May 2019 R/V Lasker Cruise Report updated 2020.05.19

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Survey Effort

Survey work during the 14-24 2019 May cruise collected a total of 232 animals which had some aspect measured. These animals consisted of 216 euphausiids (primarily *Euphausia pacifica*, *Thysanoessa spinifera*, and *Nematoscelis difficilis*) as well as squid, lanternfish, anchovies, and gelatinous animals (e.g., pyrosomes, jellyfish). Krill morphology measurements were made on 175 individual animals. Density measurements of individual krill were made on these same individuals. Approximately 150 krill were frozen and brought back to land for body mass measurements. The target strength (TS, dB re: 1 m^2) of 32 individual animals were measured at sea in an experimental aquaria. Soundspeed measurements were made on 15 different aggregations of krill (this measurement method can only be done on a group of animals).

Krill Morphology

Species information was collected from 175 individuals, which were dominated by *E. pacifica* (n = 110) and *T. spinifera* (n = 50) with few *N. difficilis* (n = 15) present. The SL2 lengths (i.e., the anterior extent of the eye-stalk to the posterior end of the sixth abdominal segment) were extracted from photographs of all animals (Figure 1).

Figure 1. Animal shapes were measured from photographs by measuring the cross-section of each krill's body at sixteen evenly spaced points between the anterior end of the eye stalk and the posterior end of the six abdominal segment (i.e., SL2 length).

There were significant differences detected in mean length among species (Kruskal-Wallis rank sum test, $\chi^2_{(df=2)} = 54.983$, $p < 0.001$; Figure 2) with *T. spinifera* (18.8 ± 0.4 mm, mean ± standard error) being significantly larger (Wilxocon rank sum test, $p < 0.001$) than both *E. pacifica* (14.3 ± 0.3 mm) and *N. difficilis* (16.6 ± 0.6 mm).

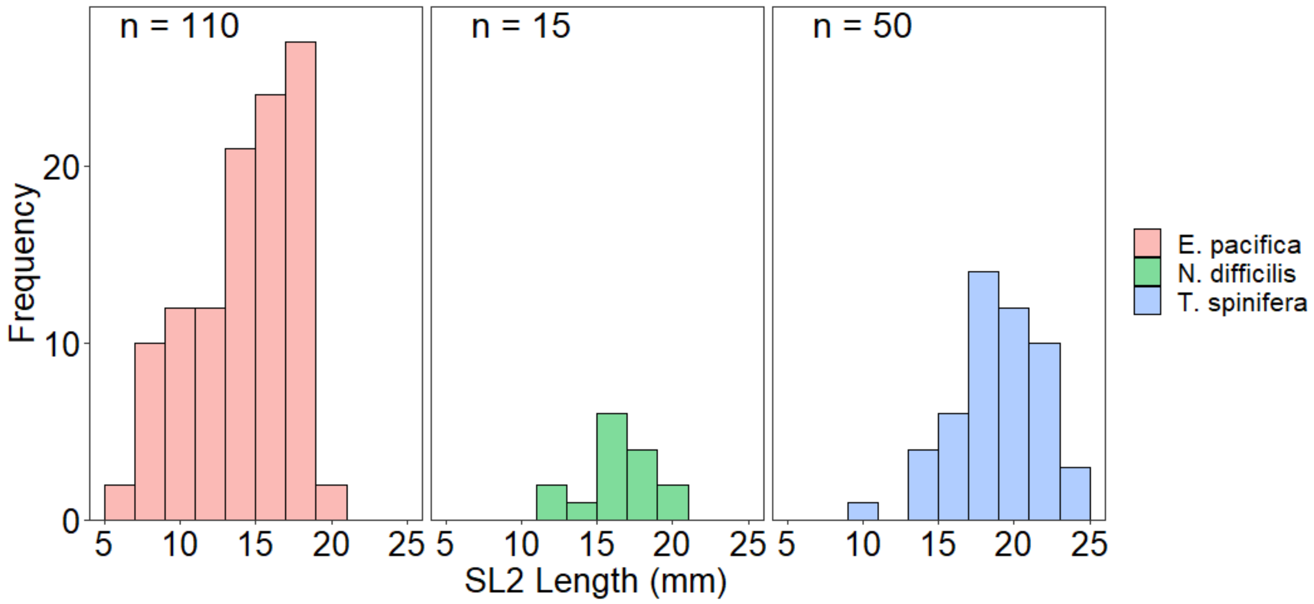

Figure 2. The SL2 length (mm) frequency distributions of *E. pacifica* (n=110, red), *N. difficilis* (n = 15, green), and *T. spinifera* (n=50, blue) from the 14-24 May 2019 survey.

Animal width and height measurements were extracted from dorsal and lateral photographs, respectively. The maximum carapace width and height were then calculated and compared to document any trends in the cross-sectional size of krill relative to length. Animal length significantly accounted for variation in maximum carapace height for *N. difficilis* ($\beta_{length} = 0.34 \pm 0.12$, $p = 0.011$), *E. pacifica* ($\beta_{length} = 0.13 \pm 0.02$, $p < 0.001$), and *T. spinifera* ($\beta_{length} = 0.13 \pm 0.04$, $p = 0.001$); however, length failed to explain the majority of the variation observed in maximum carapace height ($R^2 = 0.35$, 0.21, and 0.18, respectively; Figure 3).

