

**No evidence for interaction in the active feeding behavior in pairs of the intertidal barnacle *Balanus glandula*.**

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## **Abstract**

Barnacles are gregarious, suspension-feeding invertebrates. They are found mostly in the intertidal and sub-tidal zones, and densely aggregated to facilitate sexual reproduction by copulation. By living together in dense groups, barnacles can change their local flow environment, potentially altering the behavior or energetics of their neighbors. In this study, I recorded video of small aggregations of the barnacle *Balanus glandula* and studied whether or not individuals responded to adjacent barnacles in their active feeding behavior by either maintaining synchrony or asynchrony in cirral beating. I found that barnacles do not exhibit any behavior that suggests sensitivity to neighbors' cirral positions, and that the difference in beating phase between actively feeding individuals follows a periodic function as would be expected when comparing the phase of the two individuals beating independently.

## **Introduction**

Barnacles, due to the need to reproduce by copulation, are among the group of suspension feeding invertebrates that are not colonial but are nonetheless often found in aggregations (Brusca & Brusca 2003). The study of the effects of population density on gregarious suspension feeders has largely examined the negative effects, but recently there has been an increasing amount of attention spent on facilitation and other positive effects of high density (Leslie 2005). Among these has been the observation that, in the suspension feeding sabellid worm *Eudistylia vancouveri*, for example, local flow is altered to increase feeding efficiency in aggregations of individuals as compared to solitary ones (Merz 1984).

The effects of dense aggregation on barnacles have been examined for multiple genera, and results have been very different for each. Leslie (2005) found that individual barnacle growth rate and fecundity are reduced at high densities compared to solitary living in *Balanus glandula*, and that the benefit of living at high densities was reduced mortality. Bertness et al. (1998), studying *Semibalanus balanoides*, reported an increase in growth rate and fecundity in individuals living in groups. They report that, by crowding, barnacles provide each other with structural support (reducing the energy required for building a reinforced test) and a protective buffer from heat and desiccation stress. They observed, however, that individuals that were located in the crevices of the hummocks (commonly with complicated shapes) actually faced lower particle capture rates than solitary individuals, demonstrating the risk of being immobile in a dynamic aggregation. Other studies have also shown both with model and living animals that barnacles in different locations within an aggregation (and barnacles that are solitary) all face different local flow environments and have different particle capture rates that are functions of their positions (Pullen & LaBarbera 1991, Eckman & Duggins 1993).

It is clear that living in aggregations poses new and different challenges to individuals than being solitary, and that different types of barnacles aggregate differently. One plausible avenue of research for understanding the way that barnacles adapt to these new challenges is to measure the behavioral responses of individuals in aggregations to each other. In this study, I examined the degree to which the barnacle *Balanus glandula* responds to the cirral beats of its neighbor, either in working to maintain a constant phase difference or phase unison. Whether or not individuals respond to each other behaviorally while actively feeding gives information about their sensitivity to being in an aggregation

versus feeding as solitary individuals. Understanding this provides a modest step towards sorting out whether barnacles actively adapt and change feeding strategies as a reaction to the possible challenges or benefits of living gregariously.

### **Study System**

*Balanus glandula* (Darwin, 1854) is a common barnacle on the Pacific coast of the United States (Morris et al., 1980). It can be relatively long-lived, with a life span up to ten years, and can grow to have a diameter of 22 mm (Morris et al., 1980). It grows commonly on rocks, pier pilings, and other intertidal organisms, namely bivalves (Morris et al., 1980). Rocks were collected from various locations near the town of Friday Harbor on San Juan Island, WA, and were included on the study based on (i) the presence of a sufficient number of live barnacles to make the feeding activity of at least three closely located barnacles feasible and (ii) the somewhat planar local positions of the barnacles to normalize local flow as much as possible. Size of animals was not controlled.

### **Materials and Methods**

Two rocks containing aggregated barnacles were placed in untreated seawater in a clear acrylic viewing tank with dimensions 12.5 mm x 20 mm x 24 mm deep. A Go-Pro Hero3<sup>®</sup> camera was suspended above them, and fiber optic lights were directed at a slight upward angle from below the organism in an attempt to maximize the contrast between cirri and test in each individual. Video was taken for one to three minute intervals at either 30 (rock one) or 100 (rock two) frames per second (fps), and three individuals were selected from each rock according to adjacency and consistency of active feeding. Brine

shrimp that had been hatched one to two days prior to use were added to the water to encourage active feeding. Since number of actively feeding barnacles and their density may affect the ability of individuals to employ this behavior successfully, small aggregations of >10 actively feeding individuals were used to maximize the probability of observing an actively responsive behavior. See Figure 1 for pictures of the two rocks showing the barnacles that were measured.

