ADHESIVE STRENGTHS IN THE GASTROPOD FAMILY *NERITIDAE* ALONG AN ELEVATION GRADIENT ON MOOREA, FRENCH POLYNESIA

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Abstract. The amphidromous lifecycle of members of the gastropod family *Neritidae* provides a unique opportunity to explore the relationship between biomechanical niche adaptation and colonization of freshwater habitats by marine gastropods. Surveys conducted in the Uufau and Opunohu Rivers of Moorea, French Polynesia reinforced the neritid amphidromous lifestyle through the strong positive correlation between movement upstream and neritid size, but species distribution suggested possible anthropogenic introduction of saline conditions in the mid-reach of the Opunohu River resulting in disruption of this lifecycle. *In situ* and laboratory measurements of adhesive strength suggest no correlation between adhesive strength and size or success in upstream movement. Distribution and adhesive strengths displayed across different substrates was largely variable, prompting further questions about the relationship between adhesion to dynamic substrates and net movement of amphidromous snails.

Key words: gastropod, Neritidae*, biomechanics, freshwater ecology, Moorea, adhesion*

INTRODUCTION

An individual's survival depends not only on its intrinsic genetic potential, but also how advantageous such genetic potential is in the environment in which it lives. Genetic variation within a species which determines what parameters of the environment are tolerable, which defines the physical niche of the species. Niche partitioning refers to specialization of related co-occurring species to different parts of the overall physical environment, and is thought to be a result of competition among species over evolutionary time.

For stream organisms, factors such as salinity and flow rate can vary wildly within a general habitat and even within a microhabitat, allowing much potential for niche partitioning. However, any niche partitioning in these cases must allow for cyclical disturbances such as tide, as well as less uniform disturbances, such as rainfall and flash-flooding. Diadromous species provide a unique opportunity to study niche partitioning, because one may use the distribution of organisms along the elevation gradient as a measure of an individual's suitability for particular stream habitats. Diadromous species are also an excellent subject to study in context of the evolution of ancestral organisms' transitions from marine to freshwater habitats.

Islands in particular are useful microcosms of such evolutionary transitions (Ford and Kinzie 1982). Because the native Moorean freshwater snails are diadromous and abundant in many streams, they are ideal subjects to study niche evolution. Functional properties of shell sculpture (Yelenik 1996), morphology (Webster and Vermeij 2017), and orientation (Huryn and Denny 1997) have been extensively studied and shown to affect which parts of the stream a snail can live in. However, hydrodynamic shell morphology is only part of the equation. Adhesive properties of gastropod mucus are a major subject of interest in its unique ability to function under both wet and dry conditions despite low polymer density (Smith 2002). These properties are even more remarkable when one considers that a diadromous snail's mucus must withstand substantial hydraulic stresses under marine *and* freshwater conditions, in addition to the myriad of other bodily chemical changes necessary for osmoregulation in decreasing salinity. Adhesive ability therefore functions as a measure of adaptation to different flow regimes in the stream environment.

The *Neritidae* family of gastropods contributes substantially to freshwater snail species richness in Moorea. The *Neritidae* family is an ideal subject of study because of its amphidromous lifecycle, which is a subset of diadromy. After hatching, neritid veligers (planktonic larvae) are swept downstream to the mouth of the terminal stream, where they must mature in ocean-level saline water before

embarking on their lifelong journey upstream. Therefore, a natural age gap occurs along the elevation gradient. Snails found upstream are usually mature adults, or juveniles that hitched a ride on the back of an older, successful snail (Kano 2009). This provides an opportunity to explore both interspecies adhesive strength as well as adhesive strength across size and age within a species.