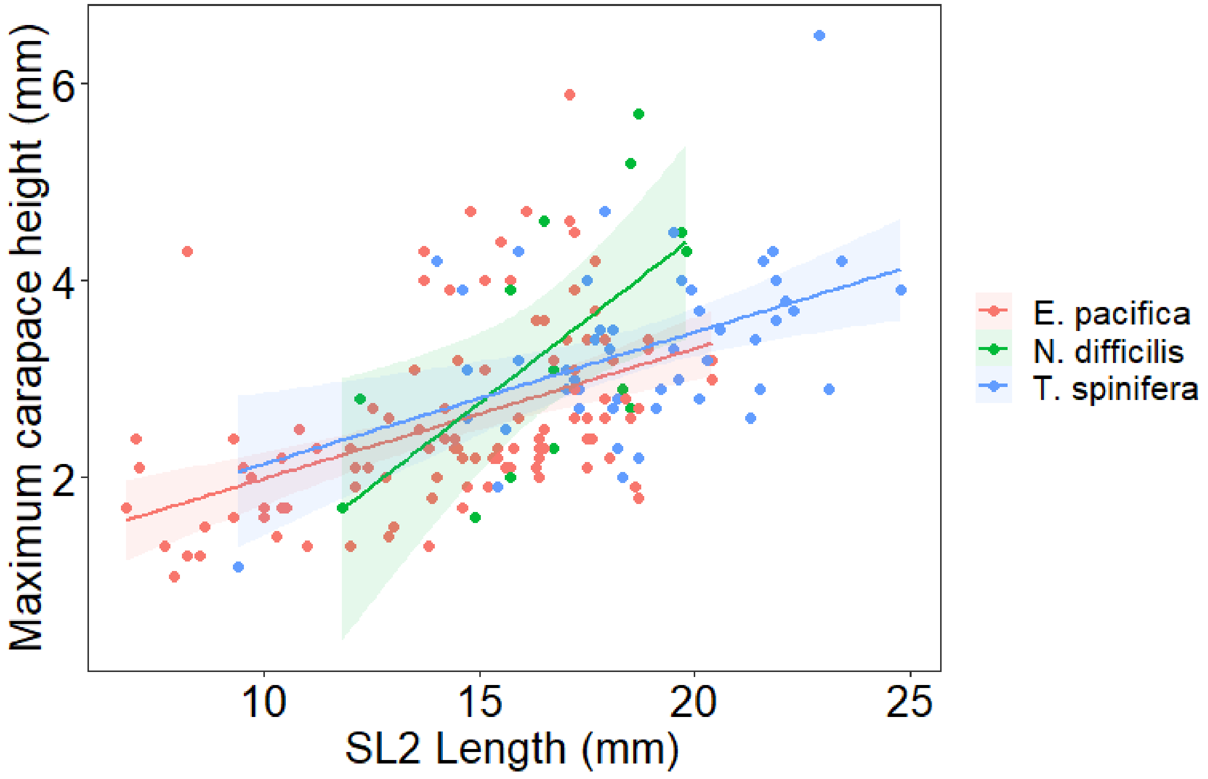

Figure 3. There was a significant positive, linear relationship between maximum carapace height (mm) and SL2 length (mm) among species. The shaded regions represent the 95th percentile confidence interval.

Similarly, animal length significantly accounted for variation in maximum carapace width for *N. difficilis* ($\beta_{\text{length}} = 0.28 \pm 0.12$, $p = 0.04$), *E. pacifica* ($\beta_{\text{length}} = 0.13 \pm 0.03$, $p < 0.001$), and *T. spinifera* ($\beta_{\text{length}} = 0.26 \pm 0.06$, $p < 0.001$); however, length failed to explain the majority of the variation observed in maximum carapace width ($R^2 = 0.23$, 0.20, and 0.23, respectively; Figure 4).

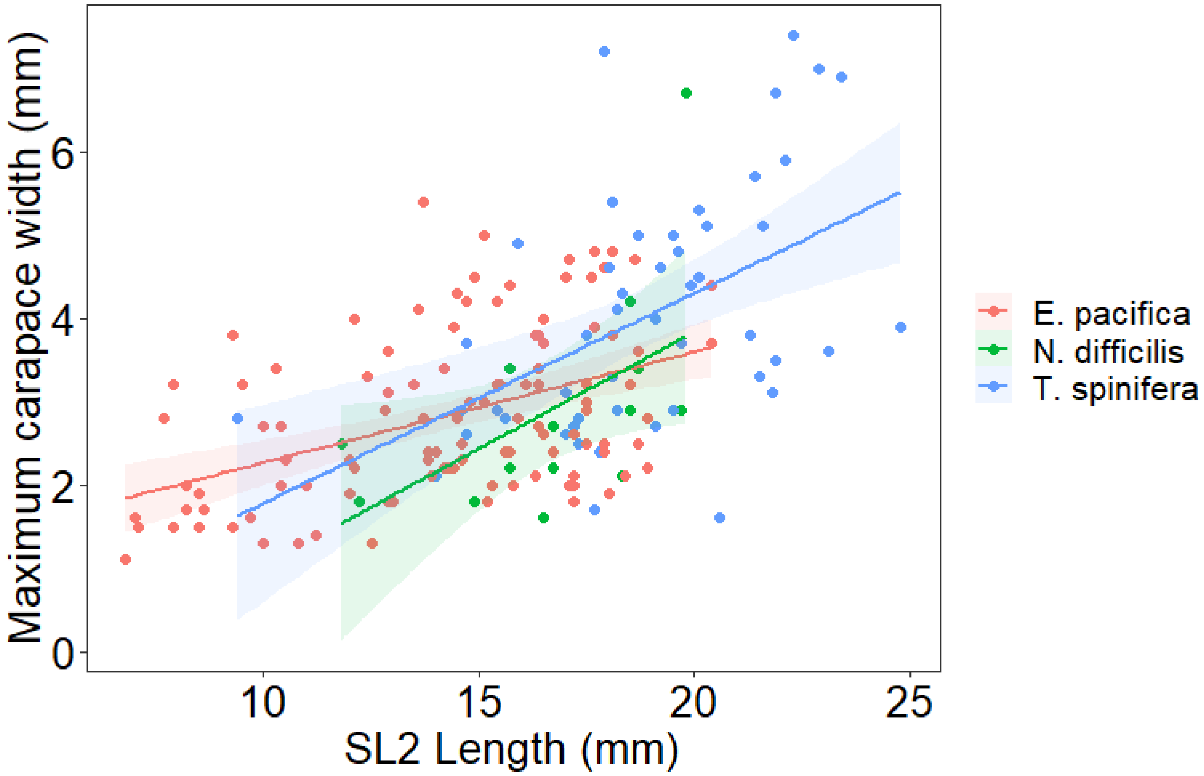

Figure 4. There was a significant, positive linear relationship between maximum carapace width (mm) and SL2 length (mm) among species. The shaded regions represent the 95th percentile confidence interval.