Following recording, video was viewed frame by frame with Quicktime Player 7<sup>®</sup>. The point in the feeding cycle at which an individual has completed bringing the cirri back and just started to sweep them in the opposite direction to capture particles was chosen to be the beginning of the cycle, and each frame where this was observed was recorded with the number one and will be referred to as a peak. Every other frame was recorded as zero, such that the total number of beats at any time can be considered as the sum of indicator functions at each frame:

$$total\ beats\ at\ frame\ i = \sum_{j=1}^i \mathbf{1}_{\{peak\ of\ phase\}}$$

This sum of indicators was then used in standard least-squares regression to fit a linear function and determine the degree to which barnacles that were actively feeding had any kind of intrinsic natural frequency. Sums of indicators for the two individuals that were being compared pairwise were plotted together to obtain a visual of how many total phases each barnacle had completed at a given frame.

Taking the frame numbers of when each individual was recorded at a peak, the data were then manipulated into vectors containing only those frames in which the peaks were observed, where the length of each beat cycle could be computed. In one instance,

because a barnacle had one irregular beat due to a disturbance at the start of filming, the first measurement of phase length was removed. Comparing between two individuals, the phase differences were computed using the value

$$D_i = \min\{|T_j - T_{k,i}|\},$$

where  $D_i$  is the difference in phase position (measured as the difference in video frames between peak frames),  $T_j$  is the set of completed-cycle times for barnacle  $j$  (frames where peak positions were observed), and  $T_{k,i}$  is the frame number where the  $i$ -th peak was observed for barnacle  $k$ . The order of subtraction is unimportant, as the absolute value is taken, making all elements of the set  $D$  with positive sign. For a given two barnacles, the set of phase differences  $D$  (with length equal to the smaller number of complete phases observed between the two barnacles being examined) was plotted against the index of how many cycles barnacle  $k$  had gone through. Note that, with this set of calculations, the differences reflect only the number of frames between peaks in the two individuals and do *not* contain any information of how many phases each individual has gone through. Further, any meaningful temporal index with respect to frame number is lost with this computation method, and the numerical index used for fitting the regression only reflects temporal rank.

To test the degree to which the phase differences  $D$  were sinusoidal, which would be reflective of non-interacting oscillatory behavior, a linear regression of sine and cosine was done to the second harmonic, such that

$$D_i = \sum_{k=1}^2 [A_k \sin\left(\frac{2\pi k}{\omega} t\right) + B_k \cos\left(\frac{2\pi k}{\omega} t\right)] + C,$$

where  $A$  and  $B$  are estimated coefficients,  $k=1$  represents the first harmonic and  $k=2$  the second,  $\omega$  is the frequency (which was picked to maximize the  $R^2$  value of the regression),  $t$  is a numerical index as discussed above, as the true position in the time series is lost in the calculation of  $D$ , and  $C$  is the vertical translation term.

If two oscillators have slightly different frequencies, then the difference in position between the two is a sinusoidal function with troughs at the point where the faster of the two has completed an integer number of cycles more than the slower oscillator (and with maxima reflecting the midpoints between each of these events). Thus, when looking at the phase differences  $D$  between two barnacles, a minimum value (not always exactly zero because of noise) reflects that the faster barnacle has reached an integer number of peaks (if the sum of peaks is considered to be a continuous function) more than the slower barnacle—in essence, that the faster barnacle has “lapped” the slower barnacle.

Following analyses of the observed data, a simulation study where data was generated using the software R (ver. 3.0.2) to simulate peak time in two oscillators was used to examine the shape and goodness-of-fit of the above model to noisy data with two oscillators that are known to be independent. This was done by modeling the number of frames between peaks as normally distributed with mean and standard deviation estimated from the data of barnacles one and two from rock one (filmed at 30 fps). After initially generating the first frame at which a peak was observed, subsequent observations were generated by adding another randomly generated value from the normal distribution with the parameters from the actual data to the previous active frame number. The same regression was applied to the pseudo-data as was applied to the actual data, and one

hundred trials were simulated to collect the maximum  $R^2$  value (with maxima treated as a function of the period parameter  $\omega$ ) of each trial.

## Results

In the analyses, it was clear that the measured phase differences  $D$  between the two individuals examined followed a somewhat periodic pattern (see Table A for a summary and Figures 2 and 3 for graphs of each). Resolution of a periodic pattern was clearer for the barnacles analyzed on rock two, which were shot at 100 fps instead of 30, which suggests that shooting at 30fps was too sensitive to measurement error and judgment of which frame constituted a peak.