The focus of this paper was on the relationship between individual adhesive ability of different species of freshwater Neritid snails and their observed distribution among microhabitats of differing flow rates and substrate types. This paper includes field and lab measurements of adhesion to fully capture both the apparent and potential adhesive abilities of the snails. The major species of interest is *Neritina turrita,* as it was the most abundant neritid species found in the Opunohu River, which was this study's main collection and survey site. Distribution of another neritid, *Neritina auriculata,* along an elevation gradient is recorded in place of adhesive ability as its shell morphology does not allow for adhesion measurements. Using adhesive ability as a measurement of adaptation, the following questions were asked: (1) How much variation is there among species in adhesive ability? (2) How does such difference among species correlate with habitat preference in streams? (3) In the case of diadromous species, how do changing parameters of habitat tolerance within a life cycle relate to the evolutionary transition of marine ancestors from saltwater to freshwater habitats?

METHODS

Study sites

All study sites were located on the island of Moorea in French Polynesia. The Uufau river site began at (-17.547783, -149.884929). There were two collection sites along the Opunohu. One was near the mouth of the river in the brackish interface (-17.520856, -149.849044) with a salinity of 34ppt. This represented the starting point of the snails' upstream journey. The second site was further upstream (-17.519493, -149.849739) with a salinity of 0ppt. Assuming the first site as the starting point, these snails successfully traveled a distance of approximately 750 m upstream, transitioning between ocean salinity to freshwater along the way.

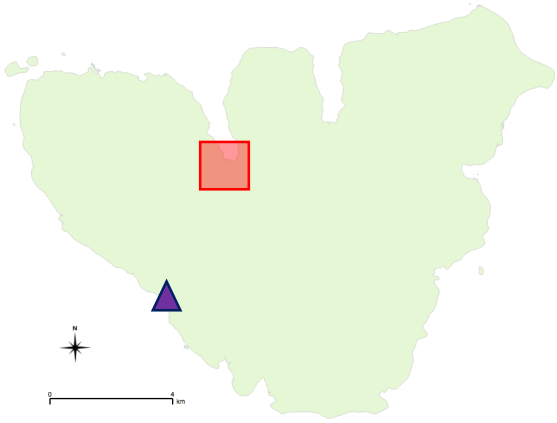

FIG. 1. Map of field sites. Red is Opunohu River, purple is Uufau River. Scale bar 4 km.

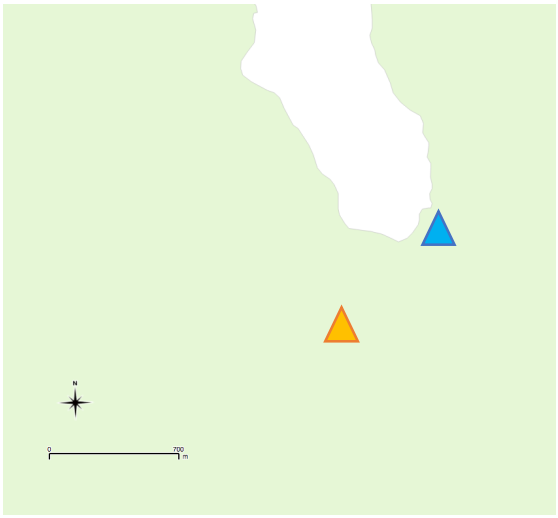

FIG. 2. Detailed map of Opunohu River field sites. Blue is downstream site, orange is upstream site. Scale bar is 700 m.

Uufau River survey

For the Neritina auriculata, a survey of the lower reaches of the Uufau River was conducted. Sites were determined along an elevation gradient with respect to variation in flow rate. All habitats were categorized as one of four major habitats: brackish, intermediate riffle, pool, and upstream. The sites were chosen in order to track both abundance and size distribution as the snails traveled upstream. Flow rate, elevation, and distance from the mouth of the stream were recorded for each site. Specimens were taken from each site and later identified. Neritina auriculata

individuals were measured across their widest diameters in millimeters using a standard ruler.