Lastly, there was a relatively weak, positive linear relationship between maximum carapace width and height for *E. pacifica* ($\beta_{\text{width}} = 0.30 \pm 0.09$, $R^2 = 0.09$, $p = 0.001$) and *T. spinifera* ($\beta_{\text{width}} = 0.24 \pm 0.12$, $R^2 = 0.14$, $p = 0.004$; Figure 5), but not *N. difficilis* ($\beta_{\text{width}} = 0.39 \pm 0.25$, $R^2 = 0.08$, $p = 0.16$).

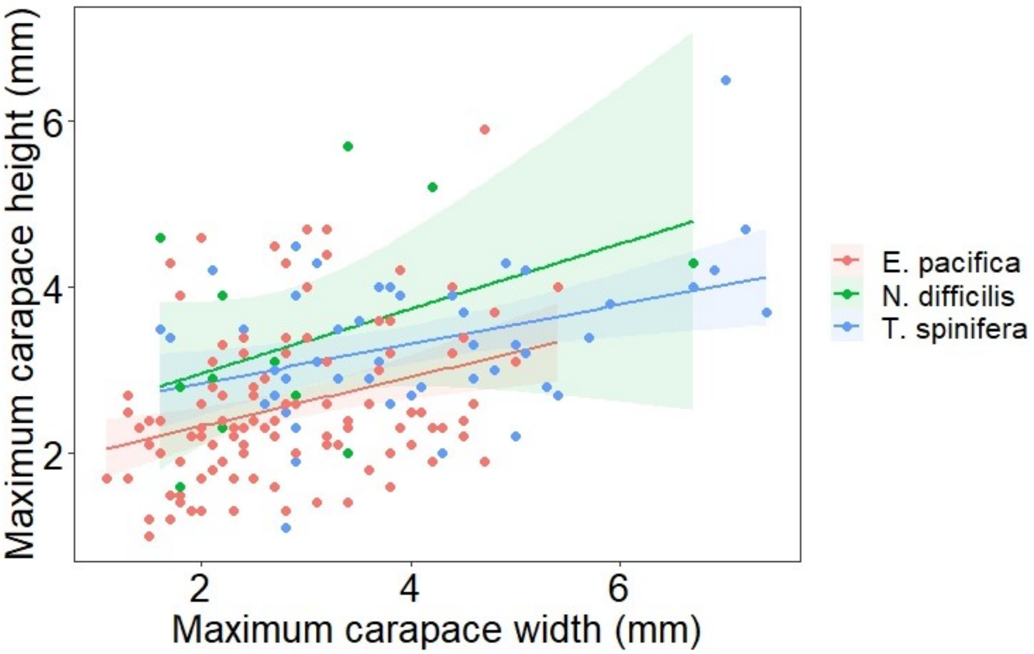

Figure 5. There was a significant, positive linear relationship between maximum carapace width (mm) and height (mm) for *E. pacifica* and *T. spinifera*, but not *N. difficilis*. The shaded regions represent the 95th percentile confidence interval.

There was a significant difference detected in mean mass of krill among species (Kruskal-Wallis rank sum test, $\chi^2_{(df=2)}$, $p < 0.001$; Figure 6) with the mean mass of *T. spinifera* (100.5 ± 6.5 mg) being significantly greater (Wilcoxon rank sum test, $p < 0.001$) than both *E. pacifica* (38.4 ± 2.0 mg) and *N. difficilis* (57.9 ± 12.1 mg).

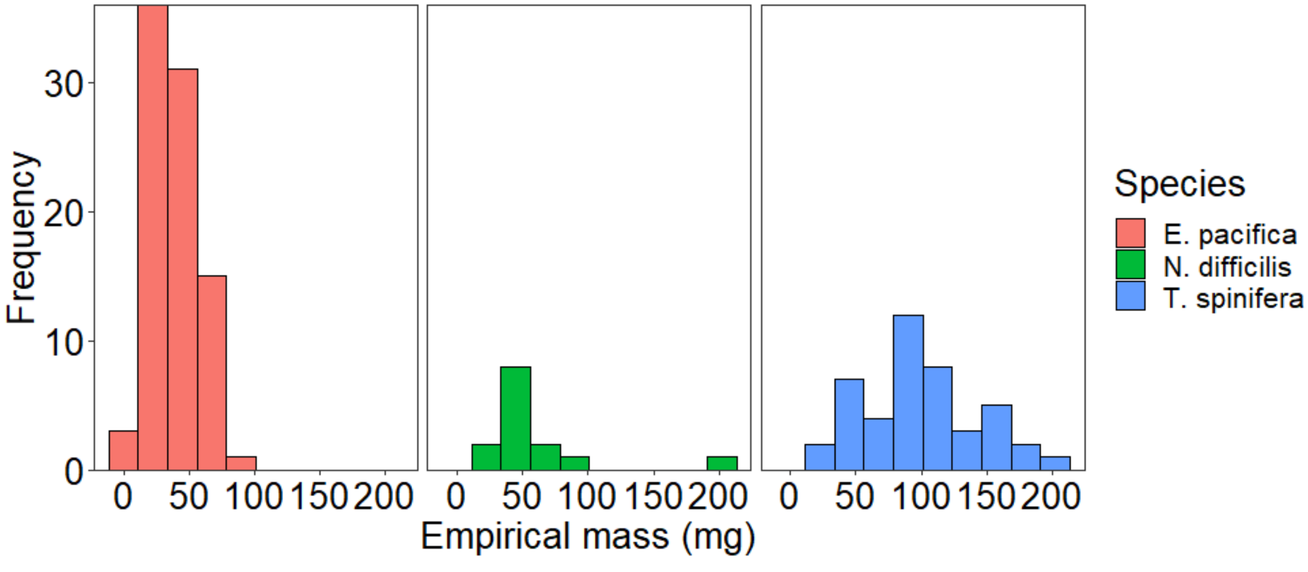

Figure 6. The measured mass (mg) frequency distributions of *E. pacifica* (red), *N. difficilis* (green), and *T. spinifera* (blue) from the 14-24 May 2019 survey. The *N. difficilis* individual with the significantly higher mass (~ 200 mg) was double-checked and that data point is correct, although this individual contained a significant number of eggs.