Quantitatively, the  $R^2$  value of the regressions used to fit the curves reflect the degree to which the first two terms of the Fourier series explained the shape of the data. In the actual barnacle data pairings,  $R^2$  values spanned from around 0.2 to 0.8, with the values for individuals examined on rock two (recorded at 100 fps) all much higher than rock one (recorded at 30 fps) (Table A). It should also be noted that, while no quantitative measures of size were involved in the selection of individuals, barnacle two on rock two is noticeably larger than neighboring barnacles one and three, and that there was neither a pattern of departure from phase walk-through between two individuals of different size nor even a reduction in  $R^2$ , implying that even individuals that are much larger than their neighbors do not influence their active feeding behavior. Similarly, the clear difference in proximity between barnacles had no meaningful correspondence with an increase or decrease  $R^2$  values (and thus the goodness-of-fit of the periodic function). If the individuals were responding to each other in any way, proximity and individual

size would very likely be factors of determining an individual's ability and will to do so, so the absence of any pattern is also indicative of a lack of active responsive behavior.

In the simulation study (simulated at 30 fps without integer rounding of frames to have increased resolution), also with periodicity  $\omega$  chosen to maximize  $R^2$ , values for  $R^2$  spanned from around 0.1 to 0.7. This serves to demonstrate that, even in a simulation study designed with oscillators that fit the assumption of independence and a constant mean frequency, a plausible amount of noise (as estimated from the data) strongly limit the ability of the regression to explain the shape of the data. Figure 4 contains three randomly selected graphs of the 100 trials of the simulation study with their regression curves to illustrate this point.

The fit of the least-squared regression on the sum of indicators showed very strong fit for all barnacles, lending strong credence to the assumption of the model that individuals had unique intrinsic frequencies (all standard errors for the slope were on the order of  $10^{-5}$  or smaller, indicating very little variance: Table B). When plotted together with the other individual in pairwise comparison, the two lines clearly show slow but consistent divergence in the number of peaks reached by a given frame, which gives further evidence to the interpretation that the individuals are both acting as entirely independent oscillators that simply have similar beating frequencies (Figures 5,6).

## **Discussion**

It is very biologically plausible that densely packed barnacles do not actively respond to their neighbors by altering their active feeding behavior. This does not give any information about the energetics of the animals, however. It is possible, for example,

that there does exist a clear advantage in terms of particle capture rate to being in or out of phase with neighbors. This could be examined both in particle capture rates and, more indirectly, by looking at flow around two organisms that are beating synchronously versus asynchronously. One possible case is that there actually is a meaningful benefit with respect to particle capture rate to beating asynchronously, and by ignoring neighbors and being out of phase for almost all of the time spent feeding barnacles come close to enjoying the same increased capture rate without expending any of the energy actively coordinating any behavior. It also may be the case that beating in unison has deleterious effects on particle capture rate because of overlap between cirri, and again may be that not having any active behavior at all approximates well enough actively avoiding phase unison at a fraction of the energetic cost.

Testing these hypotheses, and most importantly looking at flow around aggregated individuals at the small-scale local environment around cirri and how it is changed by neighboring feeding, will be the first step in understanding the way that barnacles may behaviorally adapt to the different energetic, reproductive, and physical challenges of living in groups. More generally, observing behavioral responses to density in barnacles may inform broader research on responses to aggregation in other sessile, invertebrate suspension feeders by exposing possible strategies to behaviorally adapt to depletion, reduced flow, or reduced particle capture.

## Works cited

Brusca, R.C., Brusca, G.J., 2003, *Invertebrates* (2ed), Sunderland: Sinauer Associates

Leslie, H. 2005. Positive intraspecific effects trump negative effects in high-density barnacle aggregations. *Ecology* 86: 2716-2725.

Merz, R. A. 1984. Self-generated versus environmentally produced feeding currents: a comparison for the sabellid polychaete *Eudistylia vancouveri*. *Biological Bulletin* 167: 200-209.

Bertness et al., 1998, Making mountains out of barnacles: the dynamics of acorn barnacle hummocking, *Ecology*, 79: 1382-1394

Pullen, J. & LaBarbera, M., 1991, Modes of feeding in aggregations of barnacles and the shape of aggregations. *Biological Bulletin* 181:442-452.

