Effect of Grain Size on the Burrowing Force of Pacific Sand Lance

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ABSTRACT

Pacific sand lances, *Ammodytes personatus*, are nutrient dense forage fish important to salmon recovery and orca conservation in the San Juan archipelago. Sand lances burrow into sandy sediments during quiescence and in response to threats. Ammodytes spp. choose habitats with specific sizes of sediment particles, but the biological reasons for their preferences are poorly understood. Burrowing force in different sediments is measured using resin models of sand lance and a materials testing system. The final testing methods are validated by first varying the volume of sediment, mixing procedure, and speed. Laboratory limitations do not preclude comparison with in-vivo activity. There is a non-linear relationship between force and grain size. In general, coarser sediments are more difficult to penetrate. When the scales were removed, models more easily penetrated the sediment, but produced a similar non-linear trend. Burrowing forces are correlated with observed habitat preferences, and may be useful in habitat predictions.

INTRODUCTION

The Pacific sand lance, *Ammodytes personatus*, is a forage fish previously described in the literature as *A.* hexapterus. Molecular analysis of the genus recently lead to its taxonomic reclassification (Summers pers. comm. 2011). Sand lance are crucial links in the food web because they are highly nutritious (Van Pelt et al. 1997, Robards et al. 1999a, Anthony et al. 2000), preferred prey of top predators like marine mammals and salmon (Robards et al. 1999b, Zamon 2001). Forage fish like sand lance are the primary bottleneck for energy transfer from zooplankton to larger commercially and ecologically valuable predators like king salmon (Zamon 2003, Hershberger et al. 2006, Haynes et al.

2007). In the Puget Sound, *A. personatus* comprises up to 60% of the stomach contents of juvenile Chinook Salmon (Sutherland 2008).

Salmon and orca conservation efforts seek to maintain sand lance abundance and provide food for their endangered predators by protecting Ammodytes' burrowing and spawning sediment habitats. (Bargmann 1998, Whitman 2004, Hershberger et al. 2006, Dohrmann 2006, Pentilla 2007). Sand lance aggregate around and burrow into intertidal and subtidal sediments during quiescence and in response to threats (Reay 1970, Dick and Warner 1982, Quinn 1999, Simon 2004, Greene et al. 2011). *Ammodytes spp.* burrow in habitats with specific ranges of current flow, sediment grain size, dissolved oxygen content, temperature and salinity (Van Pelt et al. 1997, Robards et al. 1999a, Anthony et al. 2000). It is unclear which of these interconnected factors are causally related to habitat selection and how they interact with each other in complex environments.

Beyond merely analyzing the distribution and abundance of wild sand lances, some aspects of sediment selectivity can be tested in laboratory experiments. In particular, two papers and a set of unpublished data show that sand lance prefer sand and avoid sediment with high proportions of silt or gravel (Pinto et al. 1984, Wright et al. 2000, Bizzarro unpub. data 2011). Preliminary lab experiments reported a positive correlation between grain size and burrowing force (Greene et al. 2011), and it has been suggested as a factor driving sediment choice multiple times (Meyer et al. 1979, Wright et al. 2000, Behrens et al. 2007). More rigorous testing is needed to determine what if any link exists between sand grain preferences and the energy required to overcome shearing forces when entering the sediments.

The experiment first tested whether using resin models and a materials testing system accurately reproduces forces experienced in-vivo. Next, five sediments were tested to determine the relationship between burrowing forces and grain size. Finally, the resin models were sanded down and retested to examine the effect of scale form on burying force and determine the adaptive or maladaptive effects of sand lance morphology. The relationship between force and grain size was compared with *Ammodytes* burrowing preferences to evaluate its potential as a predictive tool for mapping sand lance habitat.

METHODS

Specimen Preparation

A. personatus specimens were collected in October, 2011 off the docks of Friday Harbor Laboratories on San Juan island. The fish were attracted to lights in the water at night and were caught with dip nets. The fish were euthanized with MS 222, as recommended by fish care protocol (Neiffer and Stamper 2009). The flesh and scale anatomy was fixed straight by immersion in 10% formalin solution for 24hrs prior to storage in a 70% ethanol solution. The variably angled pectoral fins were removed by dissection to improve consistency amongst models. The fish that formed the basis for model #1 was 77.81 mm standard length (SL), and second model specimen was 69.26 mm SL. These lengths are near the mode found in the San Juan Channel (Blaine 2006).

Impression Molds

Molds for use with Spurr's resin were made using Coltène President light body polyvinylsiloxane (#4667). This technique produces durable, accurate models suitable for scanning electron microscopy (Mccall and Mead 2008). The burrowing motion

Ammodytes can be accurately measured by a hard model because the subterranean body is rigid during the first third of insertion (Gidmark et al. 2011). The base and catalyst were mixed in approximately equal parts for one minute. First, half of the mixture was placed inside an appropriately sized container. Next, an entire sand lance specimen was placed on its side in the mixture and covered with the rest of the material. The molds were allowed to cure for at least one hour. Subsequently, a single minimally short incision on top of the mold was made with a razor blade to facilitate specimen extraction. The molds were then washed with tap water and dried for at least two hours at 70°C. After cooling, the molds were wrapped in tape to apply pressure and seal the incision to maintain the maximum possible mold length.