When all krill species are grouped, there was a significant strong, positive log-linear relationship between mass (mg) and SL2 length (mm) (i.e., $\log_{10}(\text{Mass}_{\text{mg}}) \sim \log_{10}(\text{Length}_{\text{mm}})$, $\beta_{\text{length}} = 3.04 \pm 0.13$, $R^2 = 0.80$, $p < 0.001$; Figure 7, Table 1). When broken up by species, there were similarly strong, positive log-linear relationships between mass and length for *N. difficilis* ($\beta_{\text{length}} = 2.00 \pm 0.78$, $R^2 = 0.30$, $p = 0.025$), *E. pacifica* ($\beta_{\text{length}} = 2.56 \pm 0.16$, $R^2 = 0.74$, $p < 0.001$), and *T. spinifera* ($\beta_{\text{length}} = 3.68 \pm 0.27$, $R^2 = 0.81$, $p < 0.001$; Figure 8). However, 8 out of the 15 *N. difficilis* visibly carried eggs, which may likely have an influence on the species-specific regressions (Figure 9).

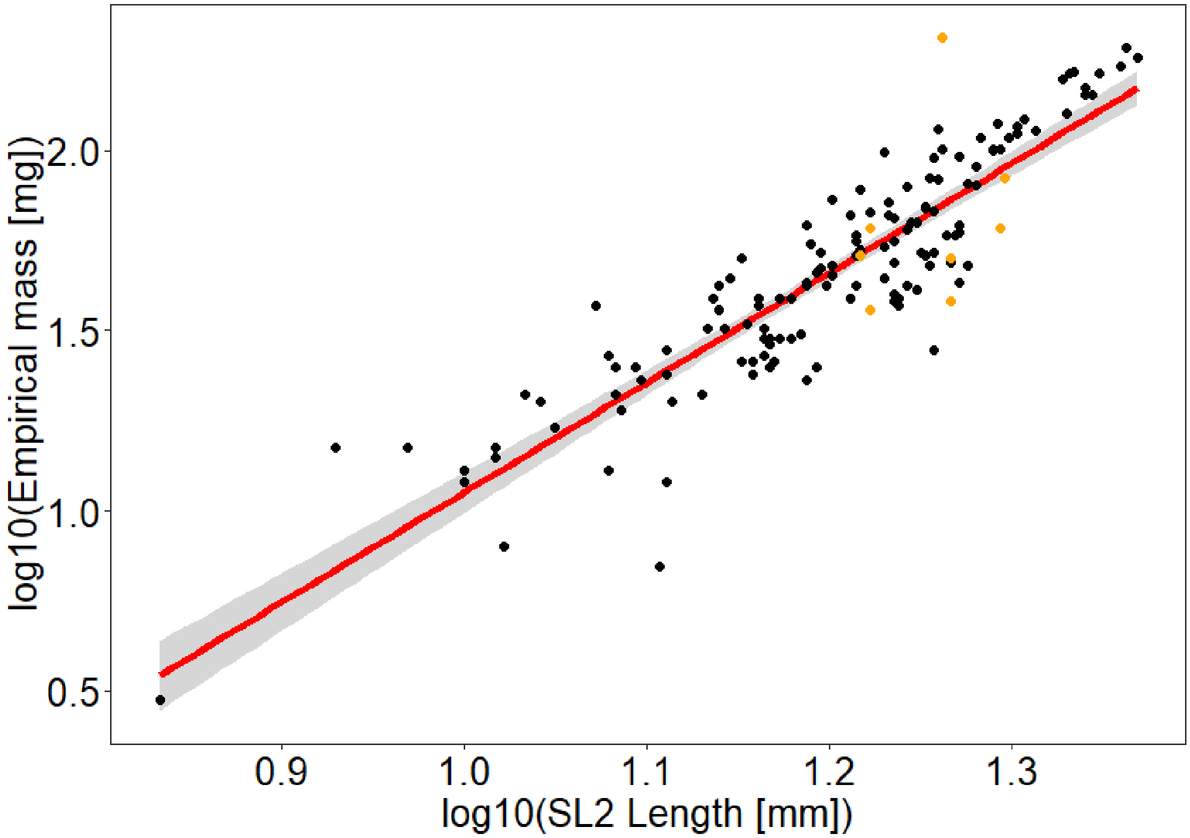

Figure 7. The log-linear relationship between the $\log_{10}$-transformed SL2 length (mm) and mass (mg) for all krill (i.e., with all species grouped together) was statistically significant. The shaded region represent the 95th percentile confidence interval. Orange points indicate *N. difficilis* with eggs.

Table 1. Mass-to-length relationships were calculated for a subset of animals for each species as well as an aggregate CA krill category. SL2 is measured in mm, and mass is measured in mg. Data from the N. difficilis may be biased as half those animals had eggs attached to their body. These regressions are based on a relatively small number of animals so proceed with caution with using the species-specific models. RMS values are provided if these equations are being used to convert a mean length to a mean mass value (per Bird, D. F., & Prairie, Y. T. (1985). Practical guidelines for the use of zooplankton length-weight regression equations. Journal of Plankton Research, 7(6), 955-960.)

