The Effects of Water Turbidity on the Re-emergence Behaviour of *Eudistylia*vancouveri

by

Finn Grundy, Lynndsay Terpsma, Rhys Pfuetzner
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Instructor: Christina Bowhay

Teaching Assistants: Dani Jakovljevic, Emily Wharin, Maggie Slein

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Home University: University of Calgary

© Lynndsay Terpsma, 07/2024 Home University: University of British Columbia - Okanagan

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Home University: University of British Columbia

Abstract

The northern feather duster worm, Eudistylia vancouveri (Order Sabillida) is a species of tube-dwelling, filter-feeding polychaete that feeds with its featherlike radioles. As a predator response, they use their hooked setae to quickly retract their delicate branchial crown into their hard-sided tubes. While there are many studies describing their physiology, limited research has been conducted on the behavioural changes of Sabellid worms in response to abiotic factors. Through laboratory experiments, we investigated whether the re-emergence time of *Eudistylia vancouveri* is affected by increased seawater turbidity. Feather duster worms, collected from Bamfield Inlet, British Columbia, were exposed to increased levels of suspended sediment for one hour before the retraction response was triggered by tactile stimulus with a human hand. After the worms' retraction response was induced, they were found to reemerge quicker in conditions with high suspended sediment levels than in clear conditions (12.9% decrease in re-emergence time). This reduced retraction response may increase predation events on the radioles of wild Sabellid populations that are exposed to high turbidity levels, either from natural or anthropomorphic sources. These results contribute to understanding the behaviour of benthic ciliary filter feeders in response to high water turbidity which is prevalent in the coastal ecosystems where they live.

Keywords

Sabellidae, Annelida, polychaete, tube worm, behaviour, invertebrate

Introduction

Sessile invertebrates have unique mechanisms and behaviours for predator avoidance. *Eudistylia vancouveri* is no exception, with its radioles forming feather-like branchial crowns, which are used for respiration and the consumption of microscopic particles (Bok et al., 2016; Merz, 1984). However, the sessile lifestyle of *E. vancouveri* leaves them vulnerable to predators when their radioles are exposed (Chen et al., 2023). To generate habitat and shelter from predators, the worms create tubes using mucus and sand (Rudy Jr. & Rudy, 1983). The worm can completely withdraw into these tubes using their hooked setae that they embed into their tube walls (Merz and Woodin, 2000). The withdrawal effect of the worms is often triggered by various stimuli, including mechanical, vibrational, water currents, and light (Nicol, 1948). The tubes provide the worms with a quick escape route from predators but when they withdraw they sacrifice the ability to respirate and feed (Giangrande, 1991).

Sabellid worms are known to decrease their retraction response to habituate to their environments. Krasne (1965) found that repeated tactile stimulation caused a slower and more delayed withdrawal until the retraction response was negated altogether. He also found that re-emergence happened quicker, and partial over complete withdrawal predominated after repeated stimulus. This suggests that the increase in mechanical stimuli generated from suspended sediment may cause the worms to decrease their reaction similar to what was found by Krasne (1965). Furthermore, E. vancouveri uptake 75% of their oxygen through their branchial crowns which indicates that they may be more likely to be exposed despite stimulus to ensure they get ample oxygen uptake (Giangrande, 1991). While many studies have described the physiology of Sabellid species, they are still an extremely understudied family. There has been very limited research done into the effects of abiotic factors on Sabellid health and behaviour. The only research done on the effects of water quality on Sabellids showed that they can bioaccumulate heavy metals (Popham & D'Auria, 1982; Young et al., 1979). However, no research has been conducted on how this affects their behaviour or what effects other aspects of water quality like turbidity have. Furthermore, the relationship between time spent hiding and tube diameter has been inconclusive in Sabellids. In other invertebrates, there is a suggestion that smaller-sized organisms spend more time hiding than their larger counterparts (Bateman & Fleming, 2015; Dill & Gillett, 1991; Markich, 2003).

Human activity such as habitat destruction, pollution, and boat activity increase water turbidity by increasing the amount of disturbed sediments (Singh et al., 2022; Safak et al., 2021). Additionally, increased water turbidity has been known to affect the behaviour and health of other benthic invertebrates with similar filter-feeding strategies to *E. vancouveri*. Ellis et al. (2002) found that increased turbidity decreased the rate that bivalves filter feed and was related to a worsening of their physiological condition. Glass sponges (hexactinellids), although they have no retraction behaviour, completely shut down their feeding when exposed to highly turbid environments to prevent the canal system from becoming clogged (Merz, 1984; Tompkins-MacDonald & Leys, 2008). No research has been done into how this would affect the behaviour of Sabellid worms, but

given their similarity to the filter-feeding and respiration behaviour of other studied organisms, they may experience similar negative effects to high levels of sediment suspended in the water column. Understanding the relationship between turbidity and behaviour is critical because Sabellid worms like *E. vancouveri* feed on particles smaller than other filter feeders consume (<6µm in size), making them a key component in the food chain of marine communities (Merz, 1984). This niche is also filled by sponges, the only other organisms to feed on particles this small (Yahel et al., 2006). However, if sedimentation causes the demise of both sponges and feather duster worms, nutrients from bacterioplankton would not be able to be cycled back into the ocean food web and would instead fall as marine snow (Frangoulis et al., 2010). Moreover, *E. vancouveri* has been described as an indicator species, meaning they can provide insight into the health of our marine ecosystems (Popham & D'Auria, 1982; Young et al., 1979).