Spurr's Resin Models

The resin was prepared from an SPI-chem "Spurr Formula Kit I" according to the manufacturer's instructions and subsequently frozen in a 60 ml syringe for long-term storage ("SPI-Chem^{TM} Low Viscosity 'Spurr' Kits" 2011). The mixture was later thawed for at least 3 hours prior to each use and refrozen thereafter. The resin was injected to into the mold using a 1.5" 16 gauge needle at slow speed to avoid bubble formation. The molds were then held directly vertically inside a vacuum oven at 70-90°C. The pressure was increased to 25lbs/in^2 for 20 minutes, then allowed to return to atmospheric pressure for 10 minutes to ensure all air escaped the liquid. The pressure cycling was repeated three times, and then the resin was cooked for at least 14 hours at room pressure. After the molds were removed and cooled to room temperature, the incision in the mold was extended to near the operculum of the fish to extract the models without damage.

Model Treatments

Models were carefully dissected with a fine scalpel to remove any obvious artifacts present in the impression mold. Each model was glued with 5-minute epoxy to a small wooden shim to facilitate stronger, consistent attachment with the materials testing system. As the dissection alterations could not be precisely replicated, the smoothed models were prepared from the originals. After all tests were completed with the original models, the scales and ventral skin folds were rendered smooth using 1200 grit sandpaper to remove about 0.1 mm of material from the surface.

Imaging

Throughout the process, pictures were taken to document the process and changes to the models using both a pocket camera and a dissecting microscope. After testing was completed, a scanning electron microscope (SEM) took higher resolution images of the molds and models from each stage of testing. The samples were coated with a 20 nm layer of gold-palladium alloy using a splutter coater prior to SEM imaging. The images were examined to determine the accuracy of the mold and model casting process and the effect of testing and treatments on the scale morphology. SEM images of sand lance scales were obtained from previous experiments performed by Adam Summers.

Sediment Isolation

Sediment was obtained from stocks isolated for previous experiments. The original sample was collected from beaches on San Juan Island, dried and sieved into subsets of uniform grain size with a RoTap™ sediment analyzer. In this experiment, five different particle size ranges were used: 0.125 mm - 0.25 mm; 0.25 mm – 0.5 mm; 0.5 mm – 1 mm; 1 mm – 2 mm; 2 mm – 4 mm. The Wentworth scale designations for these ranges are fine sand, medium sand, coarse sand, very coarse sand and granule gravel respectively (Wentworth 1922).

Testing equipment

All testing was performed using a MTS Synergie 100 with an attached 500 N load cell. The data output was recorded by Testworks 4.05 software on a connected computer. Using a modified version of the compression method, the model was inserted into a container filled with 6.0 cm sediment and 1.0 cm - 1.5 cm surface water. The vertical force on the model was measured at 100.00 Hz. The models were inserted 4.5 cm into the sediment from the surface at 1.666 cm/s. This limited depth is appropriate because sand lance only burrow five to six centimeters into the sediment (Quinn 1999, Gidmark et al. 2011). The peak load and slope of the linear portion of the curve were calculated as results. The linear slope showed the force per unit distance required to bury the model deeper than the non-linear tow region where the effects of head morphology were dominant.

Experimental design

Preliminary tests on the effects of container size, sediment rearrangement and insertion speed were performed to examine the validity of the experimental design. Model #1 was used for preliminary testing prior to its refinement via dissection. Twelve replicate strikes were performed for each condition. The subsequent examinations of different sediments were performed with 15 replicates. Three cups of sediment were placed in 14 cm diameter square Tupperware containers. The sediments were inverted and allowed to resettle prior to each testing replicate. The larger 0.50 mm, 1.00 mm and 2.00 mm sieved sediments were immediately tested, and the finer 0.125 mm and 0.25 mm sediments were left to settle for 45 - 50 minutes. There was one sediment container for each of the larger sediments, six filled 0.125 mm silt and eight with 0.25 mm. The molds were checked and adjusted with a level prior to each testing session.

Data Analysis

The raw force data and initial calculations collected by the Testworks software was analyzed visually to identify outliers and determine the slope of the linear portion of the force curve, referred to here as the penetration force. Individual replicates without a clear delineation between the tow and linear regions were discarded as outliers. The penetration force was calculated by a linear regression of least squares fitting between the selected section of data. These results were exported for further analysis and organization in Microsoft Excel 2011. The penetration force and peak load from each model were normalized to the averages with 0.5 mm sediment so the data could be pooled together. This normalized data was analyzed using two-way ANOVA (Analysis of Variance) statistical analysis amongst the different grains. As the data universally failed the Shapiro-Wilk normality test, the Kruskal-Wallis ANOVA on ranks was used followed by either Dunn's or Tukey's multiple comparison procedures.

RESULTS

Accuracy of Resin Models

The SEM images show that sand lance scales are accurately reproduced in the resin models and effectively removed by sanding (Fig. 1).

Experimental Validation

Changes in container size produced no significant effect on the penetration force, the rate of increase per unit depth, above a radius of 3.5 model widths (Fig. 2). The effect of reducing the radius further is underrepresented because the smallest container was not tested to the same depth to avoid shattering the glass tube. Deeper containers did not produce qualitatively different results. Increasing speed of insertion was associated with higher penetration forces, but the differences were only significant for speeds that differed by two orders of magnitude ($P < 0.05$; Fig. 3). Repeatedly inserting the model without mixing the sediment between tests also increased the penetration force ($P < 0.001$; Fig 4). Increasing the settling time after mixing for the finest sediments up to 24 hours produced even more variable results than when testing every 45 minutes.

Particle Size

The magnitude of forces differed between the models and was reduced after sanding (Fig. 5, 6) When the data for each model was normalized and combined into scaled and sanded data, burrowing force was non-linearly related to grain size (Fig. 7, 8). The differences between individual grain sizes were almost universally statistically significant to $P < 0.001$ for both penetration force and peak load. The larger confidence interval of the smallest sand size represents the combination of data similar to the 0.25 mm sediment and occasional force spikes that occurred deeper than 3.5cm in the sediment. The sanded and scaled models were significantly different ($P = 0.006$; Power = 0.729), but the effect of sanding on the differences among grain sizes was insignificant ($P = 0.259$; Power = 0.122).