Krill species	**# of animals**	***Length to mass equation coefficients*** **Mass (mg) = 10^intercept * SL2(mm)^β**		***R^2***	***RMS***
		intercept (mean ± SD)	***β (mean ± SD)***		
E.pacifica	86	-1.5 ± 0.2	2.56 ± 0.16	0.74	0.017
T. spinifera	*44*	-2.7 ± 0.4	3.67 ± 0.27	0.81	0.009
N. difficilis	*14*	-0.7 ± 1.0	2.00 ± 0.78	0.30	0.039
All krill combined	144	-2.0 ± 0.2	3.04 ± 0.13	0.80	0.020

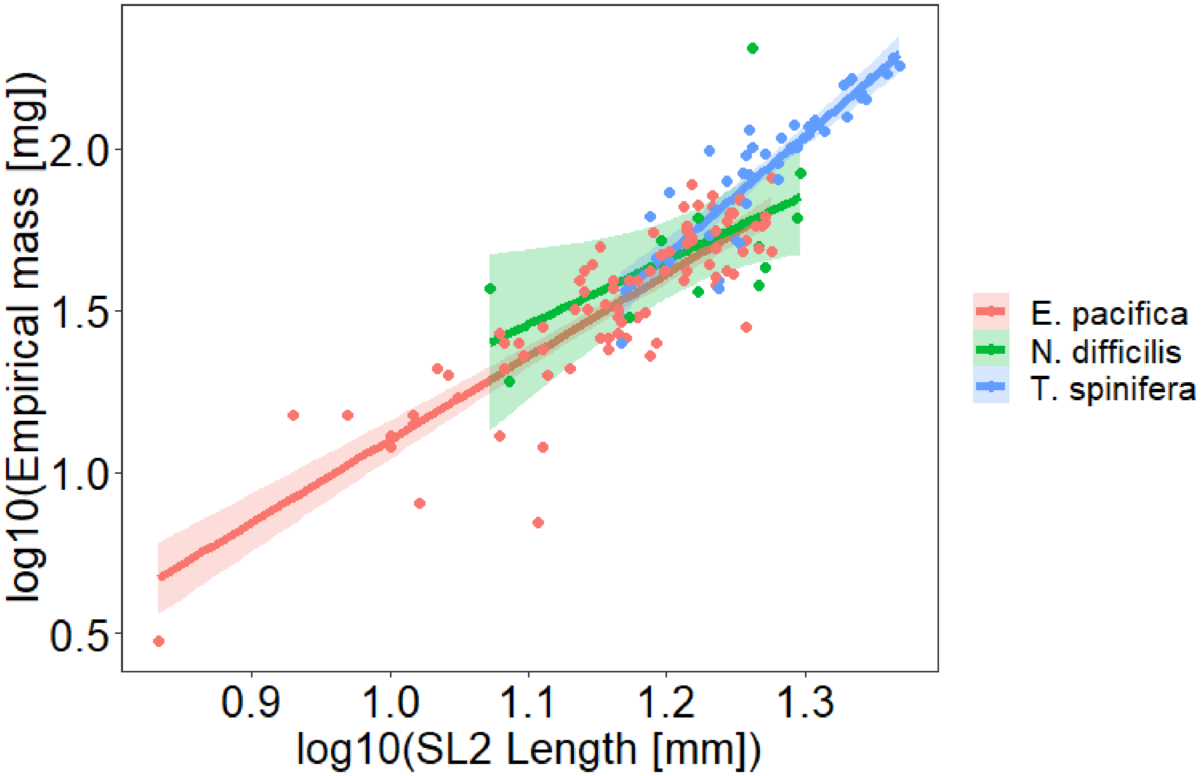

Figure 8 The log-linear relationship between the $\log_{10}$-transformed SL2 length (mm) and mass (mg) was significant for all krill species. The shaded regions represent the 95th percentile confidence interval.

Figure 9. Examples of three *N. difficilis* with eggs directly underneath their carapaces.

Material Property Measurements – Soundspeed

The speed of sound of an animal relative to seawater (sound speed contrast, h) is a necessary parameter for TS models. A PVC t-tube (volume of ~77 mL) was used with 192 kHz transducers clamped to each end. The entire system was then placed into a plastic container filled with ambient seawater (Figure 10). A total of 15 h-measurements, which measures the contrast in sound speed between an animal's body and surrounding ambient seawater, comprising 200 pings were made on bulk samples comprising krill from five sampling stations. Because of the need for many animals for this measurement, there may be small amounts of other zooplankton (e.g., chaetognaths, copepods, small shrimp, gelatinous zooplankton like salp remnants) that are in the krill assemblage that was measured. We expect the small amounts of these other species will not produce significant errors in our measured values.