In this study, we examine whether the rate of re-emergence after being startled with mechanical stimuli in E. vancouveri decreases in response to increasing seawater turbidity. We observe this behaviour in a laboratory setting with *E. vancouveri* worms after short-term (1 hour) exposure to different turbidity levels. We used 3 concentrations of suspended sediment in seawater: filtered seawater with negligible turbidity, 11.5 Nephelometric Turbidity Unit (NTU) seawater, and 20.5 NTU seawater. We hypothesized that the re-emergence time of *E. vancouveri* will decrease as water turbidity increases due to the worms becoming habituated to repeated tactile stimulation and our results support this hypothesis. We found that as water turbidity increased, the amount of time for the worms to re-emerge from their tube decreased. We also found that re-emergence time was not significantly impacted by tube diameter. Our findings could help connect benthic community health in harbours and coastal ecosystems with sediment input and disturbance. Our research shows that *E. vancouveri* are highly sensitive to at least one component of water quality and opens up potential ecological research avenues into studying the effect of other abiotic factors such as temperature, nutrient concentrations, dissolved oxygen, and pH on Sabellid physiology and behaviour.

Materials and Methods

Animal Husbandry

For this project, wild specimens were collected opportunistically from three different docks within the Bamfield Inlet, British Columbia, Canada (Figure 1). Twenty-nine worm tubes were collected at the Bamfield Marine Sciences Centre (BMSC) south docks (48.83348°N, -125.13687°W). At the Island Health dock (48.82712°N, -125.13730°W), a total of eighty-seven worms were collected, with approximately 16 individual tubes removed from the side of the wooden dock, while the other 71 worms were collected in a large hummock from the underside of a dock ladder. At the West Government dock (48.82912°N, -125.14008°W), eleven individual worm tubes were collected. A total of 127 tube worms were collected for experiments. After each collection, worms were temporarily stored in three 5-gallon buckets (one bucket per location) filled with seawater from the Bamfield inlet until they could be brought back to the lab. This water ranged from 11-13°C and was refreshed every 15 minutes by hand to maintain cold temperatures during the warm day (K. Bartlett. Conversation. 16 July 2024. pers. comm.).

Upon return to the lab, groups of tubes were separated into individuals. Each worm was labelled with colourful flagging tape marked with the worm's corresponding identification. Each worm was randomly tied to one of nine pieces of mesh (three for each experimental treatment) and weighed down by rocks in a large seatable. The mesh was used to organize the worms into experimental groups of manageable sizes to conduct trials. This seatable was filled with filtered seawater pumped from the Bamfield Inlet, 30m below sea level, which was left on a constant gentle flow. Two airstones were kept in this tank to ensure maximum oxygen supply and animal care checks were conducted twice daily to ensure proper flow and air supply. This tank was where worms were kept while experiments were not being conducted (Figure 2a). The worms were left for two days undisturbed after collection and setup. The worms were fed with 2 tablespoons of agal paste with approximately 500 mL of *Artemia* sp. two days after collection and were allowed 30 minutes without water flow so they could adequately

feed. The worms were also fed 2 hours prior to release with the same mixture of Artemia and algal paste. To return the worms, individuals were tied together into clusters using fishing line and reattached to the dock of their original collection. All northern feather duster worms were collected and released in accordance with the Fisheries and Oceans Canada (DFO) scientific collection license (XE 6 2024) and introduction or transfer (ITC) permit (138612).

Experimental Design

To determine the relationship between the water turbidity and the re-emergence behaviour of northern feather duster worms, the worms from each collection site were randomly assigned to one of three experimental groups (no sediment, moderate sediment, and high sediment) (Table 1). 4 of the collected worms were excluded from the study either due to mortality or to differences in species, resulting in a total sample size of 123. These groups were established in 45 litre (L) plastic tubs filled with the same filtered seawater (11-13°C) in the holding tank (Figure 2b). Tanks were placed in approximately 15 cm of circulating seawater in a separate sea table to maintain water temperature. Furthermore, water temperatures were checked at both the morning and evening animal checks with a digital thermometer. To create flow in the experimental tanks and keep sediment suspended in the water column, aguarium fans were placed in each group and pointed downwards. To increase water turbidity in the two non-control groups muddy, fine sediment was collected from two locations in the Bamfield Inlet (48.817517°N, -125.152287°W; 48.816028°N, -125.156111°W) and brought back to BMSC. The sediment was sieved through a 2mm and then a 0.225mm sieve while being rinsed with seawater. This sediment and water mixture was then autoclaved to remove harmful organic material (Mustapha et al., 2021). Sediment was added to the two treatment groups, with the turbidity of the moderate sediment group being approximately 11.5 NTUs and the high sediment group being approximately 20.5 NTUs. This was measured using a homemade transparency tube, constructed of a 2000mL graduated cylinder. These levels were determined based on the capabilities of our turbidity measurement instruments. One hour prior to trials, the corresponding worms were transferred to the experimental tanks. During the one-hour acclimation period,

airstones were added to the tanks to ensure ample oxygen supply and tanks were covered to limit light disturbances. After the acclimatization period, tank covers and airstones were removed and a Sony Handycam HDR-CX550V camcorder was set up over the control group. Once recording had begun, all worms in the tank were startled by the touch of a human hand, causing their retraction. Tanks were then recorded for 10 minutes to observe re-emergence. After the ten-minute period, the recording would be stopped and the process would be repeated for the moderate sediment group. Finally, the high sediment group experiment would be completed. Following the tests, the worms were returned to the large sea table with flow-through water and were allowed at least 20 hours before their next trial. Experiments were split into 3 groups per day (nine groups of worms, three per experimental group total). Video recordings were analyzed for six behavioural patterns following the completion of trials. Individuals were monitored for complete or partial retraction, complete or partial re-emergence, complete exposure, and complete hiding (Figure 3). Additionally, the opening diameter of each worm was recorded using calipers in cm to two decimal places, as previous studies have shown a correlation between the size and hiding time which we can presume E. vancouveri will follow (Bateman & Fleming, 2015; Dill & Gillett, 1991; Markich, 2003).