FIG. 3. Demonstration of site of size measurement on *N. turrita* individual

Opunohu River survey and field adhesion tests

For the Opunohu study sites, microhabitats were categorized both by flow rate and substrate type. Flow rate was separated into distinct categories: above water, pool, stream, and riffle. Substrate types consisted of: porous rock, smooth rock, branch, leaf, sand, and snail. Snails were collected from both the banks of the stream as well as from the stream itself. All snail collection occurred between the hours of 1000 and 1600. After recording its microhabitat, a given snail was secured with a harness of appropriate size was placed around its shell. The harnesses consisted of a single zip-tie tightened to an appropriate diameter and lined with a segment of rubber band to improve grip. The harness was glued to a string which was fastened to a calibrated spring scale. To measure practical adhesion, the scale was pulled in the direction of the current at that site. If this was not possible without altering the microhabitat (repositioning stones, etc.), the scale was pulled directly upward instead. For snails perched atop other snails, the top snail's adhesions were measured by holding the bottom snail in place while pulling the spring scale. The bottom snails' adhesions were not measured because excessive handling often caused them to release their grip voluntarily. After measuring adhesion strength, snails were collected and kept in aerated freshwater tanks corresponding to their collection sites for transportation to the laboratory.

FIG. 4. Snail harness used to measure adhesive power (left loop attaches to spring scale)

Controlled adhesion experiment

To record the snails' adhesion strengths under a controlled setting, a flume was constructed from three window panes cemented together to form a channel with a box at the downstream end to allow a higher water level. Water was supplied to the flume via a hose, and the flume was angled at 10 and 15 degrees to simulate low and high velocity conditions, respectively. Substrates were superglued approximately 20 cm from the water source. The substrates consisted of smooth and porous rocks collected from the Opunohu survey sites. The snails collected from the field were tested one at a time, using a simulated coin toss to determine which from site tank to select the individual. Every snail was measured in length and set on the substrate for 2 minutes undisturbed in order to acclimate to the flume and adhere to the substrate sample before commencing water flow. If the snail sealed off its operculum within 30 seconds of the current's introduction, its initial adhesion was deemed a failure. Each adhering snail was then subjected to the same adhesion measurement technique as described in the field component. A snail which did not initially retreat into its operculum upon exposure to the current, but did release its hold during the harnessing procedure, was considered successful in initial adhesion, but sensitive to physical disturbance. Therefore these snails, whose adhesive strengths were categorized as '0g,' were considered as a special adhesive category separate from the snails which failed to initially adhere or the snails which successfully maintained adhesion through the harnessing process. This adhesive success was termed 'handling adhesion'.

FIG. 5. Demonstration of controlled adhesion experiment in action, at 10 degrees.

Analyses

Results from each site of the Uufau River survey were weighed against the others in a one-way ANOVA for difference of average sizes. The same test was conducted for the Opunohu River survey results of average *N. turrita* size, as well as average adhesive strength of *N. turrita* across different substrates and flow. A Simpson's Diversity Index was taken for both upstream and downstream sites in the Opunohu River. For the controlled adhesion experiment, results underwent two-sample paired t-tests for initial, handling, and true adhesion strengths across the upstream and downstream sites. An additional two-sample paired t-test was conducted across size categories: large (≥15mm) and small (<15mm). All analyses of adhesive power were conducted excluding individuals under the size of 13 mm.

RESULTS

The general surveys conducted at the Uufau and Opunohu rivers both yielded significant results, but adhesion measurements in both the field and laboratory failed to produce significant results.

Uufau River Survey

The population of *N. auriculata* in the lower reaches of the Uufau River displayed a marked decrease in number of individuals moving up along an elevation gradient.