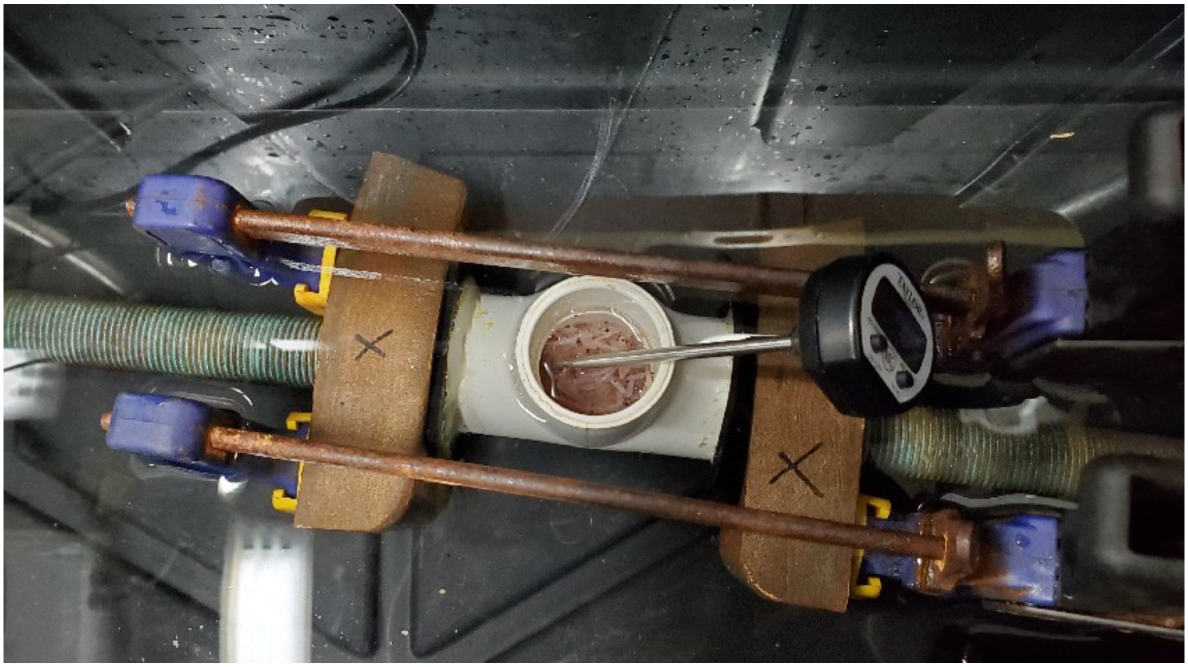

Figure 10. Sound speed measurement apparatus submerged in seawater with krill inside the chamber. Temperature measurements are taken at the start and end of measurements to account for changes in ambient sound speed due to temperature and independent of animal volume (i.e., volume fraction).

The sound speed contrast (*h*) between krill and ambient seawater was 1.024 ± 0.001 (Figure 11) with a mean volume fraction of 0.42 ± 0.01 (i.e., $\varphi_{volume\_fraction} = volume_{krill} / volume_{chamber}$). However, low volume fractions of animals may result in the transmitted soundwave passing by animals in the chamber without passing through their bodies. Therefore, setting a minimum φ_{VF} of 0.30 yields a mean sound speed contrast of 1.023 ± 0.002 (*n* = 13) with a mean φ_{VF} of 0.46 ± 0.02.

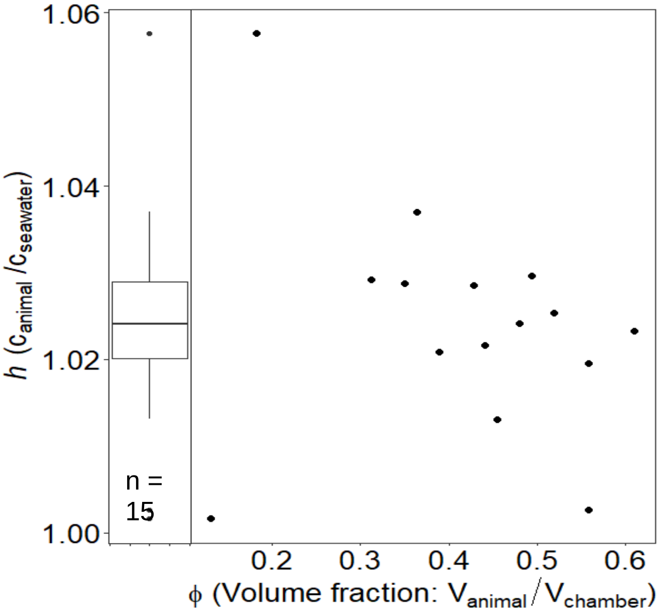

Figure 11. Mean sound speed contrast (*h*, left-panel) was 1.024 ± 0.001 with variable volume fractions. (right-panel). A threshold of $\varphi_{VF} = 0.30$ was applied to only include measurements where it was likely that the transmitted soundwave passed through the bodies of animals.

Material Property Measurements – Soundspeed

Animal density contrast (*g*) values were measured for each of the species of krill (Figure 12). There was a significant difference in mean *g* detected among species (Kruskal-Wallis rank sum test, $\chi_{(df=2)}$ = 7.28, $p = 0.03$) with mean *g* in *E. pacifica* (1.0299 ± 0.001) being significantly greater than *T. spinifera* (1.0261 ± 0.001; Wilcoxon rank sum test, $p = 0.03$), but not *N. difficilis* (1.0269 ± 0.003).

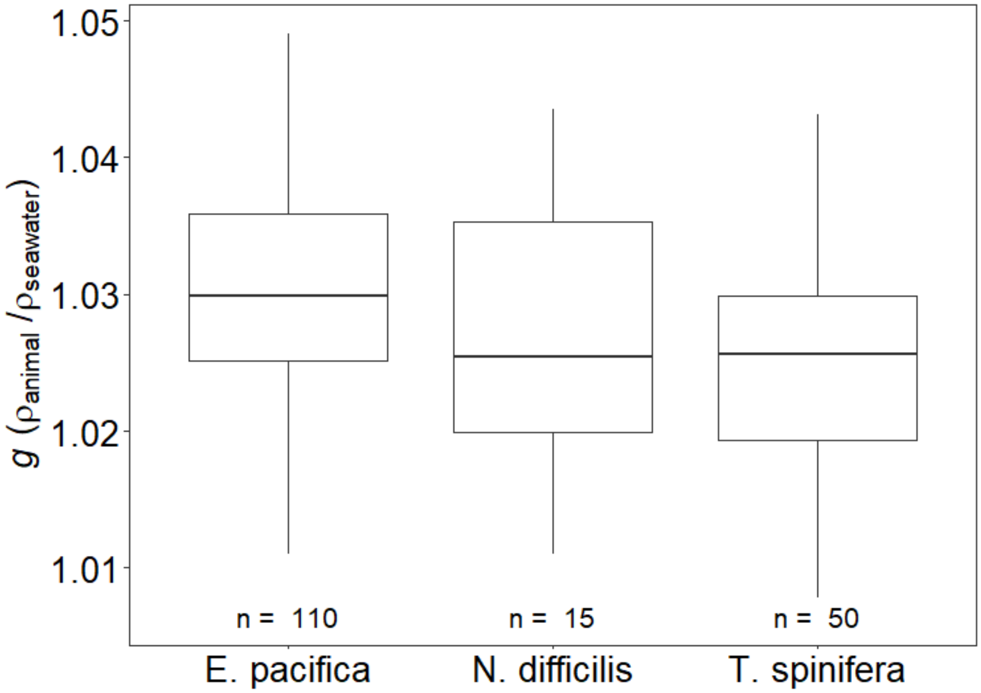

Figure 12. Animal density contrast (*g*) varied among species of krill; however, although mean *g* in *E. pacifica* was greater than *T. spinifera*, it did not significantly change between either *E. pacifica* and *N. difficilis* or *N. difficilis* and *T. spinifera*.