Statistical Analyses

Data from video recordings was inputted into a shared Google Sheets document, which was then downloaded as a comma-separated value document (.csv) for analysis. Worms that did not re-emerge within the 10 minutes of recording were assigned a time of 600 seconds (Dill & Fraser, 1997). Worms that did not retract after stimulus were assigned a time of 0s. Finally, worms that only partially retracted or re-emerged were excluded from the analysis. Time to re-emerge was compared between the three experimental groups using a mixed effects model as well as a Type II Wald chi-square ANOVA test. All data analysis and figure creation was completed in R v4.4.1 (R Core Team, 2024). The dplyr package was used to analyze the relative frequencies of behavioural categories that were not analyzed through statistical tests (Wickham, 2023). The relative frequency was calculated at the count of behaviour observations (subgroup

frequency), divided by the total number of observations for that behaviour (total frequency), for each behavioural code (Equation 1).

$$relative\ frequency = \frac{(subgroup\ frequency)}{(total\ frequency)}$$
 (Eq. 1)

The Ime4 and base statistics R packages were used to create the generalized linear model with a Poisson error distribution (Bates, 2015; R Core Team, 2024). We conducted a mixed-effects model, with fixed effects for re-emergence time, experimental groups, and tube diameter. Random effects for the model were individual worms and trial number. The car package was used to complete an ANOVA statistical test on the model data (Fox, 2019). The ggplot2 package was used to create a violin plot with the natural logarithm transformed data (Wickham, 2016). A significance level of 0.05 was used for all p-values. Tutorials from Dr. Jason Pither (UBCO) were used as supplementary to the analysis (Pither, 2022).

Results

Turbidity data was gathered in the field using a Secchi disk at the time of collection and return of the specimens. The BMSC south docks (48.83348°N, -125.13687°W) had a Secchi depth of 3.15m, the Island Health dock (48.82712°N, -125.13730°W) had a Secchi depth of 3.22m, and the West Government dock (48.82912°N, -125.14008°W) had a Secchi depth of 3.50m. However, although Secchi depth and NTUs are highly correlated, there is no conversion factor to directly relate the two, as Secchi depth provides a measure of water clarity while NTUs directly describe the amount of light scatter in a water sample (Baughman et al., 2015).

Six behaviours were observed throughout the course of the study, while only two were used in the statistical analysis. The partial retraction (PR) behaviour was observed 21-56% of the time (Control: 56%, Moderate: 23%, High: 21%) throughout all four days of experiments (Table 2). The partial re-emergence (PE) behaviour was seen between 30-36% of the time (Control: 30%, Moderate: 36%, High: 34%; Table 3). The exposed (EX) behaviour was observed between 22-56% (Control: 22%, Moderate: 56%, High:

22%, Table 4). The hidden (HI) behaviour was seen between 17-50% of the time throughout the four days of trials (Control: 50%, Moderate: 33%, High: 17%, Table 5). Further, after observing the worms for four days, it appeared that worms beside each other would begin to re-emerge at the same time.

E. vancouveri re-emergence time was significantly influenced by turbidity, with individuals in more turbid water being faster to re-emerge than those in less turbid conditions (Figure 4). Re-emergence time in the moderate and high experimental groups was 13.61% and 12.92% faster than the control group, respectively (Figure 4). The control group from the model showed an estimate of 4.72 seconds (95% CI: 4.18 -5.25 seconds). The moderate sediment group had a decrease in re-emergence time of 1.03 seconds (4.0776 seconds; p = 0.001; 95% CI: 3.69 - 4.47 seconds) when compared to the control. The high sediment group had a decrease of 1.01 seconds relative to the control (4.11 seconds; p = 0.003; 95% CI: 3.71 - 4.52 seconds). Additionally, we ran a Type II Wald chi-square test on this model, which concluded that the decrease in re-emergence time due to the treatment group was significant (χ^2 = 13.167, df = 2, p = 0.001; Table 6). Tube diameter had no significant contribution to the model (χ^2 = 0.729, df = 1, p = 0.393; Table 6). The use of a Type II Wald chi-square ANOVA test was appropriate for the data as it did not meet the assumptions of normal distribution or homogeneity of variance needed for a classic One-Way ANOVA. A Type II Wald chi-squared test allowed for more flexibility with the data analysis, given the non-normal distribution. Further, it allowed us to consider multiple predictors, such as experimental group and tube diameter.