DISCUSSION

MTS analysis is comparable to forces experienced in-vivo because the methods produce precise results similar to those expected in natural circumstances. There is no measurable difference between with larger container sizes (Fig. 2), and the measured forces should be identical those in larger sediment beds. The minimal force increases associated with faster insertion (Fig. 3) can be safely ignored when the examining relative differences between sediments. Although mixing decreased burying forces (Fig. 4), sand lance select high flow environments where similar mixing should occur (Zamon 2003, Blaine 2006), and preliminary tests found no effect of compaction on sediment selection (Greene et al. 2011). Nonetheless, future experiments should more closely examine the effects of tidal flow on mixing and compaction in sediments. The power of these conclusions could be expanded by testing different sediment mixtures, but they provide a good baseline for using hard resin models in burrowing force experiments.

In the analysis of grain size here, larger particle sizes were correlated with higher burying forces (Fig. 5,6). However, the penetration force was smaller in 2nd largest particle range than in the next largest size (Fig. 5), and this relative minimum corresponds with observed sediment selections of *Ammodytes spp.* (Wright et al. 2000, Haynes et al. 2007, Bizzarro unpub. data 2011). Further testing with a larger set of grain sizes separately and in mixtures could better determine the extent and practical significance of the relative minimum. The relative maximum produced by the highly variable forces observed in the finest sand may be caused by cracking effects in the sediment (Summers pers. comm. 2011). It would be interesting to further explore the conditions that lead to cracking and determine if sand lance behavior adjusts to its effects. The limited results of this experiment suggest that burrowing force is a factor in habitat selection.

Sand lance do not always choose the grain sizes that were easiest to penetrate in lab (Haynes et al. 2008, Bizzarro unpub. data. 2011) because there is more interstitial water and available in coarser sediments (Wright et al. 2000, Behrens et al. 2007, Hand et al. 2008). Although sand lances can alter their metabolism and undulate their bodies to bring oxygenated surface water into anoxic sediments, doing so may use more energy overall (Behrens 2007). The trade-off between ease of penetration and oxygen availability (Behrens et al. 2007) may be affected by particle size mixtures (Wright et al. 2000), the flow of oxygenated water above the sediment (Auster and Stewart 1986, Behrens 2007), and age. Larger sand lance require more oxygen (Behrens et al. 2007) and are able to penetrate coarser sediments (Bizzarro unpub. data 2011), but it may still require too much time and energy to do so in most circumstances (Holland et al. 2005). Should the trade-off between oxygen availability and energetic costs of burrowing accurately describe the driving forces behind sediment selection, it would explain much of the inconsistency in observed habitat preferences.

Although the trade-off among grain sizes is similar for sand lances without scales (Fig. 5, 6), sanding the models reduced the force required to bury into the sediment (Fig. 7, 8). Although this effect may be the result of unavoidably reducing the cross-sectional area of the body, it appears that sand lance’s scales may be maladaptive for achieving maximum burrowing efficiency. However, scales may be adaptive in the context of other selective factors. Scales may protect the sand lance from abrasion during burrowing or other environmental and biological contact. If having scales is evolutionarily beneficial, then perhaps the *A. personatus’* scales are better adapted for burrowing than larger, rougher alternatives. Testing models with various artificial scales and shapes could better explain the functional morphology of the sand lance’s exterior. Nonetheless, it appears that sand lances’ scales do not drive their sediment preferences because sanding does not affect the relative difficulty of burrowing into different sediments. Furthermore, the forces encountered in different sediments appear to largely be the result of the natural dynamics of sediment particle interactions rather than any specific morphology of the sand lance.

The range of observational and experimental results discussed here suggest that sand lance do not merely prefer sand as their common name implies, but instead choose the most energy efficient habitat given their size. The forces recorded using the resin models are a good starting point for analysis of the precise energetic costs of penetrating different sediments. Gathering both force and oxygen availability data that addresses the effects of mixing different particle diameters with varying sizes of fish would allow for direct comparisons with sand lance preferences in the wild. If there is a causal relationship between the ratio of burrowing force to oxygen availability and sediment selectivity, then data from the lab could be used to predict sand lance habitats. Developing an accurate predictive measurement is intrinsically interesting and potentially useful for conservation efforts and fishery management.

