



University of Guam Coconut Rhinoceros Beetle Biological Control Project
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<https://github.com/aubreymoore/rearing3>

Bioassay Report: PNG

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<https://github.com/aubreymoore/rearing3/raw/master/bioassay-PNG.pdf>

Contents

1 Summary	2
2 Description	2
2.1 Replicate 1	2
2.2 Replicate 2	2
2.3 Replicate 3	2
2.4 Replicate 4	2
3 Mortality	3
4 Change in Mass	4
5 Post Mortem Images	5
5.1 control	5
5.2 heat inactivated	21
5.3 virus	36

1 Summary

2 Description

Adult beetles incubated at 30°C and 80% RH for more than 2 weeks to observe possible contamination from green muscardine fungus infection were employed in a bioassay to determine the susceptibility of adults to infection by a virus isolate collected from Pupae New Guinea (**PNG**). Treatment 1 consisted of 10-20 µl sterile filtered water injected at a point on the ventral surface at the junction of the hind leg and the thoracic using a sterile needle. Treatment 2 consisted of 10-20 µl heat-inactivated virus injection while in the treatment 3, beetles were injected with 10-20 µl of untreated virus preparation. Adults were then placed in clean glass mason jars (bleach-treated) with a piece of banana added for food. Beetles were incubated at 30°C and 80% RH in a Percival incubator. All beetles were weighted every other day but monitored daily for four weeks to observe any possible signs of mortality.

2.1 Replicate 1

A total of seven adult females and eight adult males distributed among the three treatments were employed in this replicate.

2.2 Replicate 2

A total of six adult females and nine adult males distributed among the three treatments were employed in this replicate.

2.3 Replicate 3

A total of nine adult females and 12 adult males distributed among the three treatments were employed in this replicate.

2.4 Replicate 4

A total of 14 adult females and 16 adult males distributed among the three treatments were employed in this replicate.

3 Mortality

Table 1: Mortality summary.

	bioassay_treatment	ntotal	ndead	mortality	adjusted_mortality	significance
0	control	27	7	0.259259	0.00	1.000000e+00
1	heat inactivated	27	8	0.296296	0.05	1.000000e+00
2	virus	27	25	0.925926	0.90	8.193890e-07

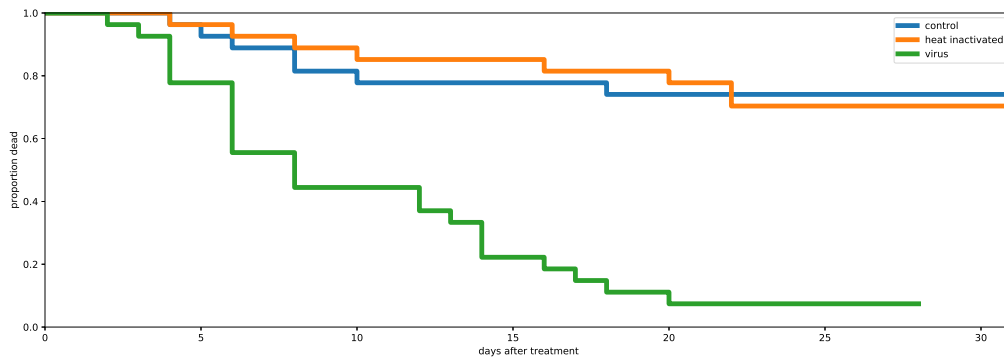


Table 2: Pairwise differences among mortality curves.

		test_statistic	p
control	heat inactivated	0.028537	8.658514e-01
	virus	24.945263	5.898128e-07
heat inactivated	virus	29.049200	7.056324e-08