Eckman, J. , Duggins, D., 1993, Effects of flow speed on benthic suspension feeders, *Biological Bulletin*, 185:28-41

Morris, R. H. et al., 1980, "Balanus glandula", *Intertidal Invertebrates of California*, Stanford: Stanford University Press. pp. 520-521

**Table A: Framerate, number of frames used in calculation, and  $R^2$  value for partial Fourier series regression. Data show goodness-of-fit ranging from moderately low to very good, with fit better uniformly at the higher framerate despite the smaller number of observations used for the fitting.**

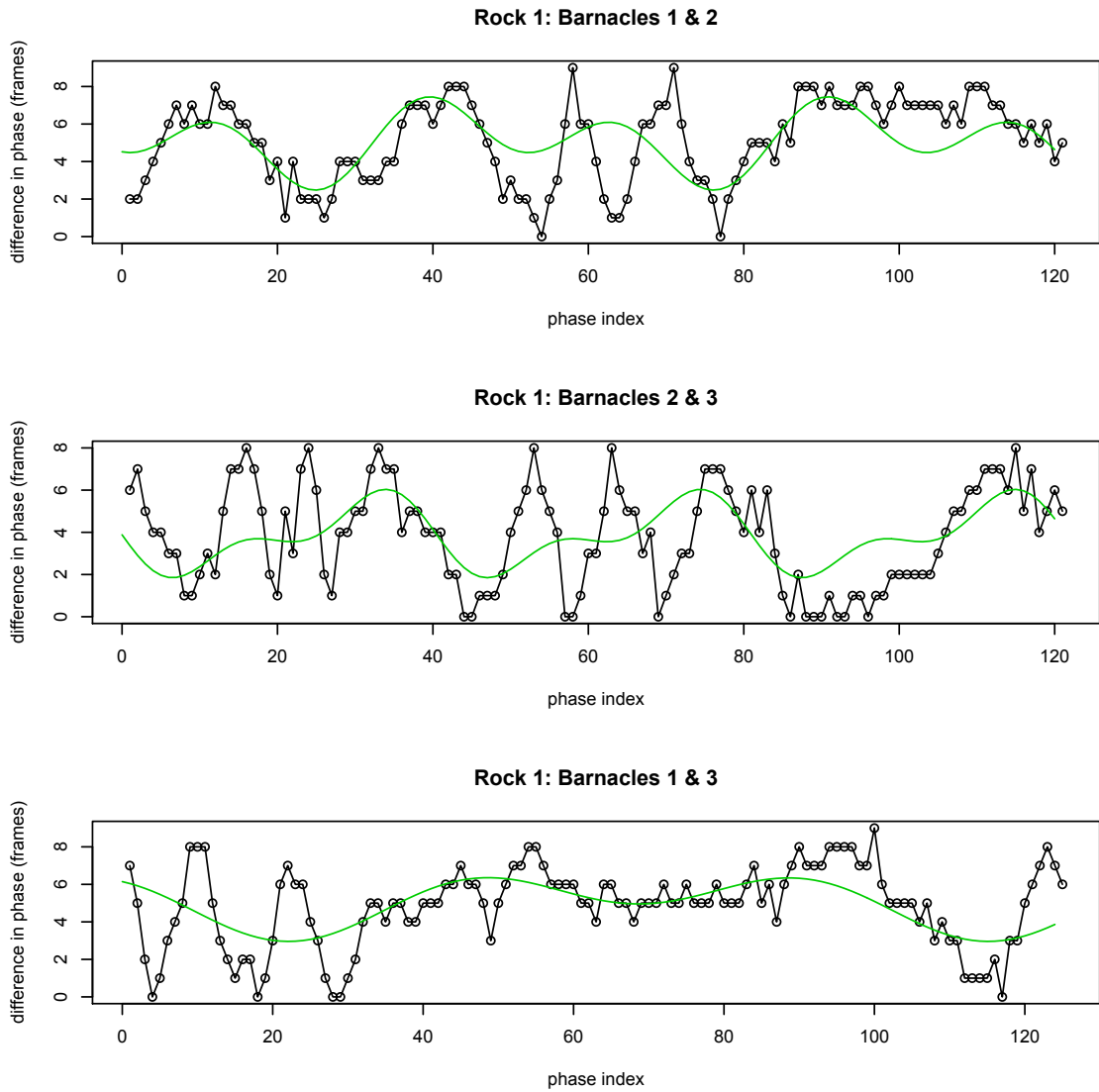
Pairing	Rock1, ind. 1 & 2	Rock1, ind. 2 & 3	Rock1, ind. 1 & 3	Rock2, ind. 1 & 2	Rock3, ind. 2 & 3	Rock2, ind. 1 & 3
Framerate	30	30	30	100	100	100
Length(D)	121	121	125	60	60	61
$R^2$ (D)	0.3376	0.2486	0.2696	0.4906	0.6872	0.7864

