and purchasing refrigerated containers for cold-air storage (Nell 2007)." (Lines 1048-1050)

Comment 33. Line 299: this is a new topic - 1st half of paragraph is about cost.

**Response 33**. I re-structured the paragraph and updated the topic sentence to more cohesively present the caveats associated with *Polydora* treatments methods developed in other regions. The paragraph now reads:

"Treating infested oysters has mitigated the effects of severe infestation in other regions, but this may not be possible for some Washington growers. First, costs can be prohibitive. Growers incur expenses associated with handling and specialized equipment, such as increasing staff hours to perform treatments, and purchasing refrigerated containers for cold-air storage (Nell 2007). Modifying grow methods to accommodate frequent mud worm treatments, or to minimize secondary stressors following treatments, may also be necessary. Treatment costs also depend on re-infection rates, which occur more readily on farms that harbor mud worm reservoirs such as dead oyster shell, and nearby wild and cultured shellfish that cannot themselves be treated (Clements et al. 2018; Lemasson & Knights 2019). Second, many of the existing treatments have been developed for species not commonly grown in Washington State. A common treatment for C. virginica is long-term cold-air storage. Maine growers have found that after 3–4 weeks at ~3°C, 100% of adult mud worms are killed, with minimal C. virginica mortality (Morse, Rawson & Kraeuter 2015). Prolonged air exposure is also commonly used for the Australian oyster S. glomerata (7–10 days, in the shade; Nell 2007). These oyster species have different physiological tolerances than C. gigas, the dominant aquaculture species in Washington, and therefore the same treatments may not be feasible for many of the state's oyster growers (Morse, Rawson & Kraeuter 2015; Nell 2007). For instance, while C. virginica can survive cold-air storage for six months with ~80% survival, no C. gigas seed or adults survived similar cold-air conditions after 20 weeks of storage (Hidu, Chapman & Mook 1988). Irrigating stored C. gigas continuously with seawater can increase survival in cold air storage (52% adults and 80% juveniles at 7°C), but whether irrigation also increases mud worm survival is not known (Seaman 1991). Finally, oyster mortality can be an issue following mud worm treatments regardless of the oyster species (Nell 2007), therefore Washington growers are highly encouraged to test treatments on a small number of oysters before applying it to large batches (Morse, Rawson & Kraeuter 2015). Making adjustments to grow methods might be necessary to improve oyster survival following treatments. For instance, increasing flow rates in a nursery upweller system can increase C. ariakensis and C. virginica survival following hypersaline and drying treatments (Bishop & Hooper

2005). More details and recommendations for treatment options are available in Morse, Rawson & Kraeuter (2015) and Nell (2007)." (Lines 1046-1133)

Comment 34. Line 321: how is this information relevant to the topic under discussion?
**Response 34**. The information regarding non-oyster shellfish host treatments has been removed.

Comment 35. Line 337: this narrative doesn't make sense - you start with the 'recent' case (which occurred 25 y ago, so is hardly recent), and THEN you mention the 1982 paper describing the movement of P. websteri to Hawaii. How do either of these sentences link to the previous one regarding the disappearance of native oysters? are they more examples of the same topic, or of a slightly different topic?
**Response 35**. This paragraph has been re-written for a more intuitive and cohesive narrative:

> "Mud worms have a long history of accompanying shellfish during translocation and becoming invasive pests. In the early 1880's, oysters believed to be infected with P. ciliata were imported from New Zealand into the George's River in Southeast Australia. Before being sold in Australian markets, they were routinely refreshed or fattened in bays adjacent to native shellfish beds (Edgar 2001; Ogburn, White & Mcphee 2007; Roughley 1922). By 1889, mud worm outbreaks had infected thirteen separate estuaries in the region, and oyster growers abandoned leases that were below the low-water mark (Roughley 1922). More recently, mud worms have been introduced to Hawaii via translocated shellfish. P. websteri was probably brought to Oahu via California oyster seed in the 1980's, which resulted in a severe infestation and caused farmers to abandon their land-locked oyster pond (Bailey-Brock & Ringwood 1982; Eldredge 1994). The non-boring Polydora species P. nuchalis was probably introduced to Hawaii in a shipment of shrimp from Mexico, fouling oyster culture ponds with masses of mud tubes (Bailey-Brock 1990). South Africa recently detected P. websteri for the first time in cultured oysters (C. gigas); the invader was probably introduced when juvenile oysters were translocated from Namibia (Simon 2011, 2015; Williams 2015). B. proboscidea has become a pest to abalone farms in South Africa since 2004 when it was first observed burrowing into cultured abalone (Simon et al. 2009). The introduced B. proboscidea presumably originated from the North American Pacific Coast where it is found in the wild benthos (Hartman 1940, 1941; Jaubet et al. 2018; Simon et al. 2009), although the species is now widely distributed throughout the world (Canada, Australia, New Zealand, Argentina, South Africa, Asia, and Europe) (Radashevsky et al. 2019). The presumed origins of introduced mud worms are, however, often based on circumstantial evidence such as documented movement of shellfish stock and the first described locations of mud worm infestations. Researchers are increasingly using molecular markers to compare the genetic structure of introduced mud worms to those in other regions (e.g., comparing mtDNA sequences) (Rice, Lindsay & Rawson 2018; Simon et al. 2009; Williams 2015). These genetic tools, which Martinelli et al. (2020) leveraged to identify the Washington State Polydora spp. in 2017, will be essential to establish the possible origin(s) of the newly identified Washington mud worms." (Lines 1136-1211)