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FIGURES

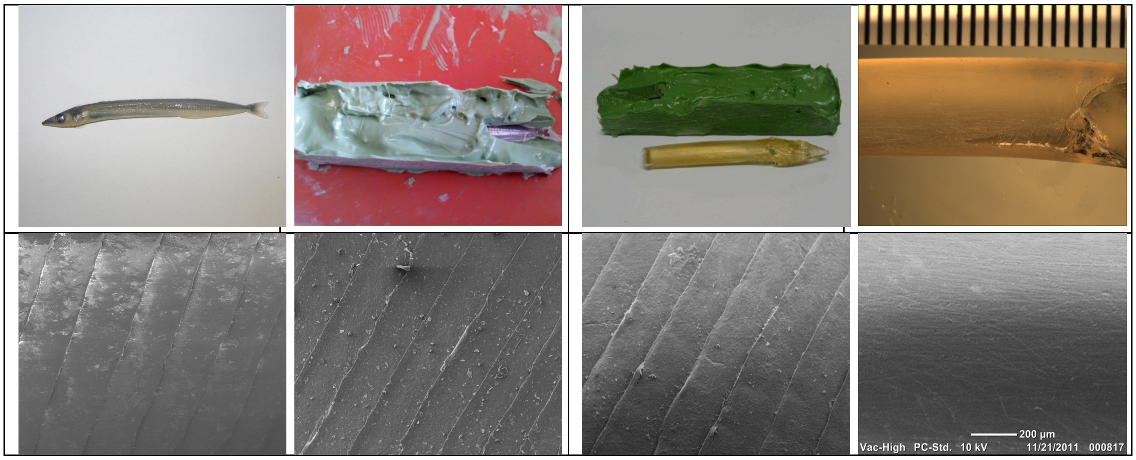

Figure 1: The top row shows macro images of each step of the process, and the bottom row shows scanning electron microscope (SEM) images of the scale form. The columns from left to right show preserved fish scales, the dental wax mold, the scales of the resin model and the abrasion leftover after sanding. The width of each scale is 0.31mm. A scale of 1 mm marks is shown on the large view of the sanded model, and a 200 um scale on its SEM counterpart because scales cannot provide a reference size.

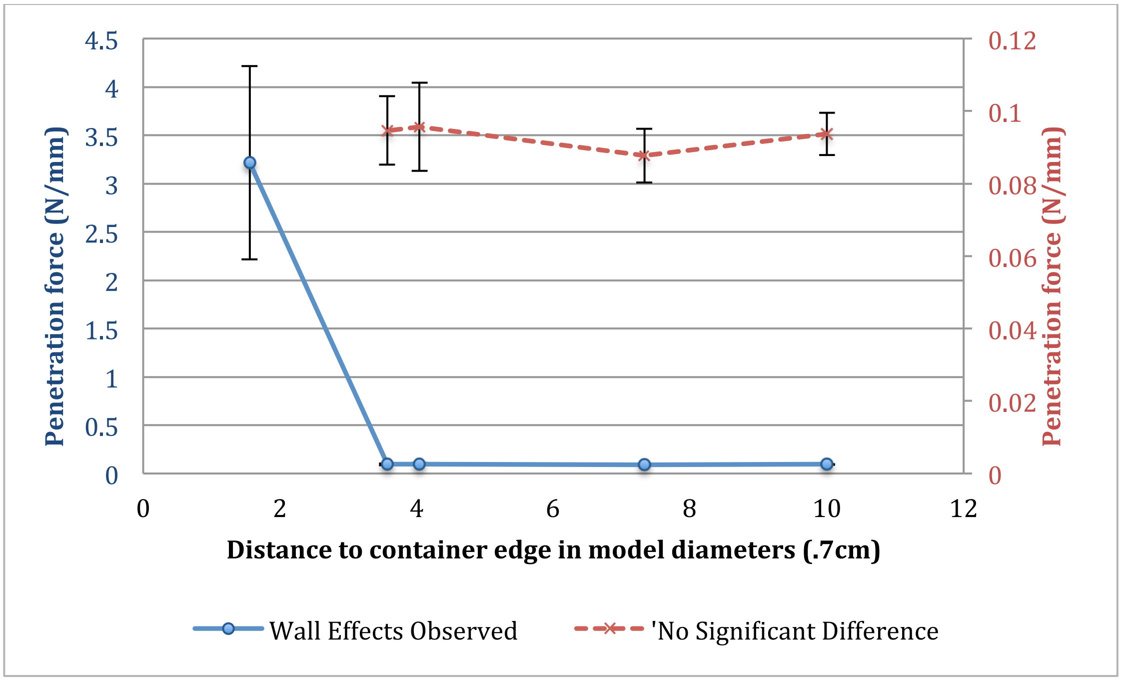

Figure 2: The penetration force is tested with different container sizes to determine if wall effects occur. Brackets show the 95% confidence interval for each container size and are obscured by data points when absent. The smallest container produced significantly higher penetration forces, but the four large containers were not significantly different ($P < 0.05$).