**Table B**

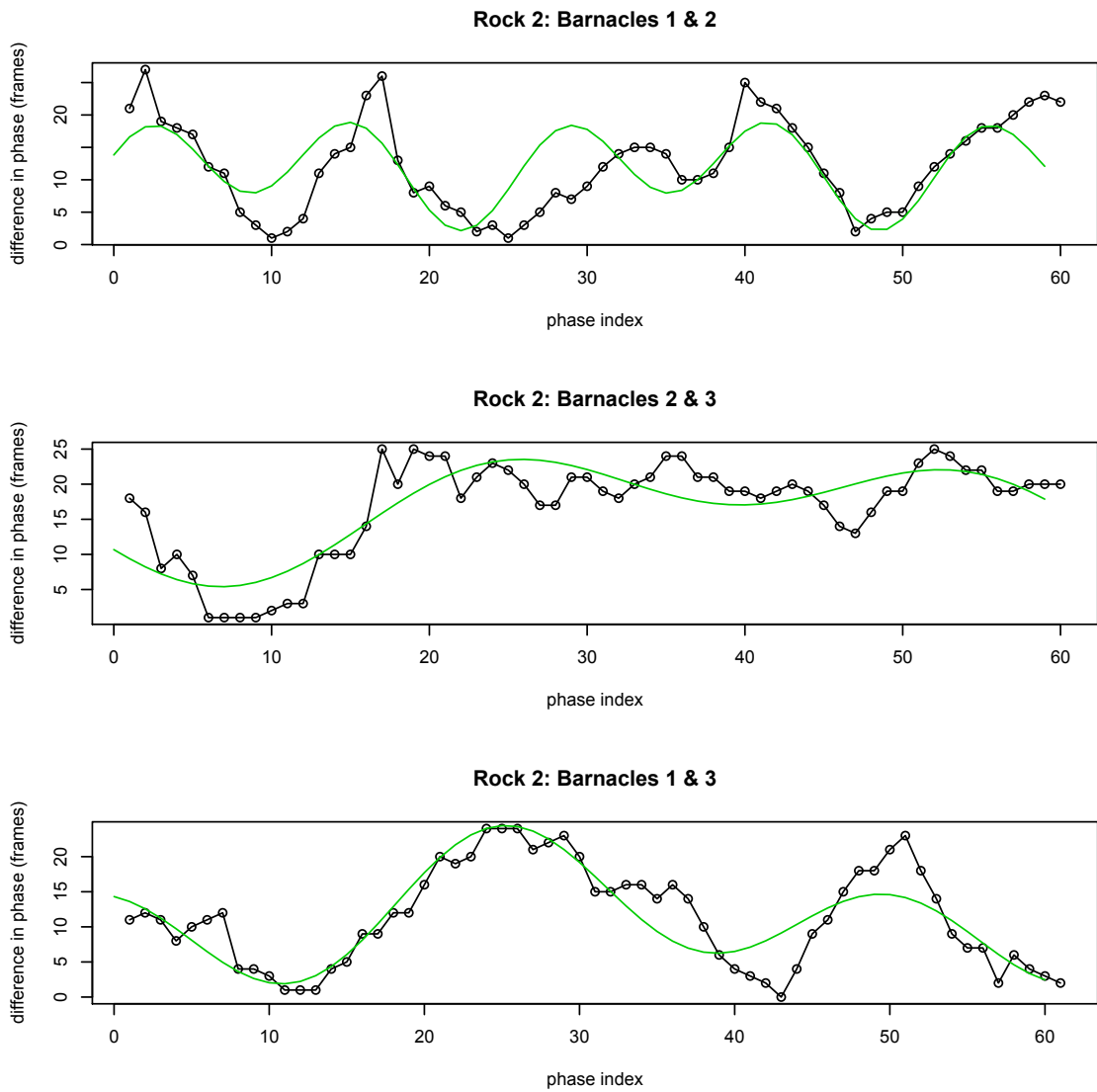
Pairing	Rock1, ind. 1	Rock1, ind. 2	Rock1, ind. 3	Rock2, ind. 1	Rock3, ind. 2	Rock2, ind. 3
Framerate	30	30	30	100	100	100
Total beats	126	122	129	64	61	62
Slope	0.06335	0.06081	0.06464	0.02135	0.02027	0.020537
$R^2$ (LSR)	0.999	0.999	1	1	0.999	1