Discussion

This study investigated the relationship between turbidity levels and re-emergence time in *E. vancouveri* and found that after complete retraction in response to mechanical stimuli, *E. vancouveri* re-emerged from their tube 12.92 to 13.61% faster than in clear seawater, supporting our hypothesis that re-emergence time inversely proportional to increasing turbidity in seawater (Table 6; Figure 4). Previous literature has demonstrated an inverse relationship between the size of an organism and the amount

of time spent hiding (Bateman & Fleming, 2015; Dill & Gillett; 1991). However, this particular study did not find a significant relationship between the diameter of *E. vancouveri* and the rate of re-emergence (Table 6). Throughout the experiment, we observed a total of six behaviours, but only two of these behaviours were statistically analyzed. While partial re-emergence (PE), exposed (EX) and hidden (HI) behaviours showed no trend in frequency, there was a pattern observed in the partial retraction (PR) behaviour. It was observed that the frequency of partial retraction decreased as the sediment content of the experiment group increased (Table 2). The other behaviours showed no identifiable trend (Table 3; Table 4; Table 5).

The Secchi depths measured at each of the collection sites cannot be directly related to NTUs as they only provide an estimate of water clarity, but they can be used to qualitatively compare the conditions of the worm's natural habitat to that of the experimental treatments (Baughman et al., 2015). However, they can be used to qualitatively assess the impact of the turbidity levels experienced by *E. vancouveri* within the Bamfield Inlet. The average Secchi depth collected at all three sites was 3.29m, while the height measured in a turbidity tube for the moderate treatment was 34.2cm, and the high turbidity treatment group had a height of 49.2cm. Using this information we can infer that the turbidity experienced and behaviours exhibited by wild specimens in the Bamfield Inlet is closer to that of the control group than the experimental treatments.

Sabellid worms like the northern feather duster worms (*E. vancouveri*) have been shown to retract into their parchment tube in response to mechanical stimuli (Chen et al., 2023). Previous research conducted on the hiding behaviours of another sessile filter feeder, the North American acorn barnacle (*Balanus glandula*) showed that the amount of time spent hiding was determined as a trade-off between the risk of being preyed upon and the requirement to extend their cirri for feeding and respiration (Dill & Gillett; 1991). No research has been done into how the retraction reflex is affected by turbidity in *E. vancouveri*. Turbidity is an important anthropogenic factor that may influence trophic interactions, species distributions, ecosystem function, and biodiversity

of many aquatic ecosystems such as those where *E. vancouveri* are found (Singh et al., 2022; Safak et al., 2021; Lunt & Smee, 2020). Our study is the first to demonstrate the effect of turbidity on the re-emergence of *E. vancouveri*.

Human activity such as habitat destruction, pollution, and boat activity is one of the driving forces influencing water turbidity as sediments are disturbed in all of these processes (Singh et al., 2022; Safak et al., 2021). Both forestry practices and wildfire disturbance in British Columbia have been shown to dramatically increase the amount of sediment deposited into river basins (Jordan, 2006; lelpi & Lapotre, 2023). Many of BC's watersheds lead into the Salish Sea and the waters of the Central Coast where E. vancouveri are abundant (Rudy Jr. & Rudy, 1983). The effect of suspended sediment on the respiration of E. vancouveri is unknown, but we have found that turbid conditions do result in a decrease in time that the worm spends with the radioles retracted (Figure 4). One potential reason for this behavioural change is that turbid water may negatively affect the respiratory abilities of *E. vancouveri*. These negative respiratory effects, such as clogging of the respiratory structures, could limit the amount of oxygen that E. vancouveri are able to absorb with their radioles. Additionally, increased turbidity is correlated with a lower dissolved oxygen concentration, as the suspended sediment limits the depth that light can penetrate to, impeding photosynthesis (United States Environmental Protection Agency, 2021). Furthermore, 75% of the total oxygen absorption in E. vancouveri is completed through the extended radioles (Giangrande, 1991). Turbid environments have been found to have higher energetic demands, requiring increased rates of respiration for invertebrates (Jones et al., 2015). Because of this, they are not able to spend as much time retracted in their tube, leading to the observed behavioural changes (Figure 3). Decreased time spent with the radioles hidden within the tube after retraction increases the risk of death by predation, as less time is given for the predator to move on, causing concern over the stability of the population.

Another potential mechanism to explain the change in re-emergence time is the habituation of the worms to mechanical stimuli from the particles in the water (Krasne,

1965). The higher turbidity causes the branchial crowns, when extended, to be in constant contact with sediment in the water. Due to the tendency of Sabellid worms to habituate to repeated stimuli, the small experimental particles may eventually be perceived as less of a potential threat and as a result, the time taken to re-emerge decreases (Ewing, 2009; Vanderleest, 2012; Nicol, 1950; Krasne, 1965). Further, there is a possibility that the constant stimulus may mask any other potential tactile stimuli or sensory cues (by decreasing the amount of light penetrating through the water column) in the water, causing the worms to re-emerge faster. Mechanisms in Sabellid worms for distinguishing between different forms of tactile stimulus are currently unknown and warrants further research into understanding how different levels of turbidity may affect these filter feeders.

Although the sediment used to increase the turbidity of the experimental treatments was autoclaved to remove organic material, the water in the plastic bins experienced a decrease in NTU value after each trial was conducted. This decrease was not experienced when the treatment water was prepared and left overnight, showing that the decrease only occurred after the worms were added to the tanks. This may be caused by a variety of reasons, including the worms consuming the particles for feeding or tube building or the sediment becoming stuck in the aquarium fans. If the decrease was caused by the worms feeding on the particles, the presence of food in the water may be causing the change in behaviour observed. *E. vancouveri* have been found to habituate to mechanical stimuli faster when exposed to high concentrations of nutrients, as the increased food availability outweighs the threat of predation (Vanderleest, 2012). However, higher turbidity has been found to reduce the foraging efficiency of suspension feeders, leaving unanswered questions about the mechanisms causing decreased suspended particles in the water (Lunt & Smee, 2020).