4 Change in Mass

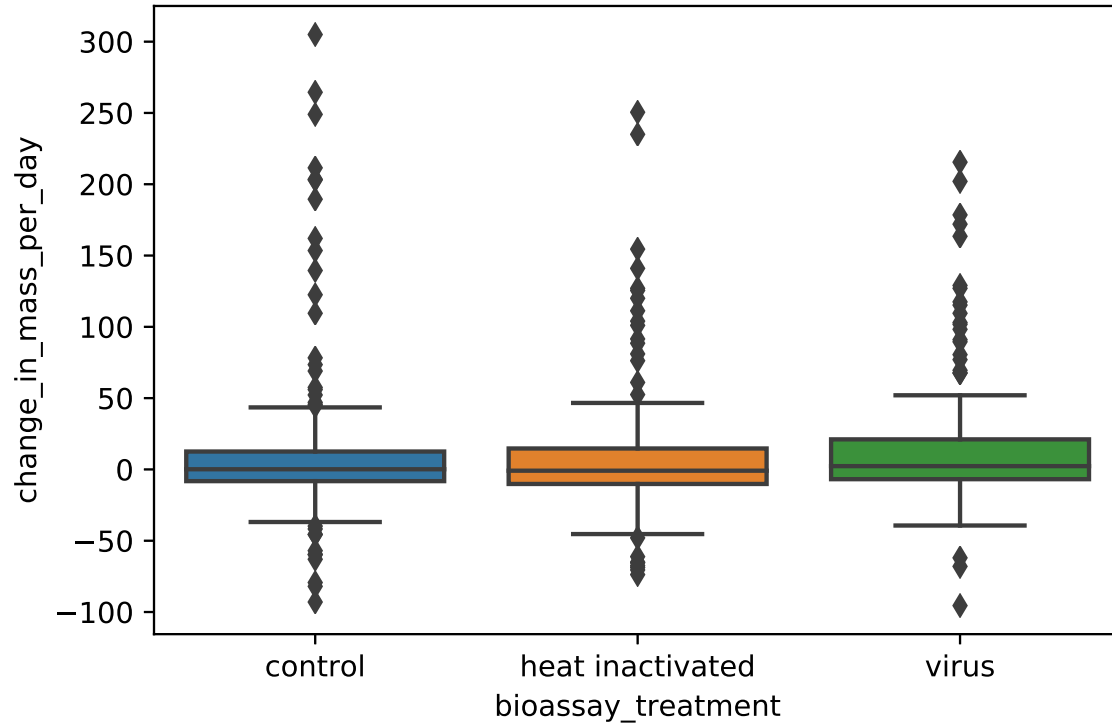


Table 3: Results of pairwise significance tests for differences in change in mass.

	control	heat inactivated	virus
control	-1.000000	0.854094	0.365357
heat inactivated	0.854094	-1.000000	0.365357
virus	0.365357	0.365357	-1.000000