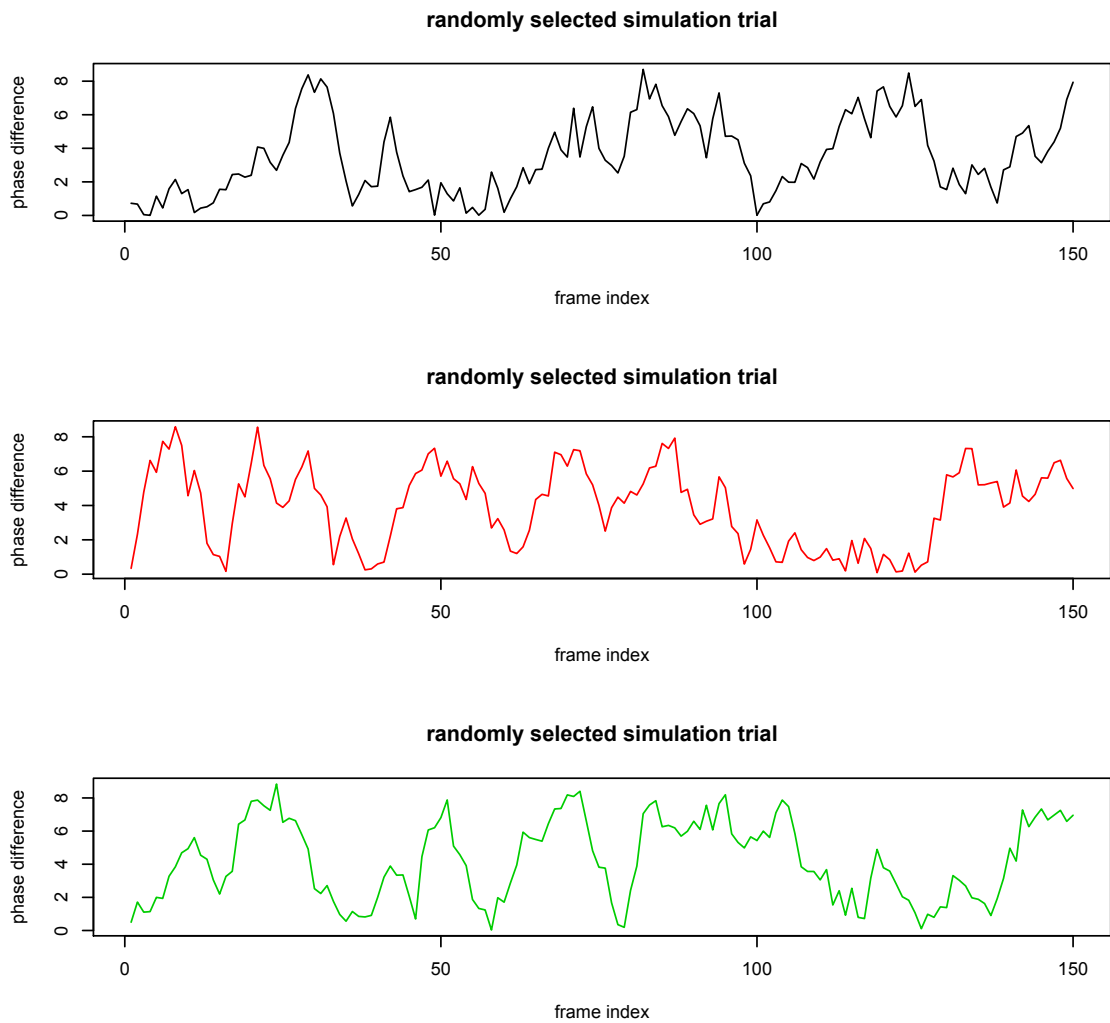
**Figure 1: Rocks one and two, respectively. For both rocks, the red dot marks barnacle one, the green dot barnacle two, and the blue dot barnacle three. Rock one was laid in the tank such that all three barnacles were on the long axis of the tank, while rock two was laid on a piece of glass to be closer to the camera and had no meaningful correspondence with tank axes.**