It is unknown whether a greater need for respiration, habituation to mechanical stimuli, or increased nutrient availability is causing the faster re-emergence time, but these findings open more questions about the cues that influence retraction and re-emergence in *E. vancouveri*. Regardless of the primary mechanism, individuals who

spend less time hiding in their parchment tube after mechanical stimuli will also be more vulnerable to predation, potentially leading to a population decrease. Significant changes to the abundance of this species may have cascading effects on the ecosystem as a whole, as *E. vancouveri* create habitats for epibiont organisms with their mucous tubes and their decline may cause a major loss in biodiversity (Bracken, 2018). It would also signify a decline in the health of the ocean ecosystem as they are considered to be an indicator species (Popham & D'Auria, 1982; Young et al., 1979)). Northern feather duster worms help cycle organic matter back into the food web by consuming particles 0.5 µm in diameter, a size range accessed only by Sabellid worms and sponges, whose loss would lead to a disturbance in trophic levels (Vijayan et al., 2019; Merz, 1984).

Worms collected from the BMSC South Dock may have been more habituated to non-harmful mechanical stimuli due to being collected from a dock used in research and field trips, and have likely experienced higher amounts of non-threatening touch from humans. However, we did not collect any individuals from lines specifically used for the field trip program, which receive frequent tactile stimulation and brief removal from the water. The organisms not specifically used by field trips likely also experienced a high amount of mechanical stimuli from BMSC students and visitors, however, at the time collections took place, it had been two months since any school programs had run. *E. vancouveri* have been shown to habituate quickly, meaning they were likely no longer accustomed to frequent mechanical stimuli at the time of this experiment (Ewing, 2009; Vanderleest, 2012; Nicol, 1950). Many individuals were also collected from other sites, limiting the possibility of this to skew the results.

Changes in light levels may have affected our results, as we were unable to maintain constant light levels during our acclimatization periods or trials. Acclimatization and trials were initially conducted with the lights off using only a red light headlamp to provide lighting during recordings but the difficulties experienced during video analysis as well as multiple instances of people other than the research team entering the room and turning the lights on caused this process to be abandoned. The decision was made

to discount light as a potential factor in the re-emergence rate as a sudden increase in light has been shown to not cause a retraction response in Sabellid tubeworms (Nicol, 1950). The potential confounding effects of light were combatted by staying away from the experimental tanks during the recordings of the trials to limit shadows. The lights were also never turned off during the trials to limit retraction from sudden decreases in light, and the tanks were covered during the acclimatization periods to limit changes in light levels experienced by the worms.

In conclusion, this investigation found that the re-emergence of northern feather duster worms was significantly affected by the turbidity of the water they reside in. The data collected provides support for the hypothesis that water turbidity affects re-emergence time. More research could be conducted in the future to determine if this trend continues with higher levels of turbidity than we were able to achieve in this study. Gaining a better understanding of how *E. vancouveri* is affected by various anthropogenic and abiotic factors is beneficial, because as a biofouling and sessile filter-feeding species, *E. vancouveri* is especially sensitive to anthropogenic forces, and is, therefore, a good model for gauging how anthropogenic forces, such as boat traffic, impact the base of the food chain (Vijayan et al., 2019; Popham & D'Auria, 1982).

EUDISTYLIA VANCOUVERI

Ephemeral, the feather-like branchial crowns

<u>U</u>ndulate with the waves

<u>D</u>evouring

<u>Insignificant</u>

Sediment and plankton.

They are meager creatures,

You are free to ignore the

Little worms tucked

In the docks under your feet

Alive; quiet but not still

Vacant tubes

Attract forgotten creatures that would be

Naked without the hollow

Covering the worm's memory provides.

Outside the tube

Uncertainty and peril drift near. Whether it be with

Valor or foolishness, the

Echo of the worm endures

Reaching out its delicate arms,

Inviting the unknown

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Author Contributions

LT directed the report writing and performed the statistical analysis, figure preparation, and camera handling. FG collected specimens, analyzed videos, and took turbidity measurements. RP led citation management and contributed to project design, writing, and group morale. All authors carried out the experimental testing, contributed to animal care and video analysis, participated in writing the report, developed the experimental design, and collected the specimens.