There was also a significant weak, negative linear relationship between SL2 length and *g* (Figure 13) for *E. pacifica* (β_{length} = -0.0009 ± 0.0002, R^2 = 0.12, $p = 0.001$) and *T. spinifera* (β_{length} = -0.0012 ± 0.0003, R^2 = 0.17, $p = 0.002$), but not *N. difficilis* (β_{length} = -0.0004 ± 0.0011, R^2 = 0.00, $p < 0.001$).

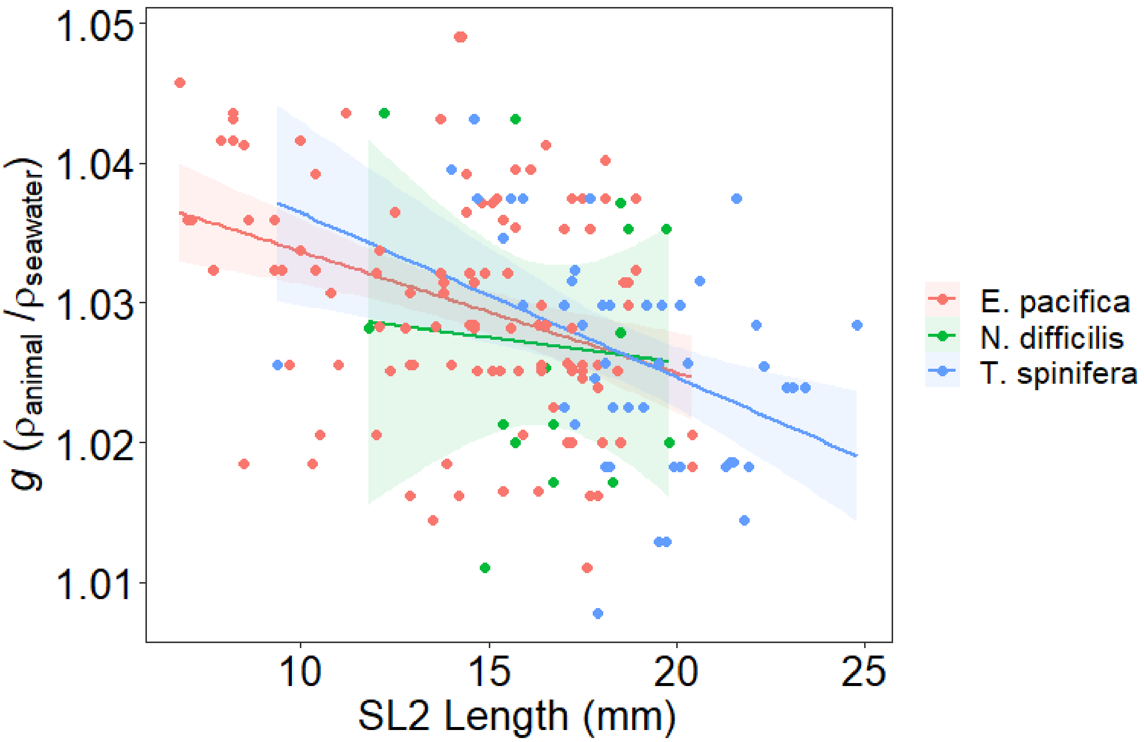

Figure 13. There was a significant, negative linear relationship between SL2 length (mm) and *g* for *E. pacifica* and *T. spinifera*, but not *N. difficilis*. The shaded regions represent the 95th percentile confidence interval.

Target Strength Measurements

A sub-set of captured animals were used for tethered TS experiments (Figures 14-15), which assist in validating theoretical TS models that are parameterized used shape, *g*, and *h* measurements, among other parameters. Animals were tethered (*n* = 32) using monofilament fishing line and were insonified at both discrete frequencies and wideband frequency sweeps. A total of 200 pings were collected for each animal at each frequency and sweep: 38, 50, 70, 120, 150, 200, 38-73, and 130-210 kHz. These measurements were calibrated both using standard methods and applying an offset based on calibration sphere measurements at the aforementioned frequencies and sweeps.

Figure 14. Animals were tethered in a 44 gallon aquarium with two transducers positioned in a bistatic setup.

Figure 15. Screenshot of a tethered krill recorded from a camera positioned inside the aquarium that is used to measure orientation (relative to the tank surface) and curvature of krill.

Empirical TS measurements were then compared to theoretical values produced by a stochastic distorted Born wave approximation (SDWBA) model originally developed for krill from other ecosystems (Figure 16). This model was parameterized using a combination of individual-specific and generalized inputs, all from measurements made during the RV Lasker cruise. Individual-specific measurements included: length, shape, *g*, orientation (relative to the transducer face, θ) within the aquarium during the tethered experiments, and body curvature. Body curvature, ρ_c, is represented by the ratio of the radius of a fitted circle that resembles the curvature of an animal to the length of the animal. For example, a 20 mm krill that is L-curved would have a generally smaller ρ_c less than 1.0. Conversely, a nearly straight-bodied 20 mm krill would have a ρ_c exceeding 6.0. Generalized measurements included: *h* and phase deviation. The SDWBA model predicted the theoretical TS of individual krill using *h* drawn from a normal distribution of values (N(1.0223, 0.0086)) and a phase deviation of 0.2. This phase deviation term attempts to account for the relative complexity of animal shape compared to the model's assumption of the body being broken up into discretized cylinders. In cases where *g* was not available due to the loss of the sample between TS and *g* measurements, the species mean *g* was used. Due to the uncertainty of orientation measurements, the model was parameterized using the measured animal orientation in the tank ± 15° to capture the range of values possible. Lastly, a total of 20 iterations of the model were ran for each animal for each frequency. A summary of parameter inputs can be found in Table 2.