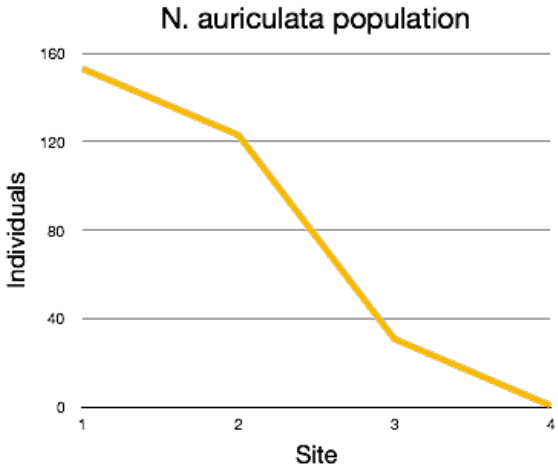

FIG. 6. Line graph of Uufau River site number against number of *N. auriculata* individuals present.

Average size of *N. auriculata* increased significantly moving upstream. The sites also demonstated a clear gradient in number of species present. Flow rate did not appear to affect number of snails, nor did salinity, as the salinity of all sites were 0ppt.

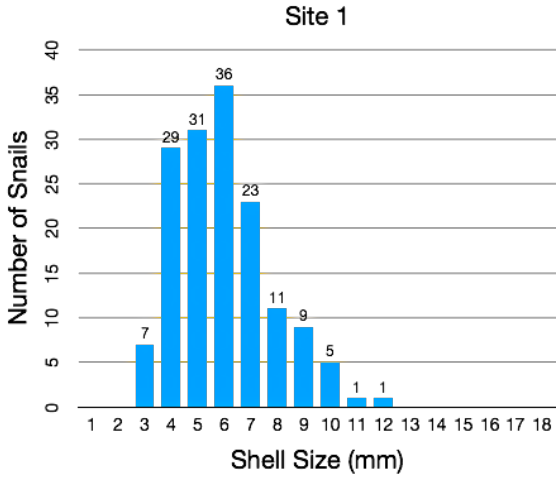

FIG. 7. Frequency table of snail sizes present in site 1.

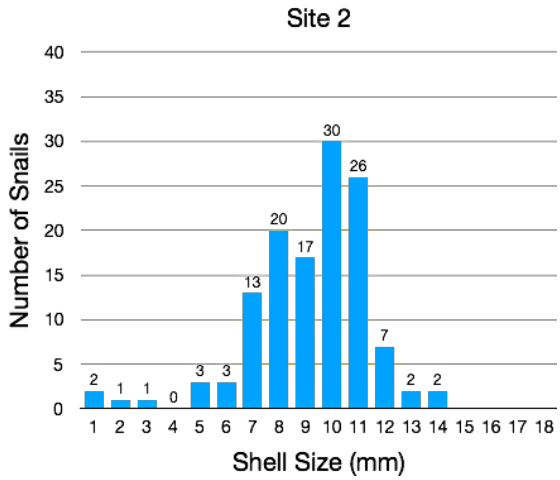

FIG. 8. Frequency table of snail sizes present in site 2.

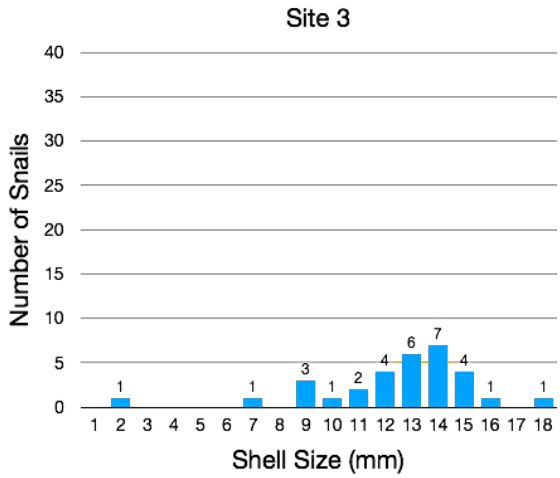

FIG. 9. Frequency table of snail sizes present in site 3.

Opunohu River Survey

Species richness and diversity increased between the downstream and upstream sites, with Simpson's Diversity Indices of 0.1057956 and 0.3132787 respectively. There was also a significant increase in average size of *N. turrita* between the downstream and upstream sites (14.90±3.11mm versus 9.30±3.37mm).