5 Post Mortem Images

5.1 control



Figure 1: Bioassay: PNG-3; Treatment: control; Beetle ID: 1244



Figure 2: Bioassay: PNG-3; Treatment: control; Beetle ID: 1245



Figure 3: Bioassay: PNG-3; Treatment: control; Beetle ID: 1246



Figure 4: Bioassay: PNG-3; Treatment: control; Beetle ID: 1247



Figure 5: Bioassay: PNG-3; Treatment: control; Beetle ID: 1248



Figure 6: Bioassay: PNG-3; Treatment: control; Beetle ID: 1249



Figure 7: Bioassay: PNG-3; Treatment: control; Beetle ID: 1250



Figure 8: Bioassay: PNG-4; Treatment: control; Beetle ID: 1265



Figure 9: Bioassay: PNG-4; Treatment: control; Beetle ID: 1268



Figure 10: Bioassay: PNG-4; Treatment: control; Beetle ID: 1274



Figure 11: Bioassay: PNG-1; Treatment: control; Beetle ID: 1365



Figure 12: Bioassay: PNG-1; Treatment: control; Beetle ID: 1366



Figure 13: Bioassay: PNG-2; Treatment: control; Beetle ID: 1379



Figure 14: Bioassay: PNG-2; Treatment: control; Beetle ID: 1383



Figure 15: Bioassay: PNG-2; Treatment: control; Beetle ID: 1385



Figure 16: Bioassay: PNG-2; Treatment: control; Beetle ID: 1387

5.2 heat inactivated



Figure 17: Bioassay: PNG-3; Treatment: heat inactivated; Beetle ID: 1251



Figure 18: Bioassay: PNG-3; Treatment: heat inactivated; Beetle ID: 1252