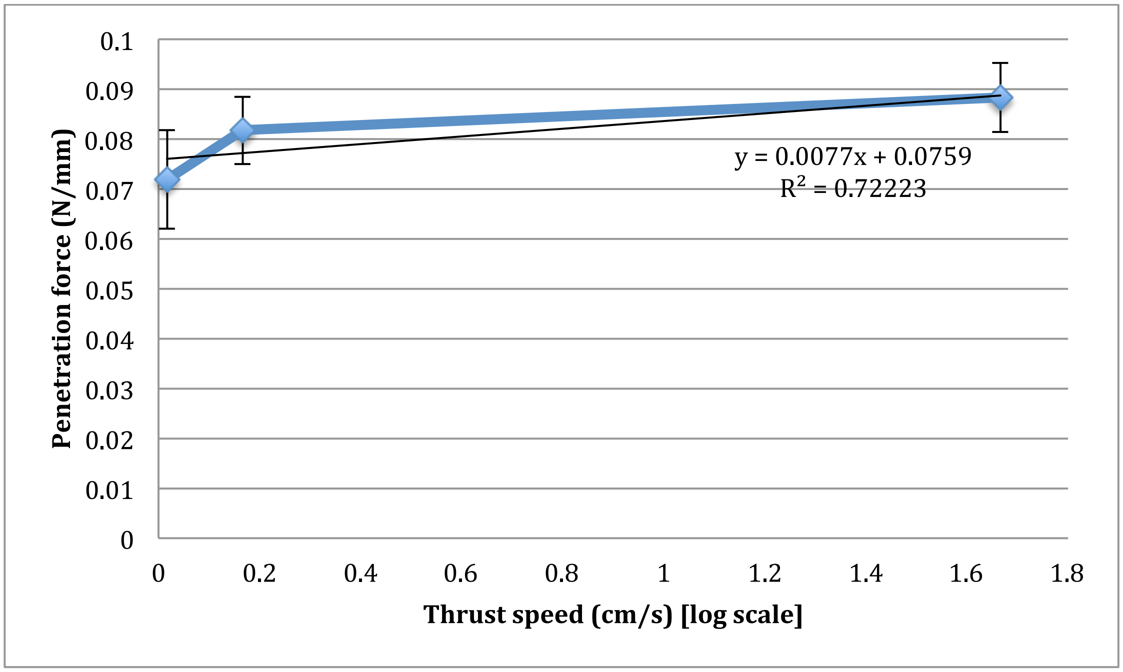

Figure 3: Thrust speed is tested with respect to penetration force in coarse sand. Minor decreases in the rate of penetration force the sand lance model is associated with order of magnitude thrust speed reductions. The 95% confidence intervals are shown as brackets, and the 0.016cm/s and 1.666cm/s trials were significantly different ($P < 0.05$).

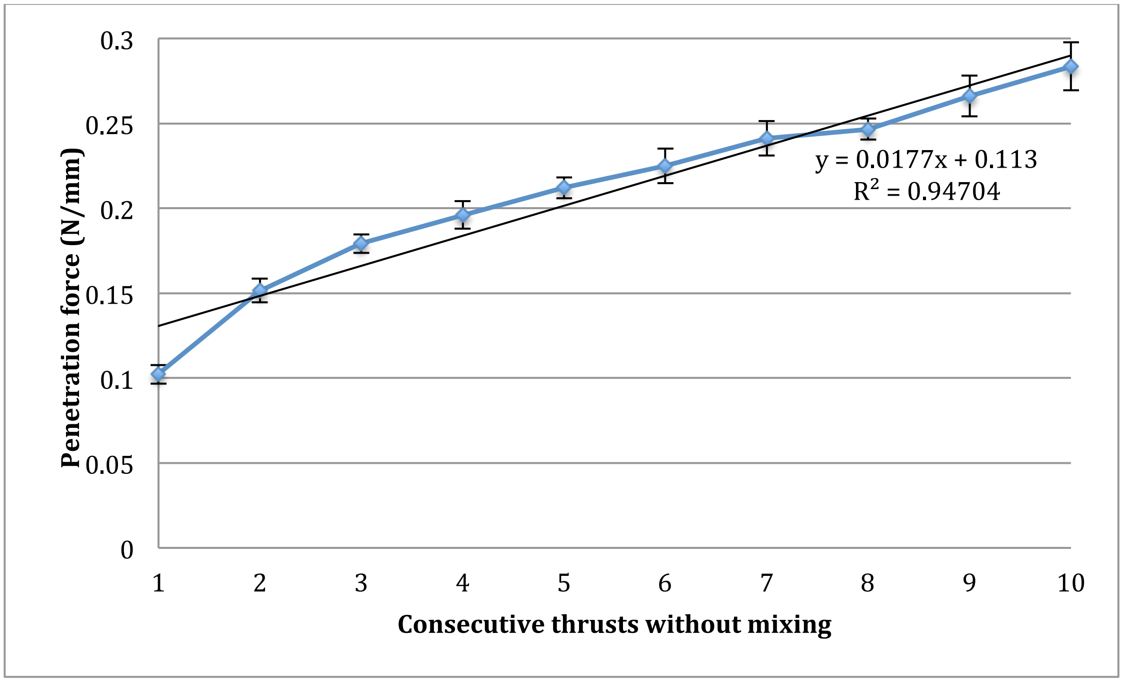

Figure 4: Penetration force is measured in sets of ten consecutive tests without inverting the sediment as is done between sets. Repeatedly inserting the Sand Lance model into the same location without mixing requires significantly ($P<0.001$) more force per unit depth, and the 95% confidence intervals are visible as brackets.