Comment 36. Line 345: inter- or intraregional transport? See also Williams et al. 2016
**Response 36**. Thank you for pointing me to Williams et al. 2016 for this section; the sentence now reads:

> “As shellfish farmers grow oysters in high-density bags, racks, or lines, a mud worm infestation can spread readily within a farm, and the subsequent movement of stock is considered the primary pathway for mud worm introductions both within and between regions (Moreno, Neill & Rozbaczylo 2006; Rice, Lindsay & Rawson 2018; Simon & Sato-Okoshi 2015; Williams, Matthee & Simon 2016).” (Lines 1250-1254)

Comment 37. Line 357: this seems like a very random subsection, with no contextualisation.
**Response 37**. There is now a broader section entitled “Status of Polydora monitoring and regulations”, which has a short introductory paragraph and which contains three subsections: 1) Examples of mitigation strategies globally, 2) Polydora status in the United States, and 3) Live shellfish regulations in Washington State.

Comment 38. Line 358: I'm not sure about this statement - the first records of loss of cultured or commercially harvested oysters to Polydora spp dates to the 1880s, but that does not meant that Polydora spp were introduced then.
**Response 38**. Much of the literature indicates that Polydora was introduced from New Zealand, however I agree that this can not be stated unequivocally, therefore the sentence has been revised to:

> “In Australia, mud worms have been common since the early 1800’s, and while they are not listed as invasive species, they are considered serious pests to abalone and oyster growers (Nell 1993; Nell 2001).” (Lines 1291-1293)

Comment 39. Line 375: citation!
**Response 39**. Nell 1993 and 2001 have been added as references.

Comment 40. Line 412: you already say this in line 409
**Response 40**. Good catch, the previous sentence (line 409) has been removed.

Comment 41. Line 416: the abbreviation must be used the first time the name is provided in full. this abbreviation has already been used multiple times in this paragraph.
**Response 41**. “Washington State Department of Fish and Wildlife” is now earlier in the paragraph at the first instance of WDFS.

Comment 42. Line 457: do you have any citations to support these statements? Additionally, Williams et al 2016 provide molecular evidence for intraregional movement of polydorid spp with infested oysters and other molluscs. You may want to cite that here too.
**Response 42**. The below references describing hatchery practices have been included. Additionally, this paper’s co-authors include authorities on aquaculture practices and translocation in Washington State: Teri King, the Aquaculture Specialist and Coordinator for Washington Sea Grant, and Brady Blake, the Shellfish Disease and Pest Prevention program specialist with Washington Department of Fish and Wildlife.

- Breese, W. P., & R. E. Malouf. 1975. Hatchery manual for the Pacific oyster. https://ir.library.oregonstate.edu/downloads/db78td066
- Toba D 2002. Small-Scale Oyster Farming for Pleasure and Profit in Washington. Washington Sea Grant program, Seattle, WA. Available at the url: https://wsg.washington.edu/wordpress/wp-content/uploads/publications/Small-Scale-Oyster-Farming.pdf

**Comments in Table 1**:

- Re: *P. uncinata* in SW Australia: *P. hoplura*
    - **Response:** Table now includes a note on P. uncinata
- It must be acknowledged somewhere that reports of P. ciliata as shell-borers are probably incorrect, since P. ciliata is a fouler, not a borer.
    - **Response:** Table now includes a note on P. ciliata. Also, please see response to Comment 7.
- Re: P. variegata in Abashiri Bay: double check - It is possible that this may in fact be P. brevipalpa (see Teramoto et al. 2013)
    - **Response:** A note on this possible misidentification has been added to the table.
- Re: P. uncinata in Japan (Sato-Okoshi & Abe 2012): Please note that P. uncinata has been synonymised with P. hoplura. See Sato-Okoshi et al. 2017 and Radashevsky et al. 2017
    - **Response:** Thank you for this clarification; a note has been added to the table.
- Re: *P. hoplura* in South Africa: These place names are used incorrectly - these are biogreographic breaks - Kleinzee, Paternoster & Saldanha Bay are to the NW of Cape Point, whereare Port Elizabeth is east of Cape Agulhas.
    - **Response:** The biogeographic breaks have been removed from the table, and only the place names remain.

Figure 3 Comment: poor image
**Figure 3 Response**: The image has been replaced with a new image, selected to best represent what a grower might see when looking at worms under the microscope. In the figure caption we point to the scanning electron microscope images published in Martinelli et al. 2020, should the reader seek higher resolution images.

Figure 4 Comment: What is LI? what do you mean by a unident. polydorin?
**Figure 4 Response**: The trees have been removed.