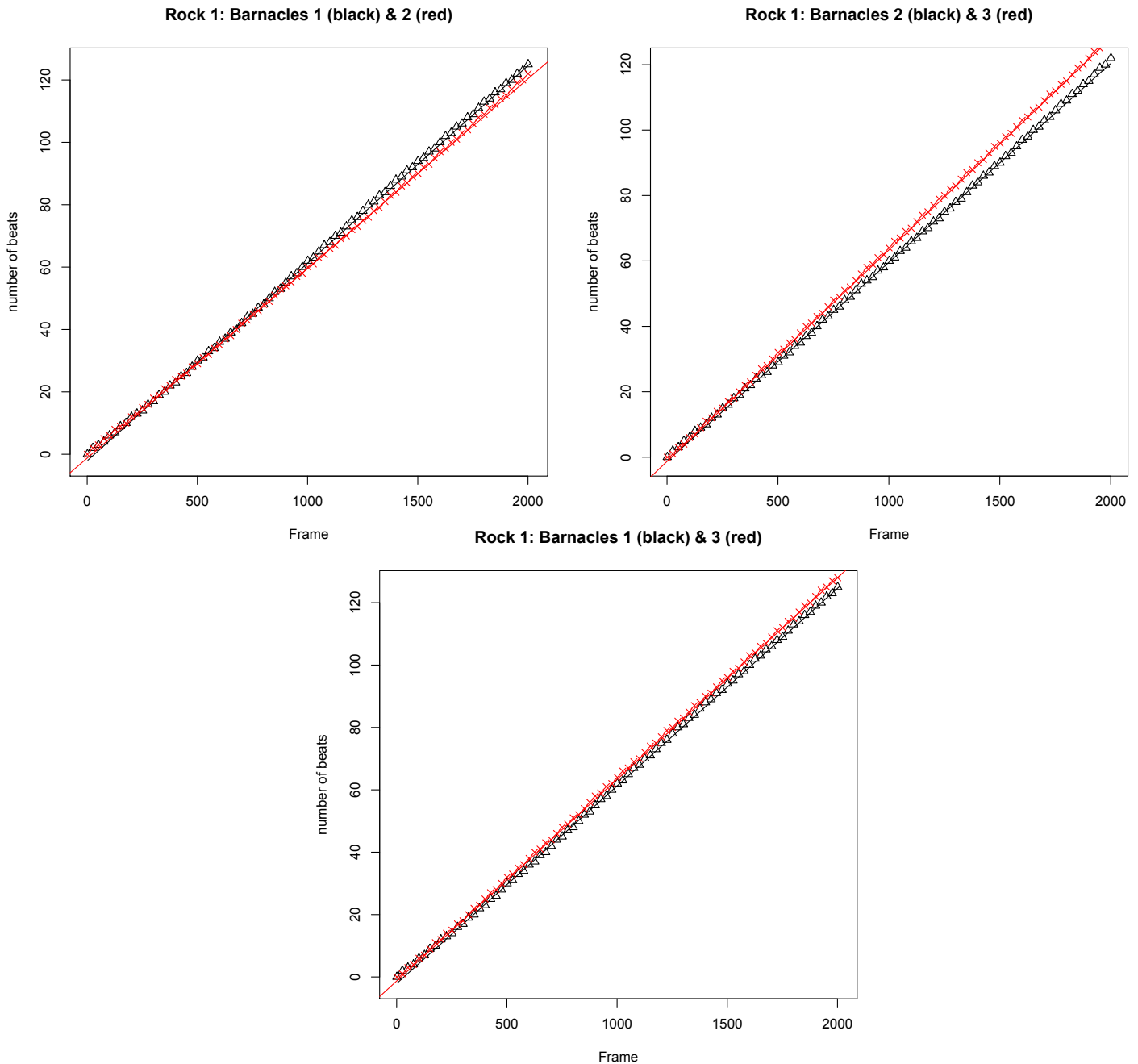
**Figure 2: phase differences for rock one. Frame rate of 30fps.  $R^2$  values for the regressions shown in table A. The X-axis indicates the phase index, where the position of  $X=i$  indicates the rank of  $D_i$  in the set of all  $D$  for a given pair, and the Y-axis gives the minimum number of frames between observed peaks in the two individuals being observed. Since all individuals had cycles that were observed to be around 16 frames in length, the largest difference that would be calculated is when the individuals are maximally out of phase with each other, which is in this case being 8 to 9 frames apart.**



**Figure 3: Rock two phase differences. Frame rate of 100 fps.  $R^2$  values for the regressions shown in table A. See figure 2 for a discussion of the axes and interpretation.**

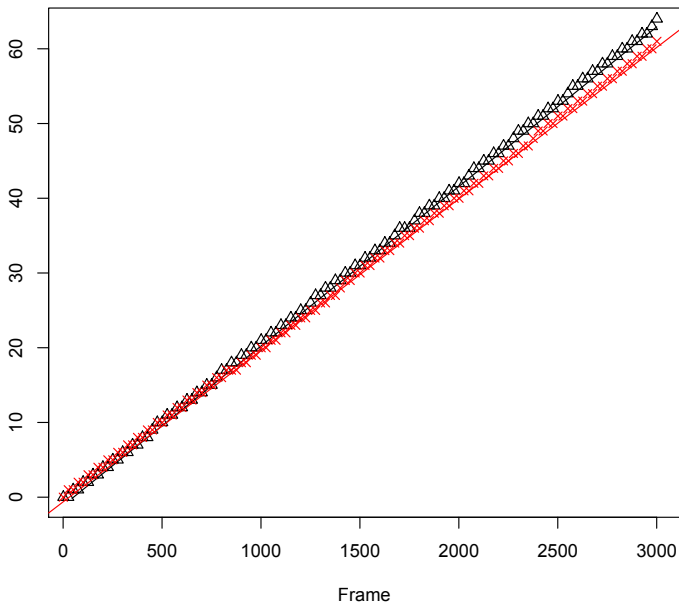


**Figure 4: Three randomly selected plots of phase difference from the simulation study.  $R^2$  values of regression lines (maximized as functions of  $\omega$ ) ranged from around 0.1 to 0.7 in 100 simulation trials. See figure 2 for a discussion of axes and interpretation, which are identical here despite the data being generated instead of observed.**