References

- Bateman, P. W., Fleming, P. A., 2015. Escape behaviour in shore crabs: constraints of body size and available shelter. Journal of Zoology. 297(4), 265–269. doi: https://doi.org/10.1111/jzo.12276
- Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using Ime4. Journal of Statistical Software. 67(1), 1-48. doi:10.18637/jss.v067.i01
- Baughman, C. A., Jones, B. M., Bartz, K. K., Young, D. B., Zimmerman, C. E., 2015. Reconstructing Turbidity in a Glacially Influenced Lake Using the Landsat TM and ETM+ Surface Reflectance Climate Data Record Archive, Lake Clark, Alaska. Remote Sensing (Basel, Switzerland). 7(10), 13692–13710. doi: https://doi.org/10.3390/rs71013692
- Bok, M. J., Capa, M., Nilsson, D.-E., 2016. Here, There and Everywhere: The Radiolar Eyes of Fan Worms (Annelida, Sabellidae). Integrative and Comparative Biology. 56(5), 784–795. doi: https://doi.org/10.1093/icb/icw089
- Bracken, M. E. S., 2018. When one foundation species supports another: Tubeworms facilitate an extensive kelp bed in a soft-sediment habitat. Ecosphere. 9(9), e02429. doi: https://doi.org/10.1002/ecs2.2429
- Chen, Z., Wei, J., Jiang, W., Gorb, S. N., Jia, Y., Zhang, Y., Wu, J., 2023. Multi-leveled locomotion system of the fan worm facilitates underwater crawling inside a slippery tube. Cell Reports Physical Science. 4(12), 101728. doi: https://doi.org/10.1016/j.xcrp.2023.101728
- Dill, L. M., Fraser, A. H. G., 1997. The worm re-turns: Hiding behavior of a tube-dwelling marine polychaete, *Serpula vermicularis*. Behavioral Ecology. 8(2), 186–193. doi: https://doi.org/10.1093/beheco/8.2.186
- Dill, L. M., Gillett, J. F., 1991. The economic logic of barnacle *Balanus glandula (Darwin)* hiding behavior. Journal of Experimental Marine Biology and Ecology. *153*(1), 115–127. doi: https://doi.org/10.1016/S0022-0981(05)80010-3
- Ellis, J., Cummings, V., Hewitt, J., Thrush, S., Norkko, A., 2002. Determining effects of suspended sediment on condition of a suspension feeding bivalve (*Atrina zelandica*): results of a survey, a laboratory experiment and a field transplant experiment. Journal of Experimental Marine Biology and Ecology. 267(2), 147–174. doi: https://doi.org/10.1016/S0022-0981(01)00355-0
- Ewing, N. 2009. Habituation of the withdrawal response to wave exposure in the tube-dwelling polychaete *Eudistylia vancouveri*. Instructor Dr. Marjorie Wonham. Fall program, *331*, Bamfield Marine Sciences Centre, Bamfield, BC. (Unpublished report on file at the BMSC Library).
- Fox J, Weisberg S., 2019. An R Companion to Applied Regression, Third edition. Sage, Thousand Oaks CA. Electronic document: https://socialsciences.mcmaster.ca/jfox/Books/Companion/ accessed 2024-07-25
- Frangoulis, C., Psarra, S., Zervakis, V., Meador, T., Mara, P., Gogou, A., Zervoudaki, S., Giannakourou, A., Pitta, P., Lagaria, A., Krasakopoulou, E., & Siokou-Frangou, I., 2010. Connecting export fluxes to plankton food-web efficiency in the Black Sea waters inflowing into the Mediterranean Sea. Journal of Plankton Research. 32(8), 1203–1216. doi: https://doi.org/10.1093/plankt/fbq010
- Giangrande, A., 1991. Behaviour, irrigation and respiration in *Eudistylia vancouveri*

- (Polychaeta: Sabellidae). Journal of the Marine Biological Association of the United Kingdom. 71(1), 27–35. doi: https://doi.org/10.1017/S002531540003736X
- H. Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.
- lelpi, A., Lapôtre, M. G. A., 2023. Modelling fire-induced perturbations in sediment flux based on stream widening and accelerated bank migration. CATENA. 228, 107173. doi: https://doi.org/10.1016/j.catena.2023.107173
- Jones, S., Carrasco, N. K., Perissinotto, R., 2015. Turbidity effects on the feeding, respiration and mortality of the copepod Pseudodiaptomus stuhlmanni in the St Lucia Estuary, South Africa. Journal of Experimental Marine Biology and Ecology. 469, 63–68. doi: https://doi.org/10.1016/j.jembe.2015.04.015
- Jordan, P., 2006. The use of sediment budget concepts to assess the impact on watersheds of forestry operations in the Southern interior of british columbia. Geomorphology. 79(1–2), 27–44. doi: https://doi.org/10.1016/j.geomorph.2005.09.019
- Krasne, F. B., 1965. Escape From Recurring Tactile Stimulation in *Branchiomma Vesiculosum*. Journal of Experimental Biology. *42*(2), 307–322. doi: https://doi.org/10.1242/jeb.42.2.307
- Lunt, J., & Smee, D. L., 2020. Turbidity alters estuarine biodiversity and species composition. ICES Journal of Marine Science. 77(1), 379–387. doi: https://doi.org/10.1093/icesjms/fsz214
- Markich, S. J., 2003. Influence of body size and gender on valve movement responses of a freshwater bivalve to uranium. Environmental Toxicology. *18*(2), 126–136. doi: https://doi.org/10.1002/tox.10109
- Mustapha, M. T., Uzun Ozsahin, D., Uzun, B., Ozsahin, I., 2021. Chapter 13 Application of fuzzy TOPSIS in the sterilization of medical devices. In I. Ozsahin, D. U. Ozsahin, B. Uzun (Eds.), Applications of Multi-Criteria Decision-Making Theories in Healthcare and Biomedical Engineering. Academic Press, pp. 197–216. doi: https://doi.org/10.1016/B978-0-12-824086-1.00013-X
- Merz, R. A., 1984. Self-Generated versus Environmentally Produced Feeding Currents: A Comparison for the Sabellid Polychaete *Eudistylia vancouveri*. Biological Bulletin. *167*(1), 200–209. doi: https://doi.org/10.2307/1541348
- Merz, R. A., Woodin, S. A., 2000. Hooked setae: tests of the anchor hypothesis. Invertebrate Biology. 119(1), 67–82. doi: https://doi.org/10.1111/j.1744-7410.2000.tb00175.x
- Nicol, J. A. C., 1948. The Giant Axons of Annelids. The Quarterly Review of Biology. 23(4), 291–323. doi: https://doi.org/10.1086/396594
- Nicol, J. A. C., 1950. Responses of *Branchiomma Vesiculosum (Montagu)* to photic stimulation. Journal of the Marine Biological Association of the United Kingdom. 29(2), 303–320. doi: https://doi.org/10.1017/S0025315400055399
- Pither, J., 2022. Tutorials for BIOL202: Introduction to Biostatistics. Electronic document: https://ubco-biology.github.io/BIOL202/index.html accessed 2024-07-25
- Popham, J. D., D'Auria, J. M., 1982. A new sentinel organism for vanadium and titanium. Marine Pollution Bulletin. *13*(1), 25–27. doi: https://doi.org/10.1016/0025-326X(82)90493-3