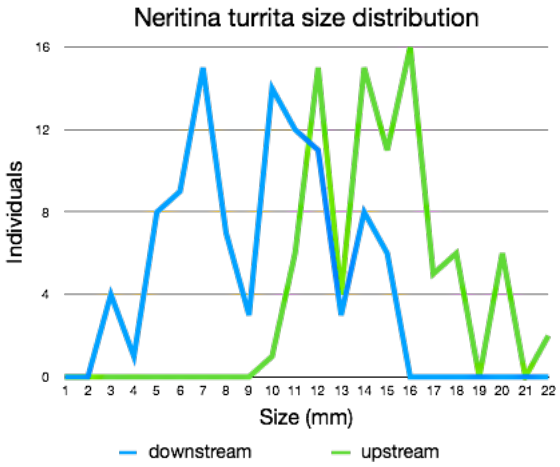

FIG. 10. Line graph depicting size distribution across upstream and downstream sites in the Opunohu River.

There was no apparent preference in *N. turrita* distribution for flow rate across sites.

TABLE 1. Table depicting number of *N. turrita* individuals found in habitats of differing flow categories across both upstream and downstream sites.

Flow Category	Individuals
estuary	28
pool	61
riffle	47
stream	23
out of water	40
bank	13

A strong preference for inorganic substrates was apparent upstream (43 of 212 individuals were observed on organic substrates). Downstream snails had only inorganic substrates available.

Field adhesion tests

Due to patterns of distribution being so skewed toward inorganic substrate preference, a conclusive comparative analysis of organic to inorganic substrate adhesive strength was not possible. No significant difference in adhesive strength was found between smooth and porous rock substrates. No significant difference in adhesive strength was found between upstream and downstream snails, even within size brackets. No significant difference in adhesive strength was discovered

across the different flow habitats. No significant difference in proportion of handling adhesions was found across the two sites. Overall the adhesive strengths of individuals were too variable to confirm nor deny trends.

Controlled adhesion experiment

Due to a relatively low population of other neritid species in both sites of the Opunohu River, a cross-species analysis of controlled adhesion yielded little to no results. Initial adhesion, handling adhesion, and adhesive power also yielded insignificant results across both size and site. Adhesive power across water velocities similarly yielded no significant differences.

DISCUSSION

Uufau River survey

The Uufau River survey's results reinforced the concept of amphidromy in Moorean neritids. The clear increase in size and decrease in population of *N. auriculata* along the elevation gradient indicated that individuals reached maturity in the lower reaches and continued to grow moving upstream. However, all sites, including the site furthest downstream (0 m from the ocean interface), were recorded to have a salinity of 0ppt, even at high tide. This may be due to the timing of this survey at the beginning of the rainy season, but it still indicates a tolerance for freshwater conditions in *N. auriculata* even relatively early in life. Further testing would be necessary to determine the exact timing of development of freshwater tolerance, possibly under controlled conditions.

Opunohu River survey

Similar to the Uufau River survey's findings, the Opunohu River survey further reinforced the concept of amphidromy through the significant increase in size of *N. turrita* between the downstream and upstream sites. Interestingly, *N. auriculata* did not appear further upstream, indicating that it may not tolerate either the higher flow of such a large river (as compared to the relatively small Uufau River) or perhaps some other factor intrinsic to the Opunohu River.