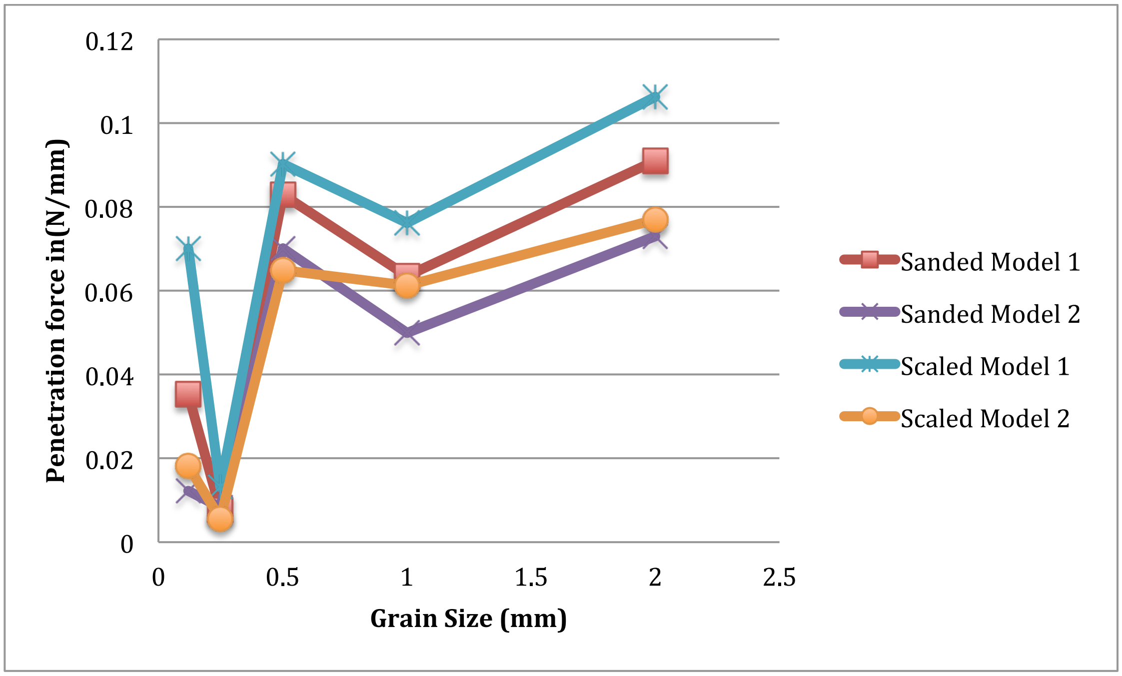

Figure 5: The average penetration forces, marginal force increase per unit depth, were recorded for each model before and after sanding. The same general pattern was observed in each case with slight differences in magnitude.

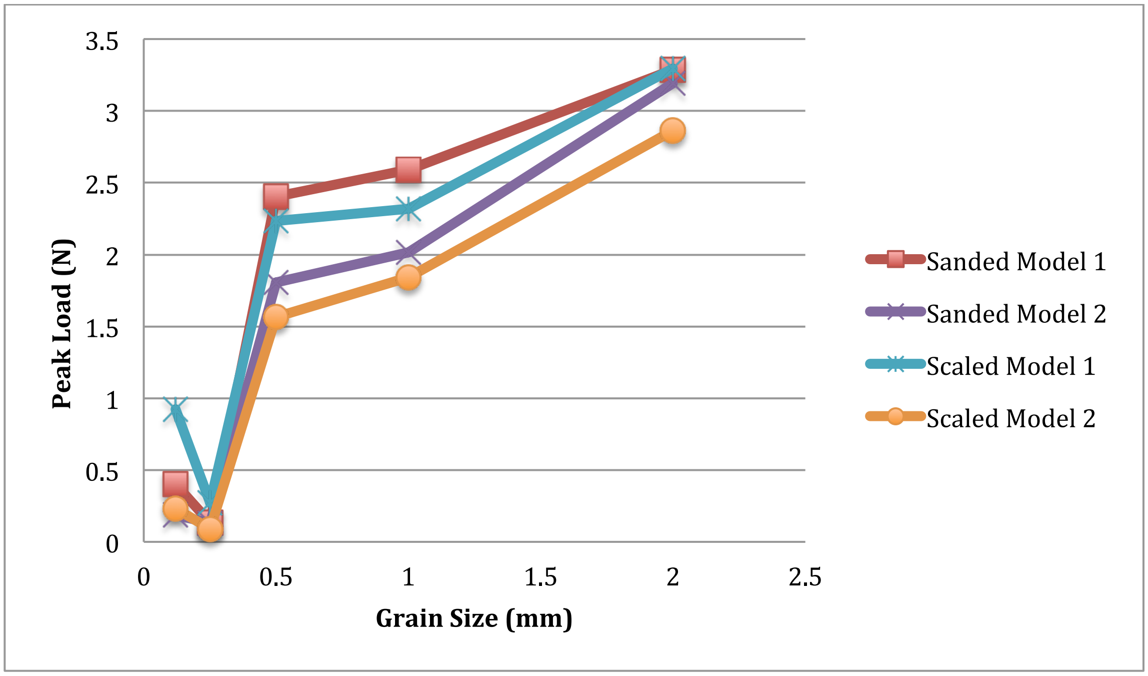

Figure 6: The average peak load, maximum force during the test, was recorded for each model before and after sanding. The same general pattern was observed in each case with slight differences in magnitude.

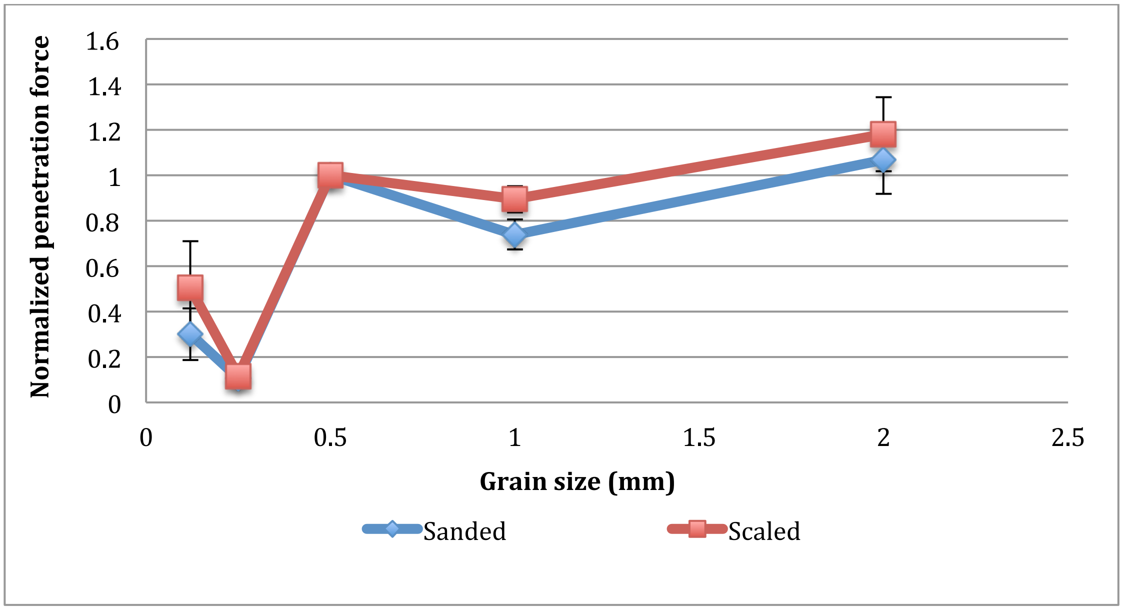

Figure 7: The individual penetration forces for each model were normalized to their respective averages in the middle sediment size and pooled together for comparison. The grain sizes were almost all significantly different from each other with $P < 0.001$, and 95% confidence intervals are shown in brackets. The treatments, sanded or scaled, were significantly different ($P < 0.001$; Power = 0.721), but the effect of treatment on grain size was not ($P = 0.259$; Power = 0.122). The 0.125 mm sediment had consistent, high outliers that increased its confidence interval.