- R Core Team, 2024. R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. Electronic document: https://www.R-project.org/ accessed 2024-07-25
- Rudy, L. H., Rudy, P., Jr, 1983. Oregon estuarine invertebrates: an illustrated guide to the common and important invertebrate animals. U.S. Fish and Wildlife Service. Electronic document: https://pubs.usgs.gov/publication/fwsobs83_16 accessed 2024-07-27
- Safak, I., Angelini, C., Sheremet, A., 2021. Boat wake effects on sediment transport in intertidal waterways. Continental Shelf Research. 222, 104422. doi: https://doi.org/10.1016/j.csr.2021.104422
- Singh, N., Choudhary, B. K., Singh, S., Kumar, R., 2022. Monitoring and assessment of anthropogenic impacts on water quality by estimating the BMWP and ASPT indices for a headwater stream in Doon Valley, India. Sustainable Water Resources Management. 8(4), 108. doi: https://doi.org/10.1007/s40899-022-00701-5
- Tompkins-MacDonald, G. J., Leys, S. P., 2008. Glass sponges arrest pumping in response to sediment: implications for the physiology of the hexactinellid conduction system. Marine Biology. *154*(6), 973–984. doi: https://doi.org/10.1007/s00227-008-0987-y
- United States Environmental Protection Agency, 2021. Fact sheet on water quality parameters. Electronic document: https://www.epa.gov/system/files/documents/2021-07/parameter-factsheet_turbid ity.pdf accessed 2024-08-01
- Vanderleest, R. 2012. Effects of food concentration and predator avoidance on the rate of habituation in *Eudistylia vancouveri Kinberg, 1866*. Instructor Dr. Hana Kucera. Fall program, 381, Bamfield Marine Sciences Centre, Bamfield, BC. (Unpublished report on file at the BMSC Library).
- Vijayan, N., Lema, K. A., Nedved, B. T., Hadfield, M. G., 2019. Microbiomes of the polychaete *Hydroides elegans* (Polychaeta: Serpulidae) across its life-history stages. Marine Biology. 166(2), 19. doi: https://doi.org/10.1007/s00227-019-3465-9
- Wickham H, François R, Henry L, Müller K, Vaughan D, 2023. dplyr: A Grammar of Data Manipulation. R package version 1.1.4, Electronic document: https://CRAN.R-project.org/package=dplyr accessed 2024-07-25
- Yahel, G., Eerkes-Medrano, D. I., & Leys, S. P., 2006. Size independent selective filtration of ultraplankton by hexactinellid glass sponges. Aquatic Microbial Ecology: International Journal. doi: https://doi.org/10.3354/ame045181
- Young, J. S., Buschbom, R. L., Gurtisen, J. M., Joyce, S. P., 1979. Effects of copper on the sabellid polychaete, Eudistylia vancouveri: I. Concentration limits for copper accumulation. Archives of Environmental Contamination and Toxicology. 8(1), 97–106. doi: https://doi.org/10.1007/BF01055144

Tables and Figures

Table 1: Worms collected from each site and in each group. Experiment group refers to which tank the worms were testing in (control, moderate sediment, high sediment). BMSC refers to the number of worms collected at the BMSC South docks. Similarly, the Health Center column represents the number of worms collected from the Island Health Dock, and the West Gov. column represents the number of worms collected at the West Government Dock.

Site of Tube Worm Collections

Experimental group	BMSC	Health Center	West Gov.
Control	5	31	5
Moderate sediment	9	31	4
High sediment	11	25	2

Table 2: Frequency table of the number of times the Partial Retraction (PR) behaviour in *E. vancouveri* was observed throughout the experiments. The testing_group column represents the experimental sediment level.

Relative Frequency of Partial Retraction Behaviour

testing_group	Count_PR	Total_count_PR	Relative_frequency_PR
Control	32	57	0.5614
Moderate	13	57	0.2281
High	12	57	0.2105

Table 3: Frequency table of the number of times the Partial Re-Emergence (PE) behaviour in *E. vancouveri* was observed throughout the experiments. The testing_group column represents the experimental sediment level.