While the decreased presence of *N. auriculata* moving upstream does reinforce the concept of neritid amphidromy, species richness of neritids in general increased moving upstream, which detracts from this theory. One would expect neritid species richness to decrease moving upstream, as mature snails must undergo the relatively unstable environment of an increasingly dynamic habitat. However, the opposite seems to be true: there were some species (namely, *N. canalis* and *C. spinosa*) which *only* appeared upstream, indicating either an extreme selective pressure for upstream movement or some other force at work which reduces the population downstream. As neritid reproduction is mostly constant year-round under tropical conditions (Berry et al 1973), there should theoretically be juvenile or sub-adult *N. canalis* and *C. spinosa* present in greater numbers downstream than up. The upstream site also only feeds into the greater Opunohu River, so there is presumably no other sufficiently saline natural origin at which these veligers may mature. However, there is a shrimp farm along the river which may be artificially salinizing the water, forming estuarine conditions where there once was freshwater, and providing an anthropogenic site of maturation for certain neritid veligers. Further investigation into whether this farm is affecting the salinity of the Opunohu River and its subsequent impacts on the freshwater ecology of the river could help shed light on anthropogenic effects of agriculture on freshwater ecosystems.

This still begs the question of why *N. turrita* was distributed among both sites, while no other neritid species was. They may simply outnumber the other species to such a degree as to overcome whatever ecological barrier prevents the other species from dispersing along the river. Perhaps more interestingly, something physiological about *N. turrita* specifically may allow it to tolerate or even flourish under these conditions. Again, further investigation into the areas between the downstream and upstream sites may be conducted to explore the reasons behind this strange distribution pattern.

Substrate distribution yielded a strong preference in neritids for inorganic substrates. This makes sense, because neritids are largely benthic and are frequently found on the sides of and underneath rocks. However, the snails found on organic substrates are afforded a unique situation generally unavailable to the rock-adhering snails: organic substrates are often times dynamic, or possess a greater likelihood of becoming dynamic under stream conditions. Many snails were found on leaves which floated closer to the surface of the water or floating downstream, which may result in a

net downstream movement of these snails, especially in periods of heavy rainfall. Conversely, snails found on the backs of other gastropods (generally neritids, sometimes limpets or similar) indicate that adhesion to certain organic substrates (i.e., other shells) may result in a net upstream movement for some snails. This hitchhiking behavior has been studied before (Kano 2009), but not within context of strength of adhesion of the hitchhiking snail to its ride. Further studies into the relationship between adhesive strength and hitchhiking behaviors may shed light on the symbiosis of amphidromous gastropods.

Field adhesion tests

Field adhesion tests yielded little to no significant results due to dearth of non-*turrita* neritid species, as discussed above. *In situ* adhesion across size also yielded no significant results either, which limits the scope of this study by eliminating this potential control variable. Adhesion across flow categories and substrate types being largely variable, no conclusions can be drawn about the relationship between adhesive strength and microhabitat selection. It is possible that these are weakly linked, but there were too few useable samples (snails with both successful initial and handling adhesions) to reach conclusions.

Controlled adhesion experiment

Results from the flume experiment generally mirrored field findings. A lack of other neritid species made analysis of controlled adhesion measurement across species largely dependent on pseudo-replicates, resulting in insufficient data. As mentioned above, adhesive strength did not appear to trend one way or the other as size increased, suggesting that snails found upstream may be larger by virtue of age, rather than selective pressure, further reinforcing the amphidromous lifecycle of neritids. Wide range of adhesives strengths displayed under both high and low velocity conditions across sites similarly suggests that successful upstream journeys depend on chance rather than adhesive strength.

Because adhesive strength of neritids was incredibly variable across size, species, and microhabitat, it cannot be concluded that adhesive strength is an adequate tool to measure niche adaptation in neritids. Development of greater adhesives strengths under freshwater conditions may or may not have played a role in the initial colonization of freshwater streams by gastropods. Further testing of neritid adhesion under saline conditions across different sizes and collection sites may reveal whether or not the adhesive abilities of gastropods are closely linked to the evolutionary development of neritid freshwater tolerance.

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APPENDIX A

TABLE 2. Uufau River survey data summary.