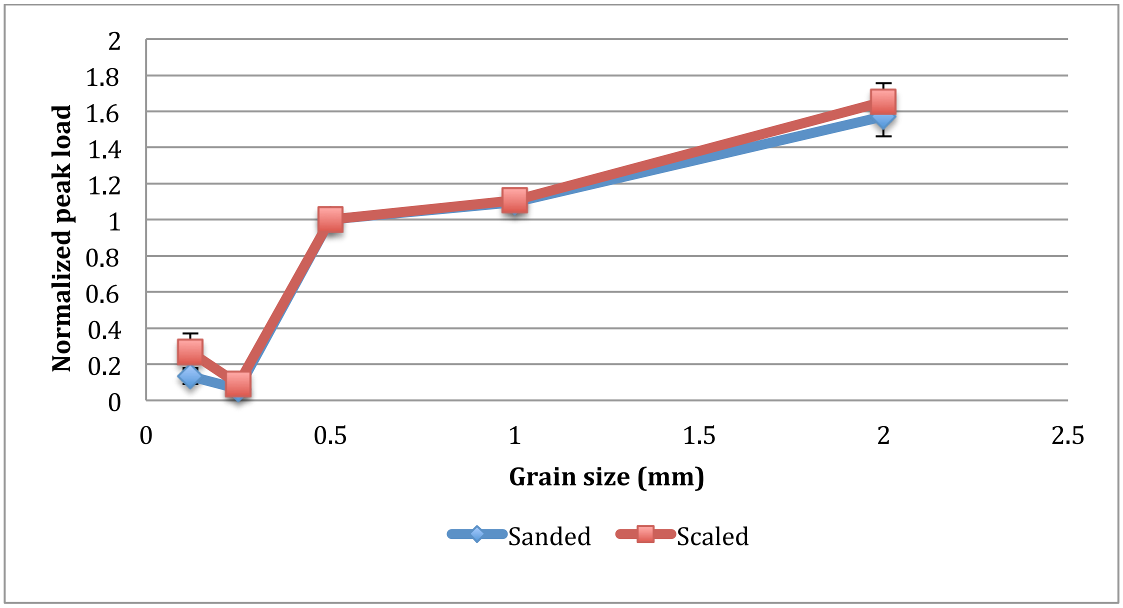

Figure 8: The individual peak loads for each model were normalized to their respective averages in the middle sediment size and pooled together for comparison. The grain sizes were almost all significantly different from each other with $P < 0.001$, and 95% confidence intervals are shown in brackets. The treatments, sanded or scaled, were significantly different ($P < 0.001$; Power = 0.636), but the effect of treatment on grain size was not ($P = 0.167$; Power = 0.200). The 0.125 mm sediment had consistent, high outliers that increased its confidence interval.

REFERENCES

Anthony, J., D. Roby, and K. Turco. 2000. Lipid content and energy density of forage fishes from the northern Gulf of Alaska. Journal of Experimental Marine Biology and Ecology 248:53–78.

Auster, P. J., and L. L. Stewart. 1986. Species profiles: Life histories and environmental requirements of coastal fishes and invertebrates (North Atlantic): Sand lance.[Ammodytes spp]. Connecticut Univ., Groton (USA).

Bargmann, G. 1998. Forage Fish Management Plan. Washington Fish and Wildlife Commission.

Behrens, J. W. 2007, April. Effect of Global Change related oxygen depletion on fish; lesser sandeel (Ammodytes tobianus) as model organism. University of Copenhagen.

Behrens, J. W., H. J. Stahl, J. F. Steffensen, and R. N. Glud. 2007. Oxygen dynamics around buried lesser sandeels Ammodytes tobianus (Linnaeus 1785): mode of ventilation and oxygen requirements. Journal of Experimental Biology 210:1006 -1014. doi: 10.1242/jeb.000570.

Bizzarro, J. 2011, December 4. Unpublished data.

Blaine, J. 2006. Pacific sand lance (Ammodytes hexapterus) present in the sandwave field of central San Juan Channel, WA: Abundance, density, maturity, and sediment association. Apprenticeship Project Report, FISH 492.

Dick, M. H., and I. M. Warner. 1982. Pacific Sand Lance, Ammodytes hexapterus Pallas, in the Kodiak Island Group, Alaska.

Dohrmann, J. 2006, August 14. Comments on Designation of Critical Habitat for the Southern Resident Killer Whale. Puget Sound Action Team.

Gidmark, N. J., J. A. Strother, J. M. Horton, A. P. Summers, and E. L. Brainerd. 2011. Locomotory transition from water to sand and its effects on undulatory kinematics in sand lances (Ammodytidae). Journal of Experimental Biology 214:657–664.

Greene, G. H., T. Wyllie-Echeverria, D. Gunderson, J. Bizzarro, V. Barrie, K. Fresh, C. Robinson, D. Cacchione, D. Penttila, M. Hampton, and A. Summers. 2011. Deep-Water Pacific Sand Lance (Ammodytes hexapterus) Habitat Evaluation and Prediction for the Northwest Straits Region. Northwest Straits Commission.