Relative Frequency of Partial Re-Emergence Behaviour

testing_group	Count_PE	Total_count_PE	Relative_frequency_PE
Control	110	362	0.3039
Moderate	129	362	0.3564
High	123	362	0.3398

Table 4: Frequency table of the number of times the Exposed (EX) behaviour in *E. vancouveri* was observed throughout the experiments. The testing_group column represents the experimental sediment level.

Relative Frequency of Exposed Behaviour

testing_group	Count_EX	Total_count_EX	Relative_frequency_EX
Control	4	18	0.2222
Moderate	10	18	0.5556
High	4	18	0.2222

Table 5: Frequency table of the number of times the Hidden (HI) behaviour in *E. vancouveri* was observed throughout the experiments. The testing_group column represents the experimental sediment level.

Relative Frequency of Hidden Behaviour

testing_group	Count_HI	Total_count_HI	Relative_frequency_HI
Control	15	30	0.5000
Moderate	10	30	0.3333
High	5	30	0.1667

Table 6. Results of ANOVA test from the GLM of the northern feather duster worm experiment. Response includes variables "testing_group" which represents the three experimental groups used in the project and "tube_diameter_cm" which is the diameter of each individual tube. The chi-squared value quantifies how much of the data deviates from the expected values under the null hypothesis. The degrees of freedom refers to the number of independent pieces of information used to estimate the parameters tested. Finally, the p-value indicates the probability of observing a chi-squared value as extreme as, or more extreme, than the one calculated assuming the null hypothesis is true.

Type II Wald Chi-square ANOVA Results

Response	Chisq	df	Pr(>Chisq)
testing_group	13.1674	2	0.0014
tube_diameter_cm	0.7294	1	0.3931

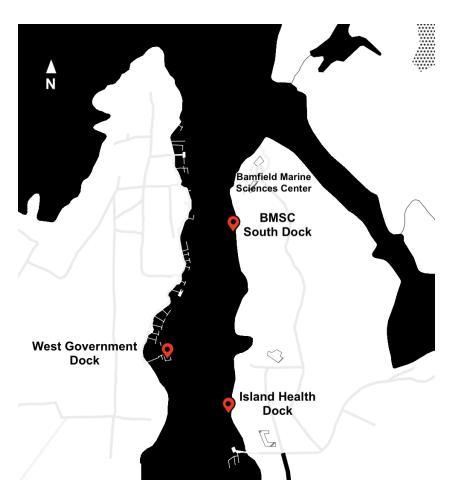


Figure 1: Map of the Bamfield Inlet with placemarks on the three sites of collection. Map courtesy of Stadia Maps.

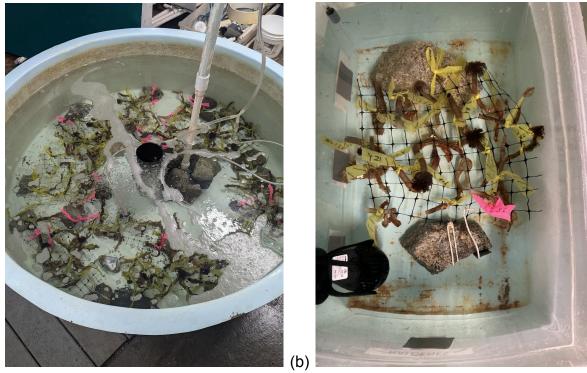


Figure 2: (a) Holding tank conditions for northern feather duster worms (*Eudistylia vancouveri*). (b) Control experimental tank (45L) during experimental trial. Both tanks were kept at the Bamfield Marine Science Center in Bamfield, British Columbia, Canada.

(a)

Behaviour	Behaviour Code	Description
Complete		All radioles of the worm are completely hidden within the
retracted	CR	tube of the worm: No radioles remain outside of the tube
Partial		Radioles were retracted, but not completely within the
retraction	PR	tube (some are still visible)
Complete		The entirety of the radioles have been re-exposed after
re-emergence	CE	retraction
Partial		A partial amount of the radioles have been re-exposed
re-emergence	PE	after retraction
		Worm was retracted in tube at the beginning of trials
Hidden	HI	
		Worm did not retract in response to stimuli at the
		beginning
Exposed	EX	of the trial

Figure 3: Ethogram of behaviours recorded during analysis of video recordings of experiments in varying water turbidity.

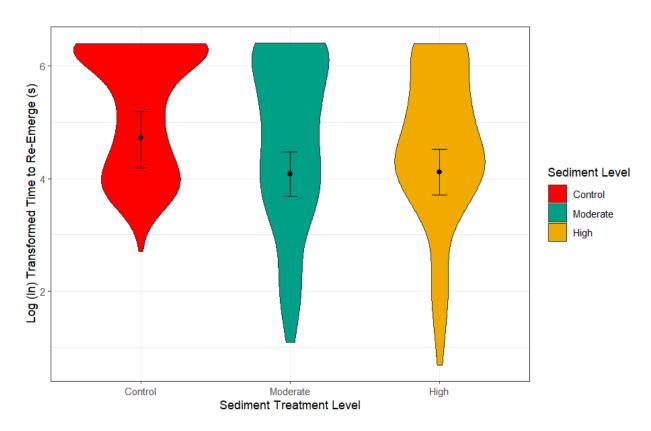


Figure 4: Violin plot of the natural log transformed re-emergence time (seconds) in *E. vancouveri* (n = 123) in comparison to sediment treatment levels. Solid dots denote estimates with bars showing the 95% confidence interval.