Figure 19: Bioassay: PNG-3; Treatment: heat inactivated; Beetle ID: 1253



Figure 20: Bioassay: PNG-3; Treatment: heat inactivated; Beetle ID: 1254



Figure 21: Bioassay: PNG-3; Treatment: heat inactivated; Beetle ID: 1255



Figure 22: Bioassay: PNG-3; Treatment: heat inactivated; Beetle ID: 1256



Figure 23: Bioassay: PNG-3; Treatment: heat inactivated; Beetle ID: 1257



Figure 24: Bioassay: PNG-4; Treatment: heat inactivated; Beetle ID: 1278



Figure 25: Bioassay: PNG-4; Treatment: heat inactivated; Beetle ID: 1280



Figure 26: Bioassay: PNG-4; Treatment: heat inactivated; Beetle ID: 1281



Figure 27: Bioassay: PNG-4; Treatment: heat inactivated; Beetle ID: 1283



Figure 28: Bioassay: PNG-4; Treatment: heat inactivated; Beetle ID: 1284



Figure 29: Bioassay: PNG-2; Treatment: heat inactivated; Beetle ID: 1384



Figure 30: Bioassay: PNG-2; Treatment: heat inactivated; Beetle ID: 1386



Figure 31: Bioassay: PNG-2; Treatment: heat inactivated; Beetle ID: 1389

5.3 virus



Figure 32: Bioassay: PNG-3; Treatment: virus; Beetle ID: 1258



Figure 33: Bioassay: PNG-3; Treatment: virus; Beetle ID: 1259



Figure 34: Bioassay: PNG-3; Treatment: virus; Beetle ID: 1260



Figure 35: Bioassay: PNG-3; Treatment: virus; Beetle ID: 1261



Figure 36: Bioassay: PNG-3; Treatment: virus; Beetle ID: 1262