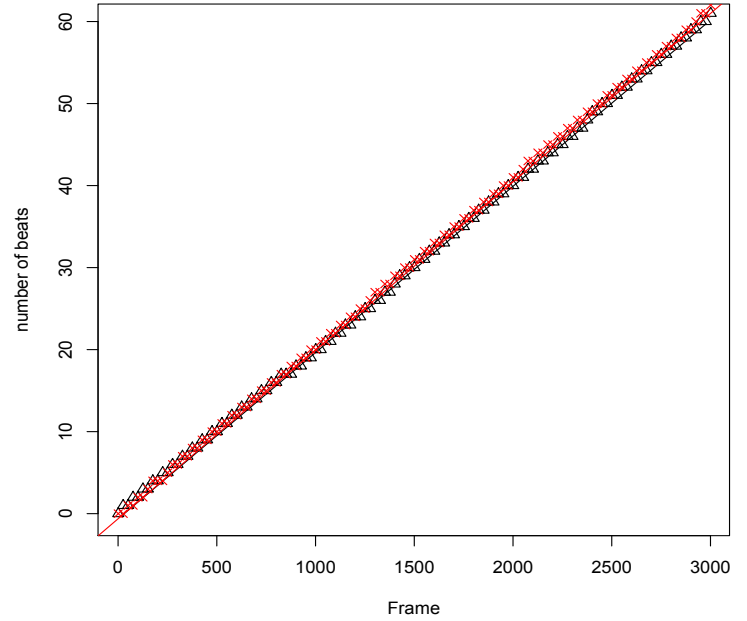


**Figure 5: Rock one linear models reflecting constancy in rate.  $R^2$  values of regression summarized in table B. Here the X-axis gives the frame number, and the Y-axis gives the number of peaks observed at that frame number. The red and black Xs indicate actual observations, which the solid red and black lines show the fitted linear model. While difficult to see when the graph is compressed vertically, there is slow but strong divergence in the number of peaks observed. This shows that the slower barnacle is “lapped” occasionally, which on the graph is when the difference between the Y-value of the two lines is an integer. The X-values at which this occurs would theoretically be marked by a trough on the above graphs, although noise in the data and lappings happening between frames make most of these events difficult to observe visually in the data.**

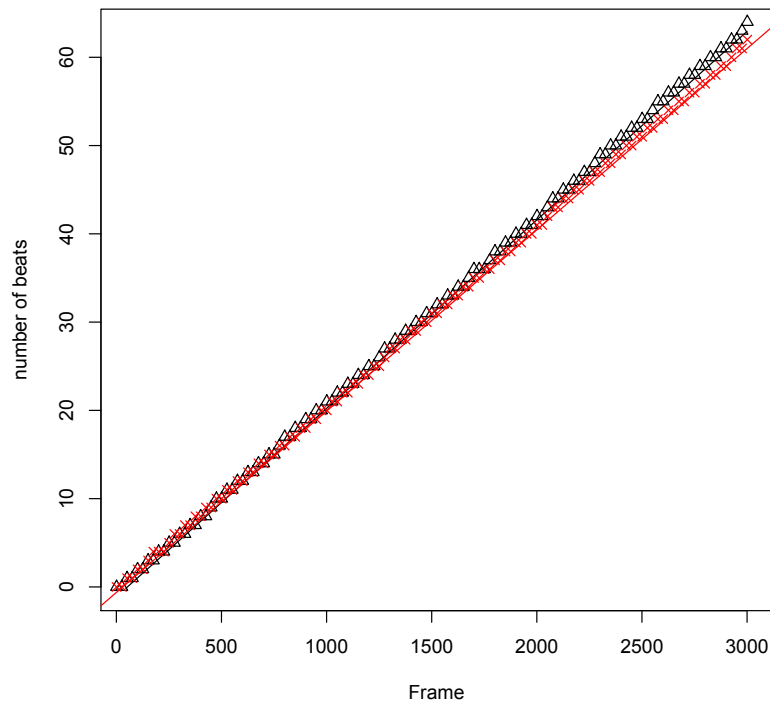
Rock 2: Barnacles 1 (black) & 2 (red)



Rock 2: Barnacles 2 (black) & 3 (red)



Rock 2: Barnacles 1 (black) & 3 (red)



**Figure 6: Rock two linear models reflecting constancy in rate.  $R^2$  values of regression summarized in table B. See figure 5 for a discussion of the axes and interpretation.**