Site	*Site category*	*Distance from ocean (m)*	*Elevation (m)*	*Water velocity (m/s)*	*Number of N. auriculata (individuals)*	*Average size of N. auriculata (mm)*	*Total number of species present*
1	brackish	0-10	0	0	153	5.95±1.8	1
2	intermediate riffle	21-24	1	0.3	123	9.27±2.2	2
3	pool	32-35	2	0	31	12.45±3.0	5
4	upstream	42.6-50	3	0.3	1	7	5

TABLE 3. Data summary and one-way ANOVA for average *N. auriculata* size across sites in Uufau River survey.

Groups	*Count*	*Sum*	*Average*	*Variance*
Site 1	153	911	5.954248366	3.280787754
Site 2	123	1140	9.268292683	5.033986405
Site 3	31	386	12.4516129	9.122580645

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1451.320605	2	725.6603024	159.1057881	5.22631E-48	3.025448266
Within Groups	1386.503499	304	4.560866774			
Total	2837.824104	306				

APPENDIX B

TABLE 4. Statistical overview and single-factor ANOVA for average force of *N. turrita* >10mm across upstream and downstream sites in Opunohu River.

Groups	*Count*	*Sum*	*Average*	*Variance*		
Downstream	29	3050	105.1724138	4990.147783		
Upstream	70	7270	103.8571429	4189.254658		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	35.47245858	1	35.47245858	0.008024644	0.928805385	3.939126125
Within Groups	428782.7094	97	4420.440303			
Total	428818.1818	98				

TABLE 5. Difference of proportion power calculation for binomial distribution (arcsine transformation) for handling adhesions across size brackets for *N. turrita* in Opunohu River field adhesion survey:

h	0.1606374
n1	98
n2	37
Significance Level	0.7211945
Power	0.8
Alternative	Two-sided

APPENDIX C

TABLE 6. Statistical overview and one-way ANOVA for average force across species in controlled adhesion experiment:

Species	*Count*	*Sum*	*Average*	*Variance*
C. spinosa	8	260	32.5	221.4285714
N. canalis	3	120	40	300
N. turrita	27	1080	40	384.6153846

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	355.2631579	2	177.6315789	0.511695906	0.603893347	3.267423525
Within Groups	12150	35	347.1428571			
Total	12505.26316	37				

TABLE 7. Statistical overview and one-way ANOVA for average force across size of *N. turrita* in controlled adhesion experiment.

Size	*Count*	*Sum*	*Average*	*Variance*
13 mm	6	210	35	350
14 mm	8	280	35	171.4285714
15 mm	2	50	25	50
16 mm	8	440	55	571.4285714
17 mm	3	100	33.33333333	133.3333333

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2733.333333	4	683.3333333	2.068807339	0.119528327	2.81670834
Within Groups	7266.666667	22	330.3030303			
Total	10000	26				

TABLE 8. Statistical overview and one-way ANOVA for average force of *N. turrita* across size brackets in controlled adhesion experiment:

Size Bracket	*Count*	*Sum*	*Average*	*Variance*
<15 mm	12	420	35	318.1818182
≥15 mm	13	590	45.38461538	526.9230769

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	672.9230769	1	672.9230769	1.57559906	0.221999905	4.279344309
Within Groups	9823.076923	23	427.090301			
Total	10496	24				

TABLE 9. Statistical overview and one-way ANOVA for average adhesive force of *N. turrita* across sites in controlled adhesion experiment.

Site	*Count*	*Sum*	*Average*	*Variance*
Downstream	9	390	43.33333333	775
Upstream	12	480	40	163.6363636

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	57.14285714	1	57.14285714	0.135714286	0.716651938	4.380749692
Within Groups	8000	19	421.0526316			
Total	8057.142857	20				

TABLE 10. Statistical overview and one-way ANOVA for average adhesive force of *N. turrita* across high and low water velocities in controlled adhesion experiment.

Velocity	*Count*	*Sum*	*Average*	*Variance*
Low	13	540	41.53846154	530.7692308
High	8	330	41.25	241.0714286

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.412087912	1	0.412087912	0.000971817	0.975455931	4.380749692
Within Groups	8056.730769	19	424.0384615			
Total	8057.142857	20				