Figure 37: Bioassay: PNG-3; Treatment: virus; Beetle ID: 1263



Figure 38: Bioassay: PNG-3; Treatment: virus; Beetle ID: 1264



Figure 39: Bioassay: PNG-4; Treatment: virus; Beetle ID: 1285

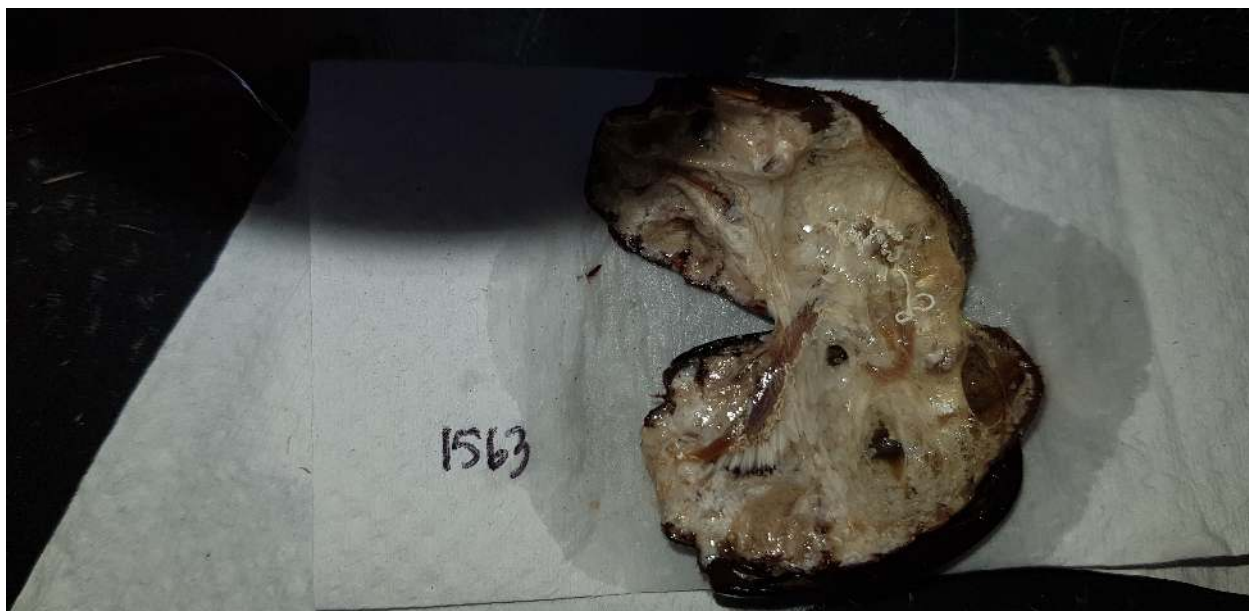


Figure 40: Bioassay: PNG-4; Treatment: virus; Beetle ID: 1286



Figure 41: Bioassay: PNG-4; Treatment: virus; Beetle ID: 1289



Figure 42: Bioassay: PNG-4; Treatment: virus; Beetle ID: 1290



Figure 43: Bioassay: PNG-4; Treatment: virus; Beetle ID: 1291



Figure 44: Bioassay: PNG-4; Treatment: virus; Beetle ID: 1292



Figure 45: Bioassay: PNG-4; Treatment: virus; Beetle ID: 1293



Figure 46: Bioassay: PNG-1; Treatment: virus; Beetle ID: 1373



Figure 47: Bioassay: PNG-1; Treatment: virus; Beetle ID: 1374



Figure 48: Bioassay: PNG-1; Treatment: virus; Beetle ID: 1375



Figure 49: Bioassay: PNG-1; Treatment: virus; Beetle ID: 1376



Figure 50: Bioassay: PNG-1; Treatment: virus; Beetle ID: 1377

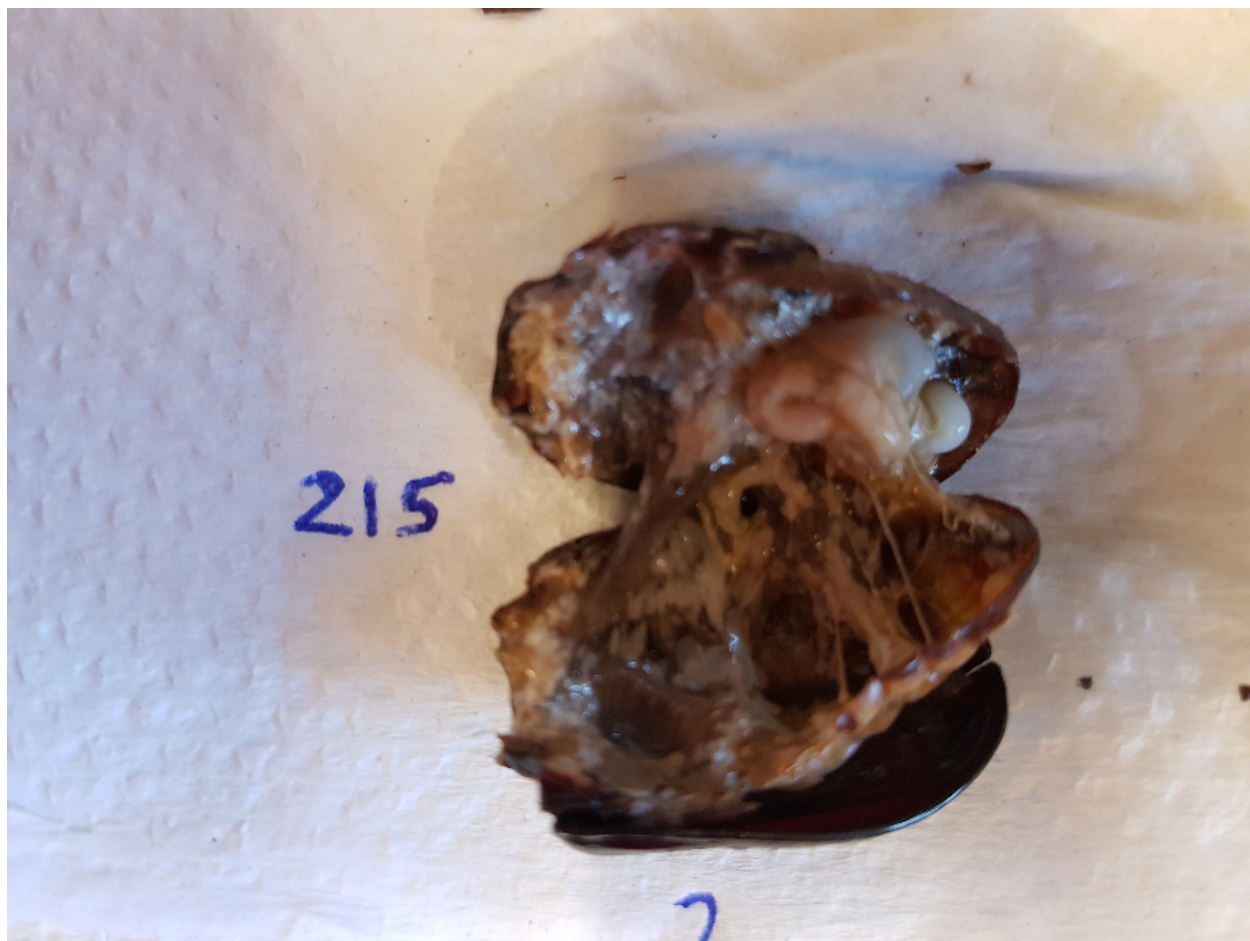


Figure 51: Bioassay: PNG-2; Treatment: virus; Beetle ID: 1380

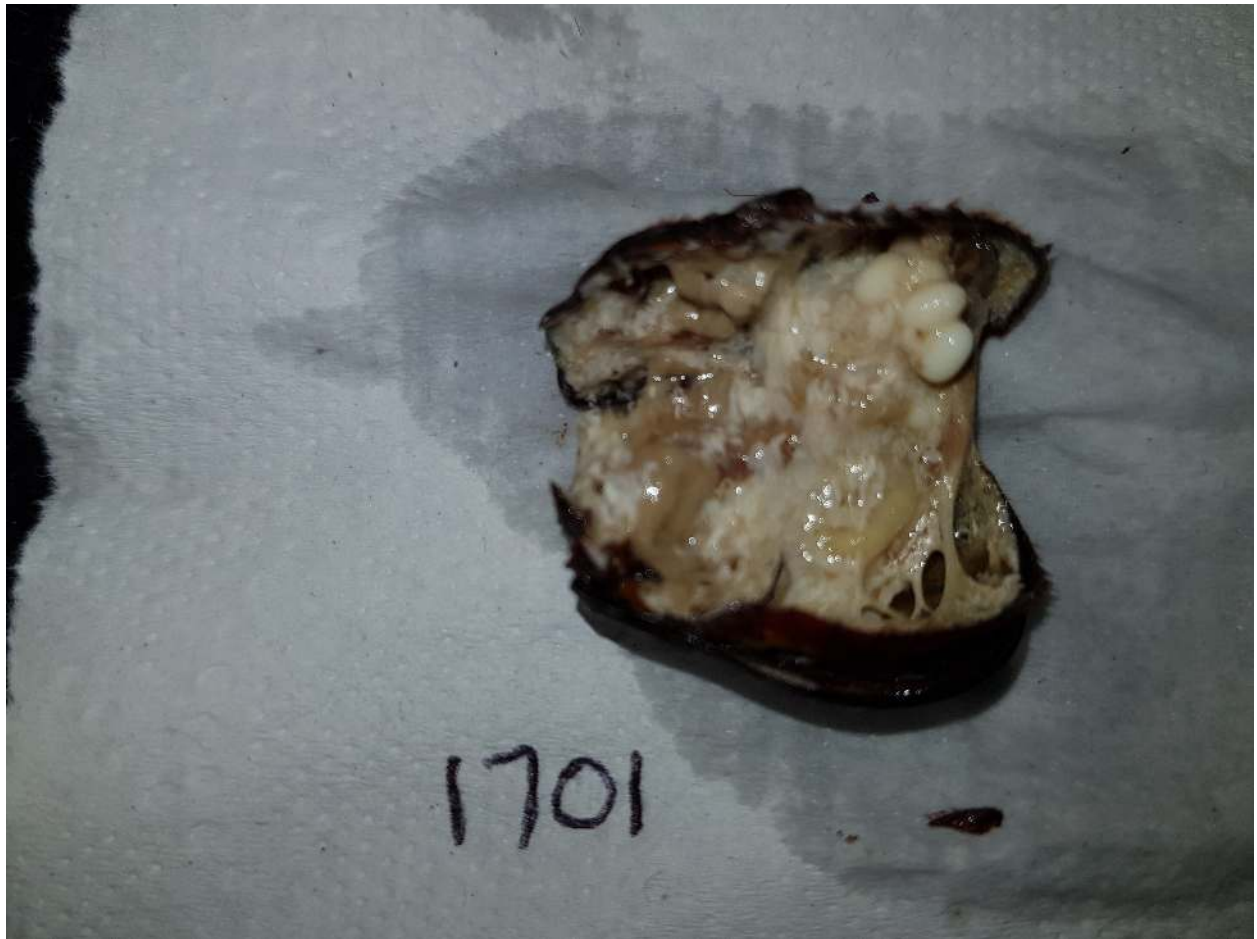


Figure 52: Bioassay: PNG-2; Treatment: virus; Beetle ID: 1388



Figure 53: Bioassay: PNG-2; Treatment: virus; Beetle ID: 1391



Figure 54: Bioassay: PNG-2; Treatment: virus; Beetle ID: 1392