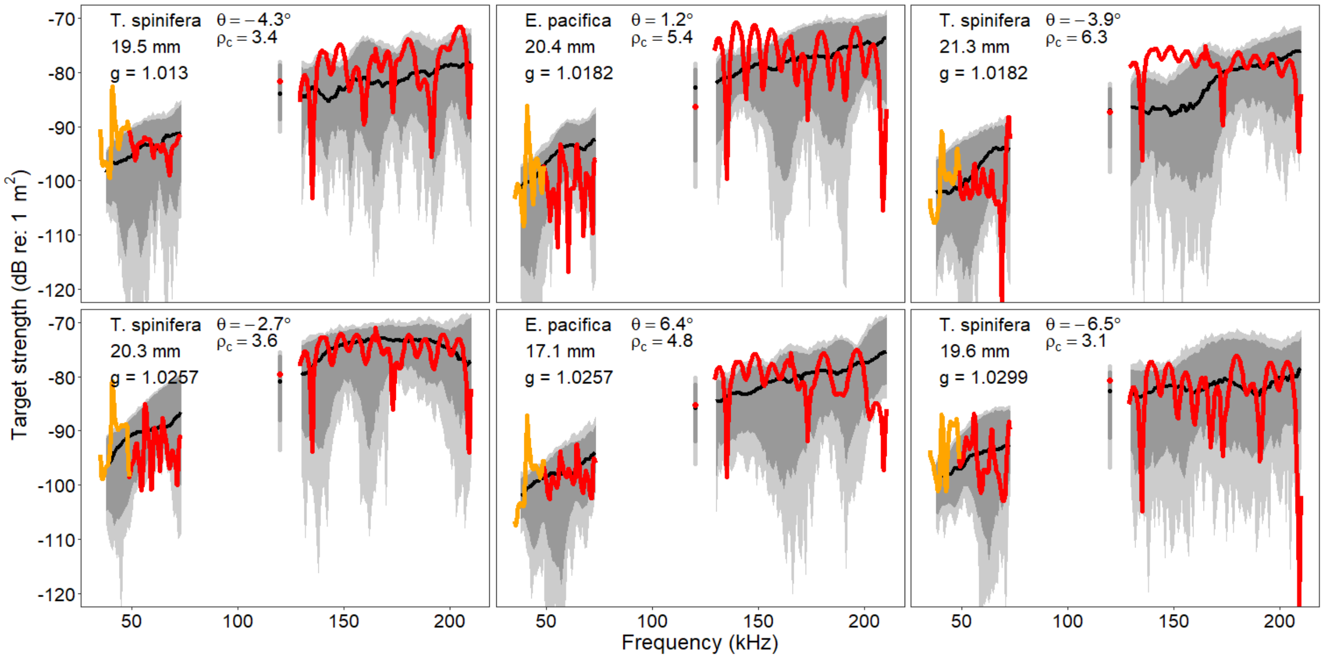

Figure 16. Comparisons between the empirical tethered tank (red) and median SDWBA theoretical TS measurements (black) were generally in agreement at higher frequencies and at 120 kHz (the single point between the two wideband sweeps). Individual animal/model parameters are provided in each sub-panel. Lengths are SL2. The dark gray region represents the 95th percentile confidence interval. The light gray region represents the prediction interval (i.e., minimum and maximum values from all model iterations at each frequency). The orange line represents empirical measurements below 49 kHz where it appeared the signal-to-noise ratio was low, resulting in TS values being too polluted by noise and not being able to be fully isolated.

Table 2. Summary of SDWBA model inputs for each krill species with distributions (gaussian, mean value ± standard deviation) reported where appropriate. Although some modeling parameters such as length and *g* may be individual-specific, they can also be generalized for species-level models. Parameters such as θ and ρ_c are specific to experimental conditions of tethered individuals.

Parameters	***E. pacifica***	***N. difficilis***	***T. spinifera***
Shape	Individual-specific	Individual-specific	Individual-specific
Length (SL2)	N(14.3, 3.4)	N(16.6, 2.4)	N(18.8, 2.9)
g	N(1.0299, 0.0082)	N(1.0269, 0.0099)	N(1.0261, 0.0078)
h	N(1.0233, 0.0086)	N(1.0233, 0.0086)	N(1.0233, 0.0086)
ρ_c	Individual-specific	Individual-specific	Individual-specific
θ	Individual-specific	Individual-specific	Individual-specific
φ	0.2	0.2	0.2
Number of iterations	20	20	20

Conversion of krill lengths

There are several different krill lengths that can be measured. We measured both SL1 and SL2 for the krill that were collected and calculated regression relationships for each species as well as an aggregate of all krill measured during the cruise (Table 3). SL1 is measured from the anterior tip of the rostrum to the posterior end of the uropods. SL2 is measured from the anterior of the eye to the end of the 6th abdominal segment. There is also SL3 (not measured in this work) which is the posterior base of eye stalk to end of the 6th abdominal segment. Note that the number of animals used in the SL1 and SL2 regressions differs slightly from the number of each species used in the other measurements in this report.

Table 3. Regression relationships between SL1 and SL2 for krill photographed and measured during the cruise. SL1 is measured from the anterior tip of the rostrum to the posterior end of the uropods. SL2 is measured from the anterior of the eye to the end of the 6th abdominal segment. Conversions between the two values can be done using the formula: SL2 = β * SL1 + intercept.

Krill species	**# of animals**	***Linear regression coefficients (mean ± SD)*** **SL2 = β * SL1 + intercept**		***R^2***
		β	***intercept***	
E.pacifica	115	0.82 ± 0.007	0.08 ± 0.13	0.99
T. spinifera	*47*	0.88 ± 0.018	-0.88 ± 0.43	0.98
N. difficilis	*18*	0.81 ± 0.029	0.18 ± 0.61	0.98
All krill combined	188	0.84 ± 0.006	0.33 ± 0.13	0.99