Hand, V. L., J. R. Lloyd, D. J. Vaughan, M. J. Wilkins, and S. Boult. 2008. Experimental Studies of the Influence of Grain Size, Oxygen Availability and Organic Carbon Availability on Bioclogging in Porous Media. Environmental Science & Technology 42:1485-1491. doi: 10.1021/es072022s.

Haynes, T. B., C. K. L. Robinson, and P. Dearden. 2008. Modelling nearshore intertidal habitat use of young-of-the-year Pacific sand lance (Ammodytes hexapterus) in Barkley Sound, British Columbia, Canada. Environmental Biology of Fishes 83:473-484. doi: 10.1007/s10641-008-9374-2.

Haynes, T. B., R. A. Ronconi, and A. E. Burger. 2007. Habitat Use And Behavior Of The Pacific Sand Lance (Ammodytes Hexapterus) In The Shallow Subtidal Region Of Southwestern Vancouver Island. Northwestern Naturalist 88:155-167. doi: 10.1898/1051-1733(2007)88[155:HUABOT]2.0.CO;2.

Hershberger, P., D. Fagergren, P. S. A. Team, P. Frazier, K. Stick, M. O'Toole, G. Bargman, G. Kirby, P. Dorn, L. Thorsteinson, and others. 2006. Puget Sound Forage Fish Plan. Puget Sound Action Team.

Holland, G. J., S. P. R. Greenstreet, I. M. Gibb, H. M. Fraser, and M. R. Robertson. 2005. Identifying sandeel Ammodytes marinus sediment habitat preferences in the marine environment. Marine Ecology Progress Series 303:269–282.

Mccall, J. R., and K. S. Mead. 2008. Structural and Functional Changes in Regenerating Antennules in the Crayfish Orconectes sanborni. The Biological Bulletin 214:99 -110. Retrieved November 23, 2011, .

Meyer, T. L., R. A. Cooper, and R. W. Langton. 1979. Relative Abundance, Behavior, and Food-Habits of the American Sand Lance, Ammodytes-Americanus, from the Gulf of Maine. Fishery Bulletin 77:243-253.

Neiffer, D. L., and M. A. Stamper. 2009. Fish sedation, anesthesia, analgesia, and euthanasia: Considerations, methods, and types of drugs. ILAR Journal 50:343–360.

Van Pelt, T. I., J. F. Piatt, B. K. Lance, and D. D. Roby. 1997. Proximate composition and energy density of some north pacific forage fishes. Comparative Biochemistry and Physiology Part A: Physiology 118:1393-1398. doi: 10.1016/S0300-9629(97)00240-5.

Pentilla, D. 2007. Marine Forage Fishes in Puget Sound. Washington Department of Fish and Wildlife.

Pinto, J., W. Pearson, and J. Anderson. 1984. Sediment preferences and oil contamination in the Pacific sand lance Ammodytes hexapterus. Marine Biology 83:193–204.

Quinn, T. 1999. Habitat Characteristics of an Intertidal Aggregation of Pacific Sandlance (Ammodytes hexapterus) at a North Puget Sound Beach in Washington. Northwest Science 73:44-49.

Reay, P. J. 1970. Synopsis of biological data on North Atlantic sandeels of the genus Ammodytes (A. tobianus, A. dubius, A. americanus and A. marinus). Food and Agriculture Organization of the United Nations.

Robards, M. D., J. A. Anthony, G. A. Rose, and J. F. Piatt. 1999a. Changes in proximate composition and somatic energy content for Pacific sand lance (Ammodytes hexapterus) from Kachemak Bay, Alaska relative to maturity and season. Journal of Experimental Marine Biology and Ecology 242:245–258.

Robards, M. D., M. F. Willson, R. H. Armstrong, J. F. Piatt, and others. 1999b. Sand Lance: a review of biology and predator relations and annotated bibliography: Exxon Valdez Oil Spill Restoration Project 99346 Final Report. Research Paper.

Simon, M. J. 2004. Preferred burrowing habits of the Pacific sand lance (Ammodytes hexapterus); a comparison of various sites on San Juan Island, Washington. University of Washington Friday Harbor Labs, Friday Harbor, WA.

SPI-Chem™ Low Viscosity "Spurr" Kits. 2011. . Retrieved from http://www.2spi.com/catalog/chem/instructions_spurr_kits.html.

Summers, A. 2011, December 5. .

Sutherland, D. 2008. Habitat Conservation Plan for Washington Department of Natural Resources' Geoduck Fishery. Washington State DNR Aquatic Resources.

Wentworth, C. K. 1922. A scale of grade and class terms for clastic sediments. The Journal of Geology 30:377–392.

Whitman, T. 2004. Documented Surf Smelt and Pacific Sand Lance Spawning Beaches in San Juan County with a Summary of Protection and Restoration Priorities for Forage FIsh Habitat. Friends of the San Juans.

Wright, P. J., H. Jensen, and I. Tuck. 2000. The influence of sediment type on the distribution of the lesser sandeel, Ammodytes marinus. Journal of Sea Research 44:243-256. doi: 10.1016/S1385-1101(00)00050-2.

Zamon, J. E. 2001. Seal predation on salmon and forage fish schools as a function of tidal currents in the San Juan Islands, Washington, USA. Fisheries Oceanography 10:353–366.

Zamon, J. E. 2003. Mixed species aggregations feeding upon herring and sandlance schools in a nearshore archipelago depend on flooding tidal currents. Marine Ecology Progress Series 261:243–255.