Guana Island Wildlife Sanctuary

Marine Science Month Report

2001— 2002

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Introduction

This report presents the activities of the Marine Science Month Program (MSM) on Guana Island, British Virgin Islands, during July and August 2001 and 2002. This program has run annually since 1991, and it complements a larger terrestrial research program, headed by James (Skip) Lazell, that take place in October each year. Research efforts on Guana are supported by The Falconwood Corporation and the Guana Island Wildlife Sanctuary in addition to granting agencies that fund individual research projects.

MSM provides research facilities, logistical support and accommodation to scientists conducting basic research in marine biology. The scientists donate part of their time to education programs in which BVI students participate in field research on Guana. In 2001, the scientists presented their work in a public symposium at the H. Lavity Stoutt Community College (see Appendix 1). Local news articles covering Guana's scientific and educational activities between 2001 and 2002 are shown in Appendix 2.

Publications and research summaries from individual scientific teams are presented here, followed by student reports.

List of new publications 2001 – 2002

Martin, Joel W, 2002. *Microptrosthema jareckii,* a new species of stenopodidean shrimp (Crustacea: Decapoda: Stenopodidea: Spongicolidae) from Guana Island, British Virgin Islands. Proceedings of the Biological Society of Washington. Vol. 115(1): 108-117

Martin, Joel. W., Donald B. Cadien, and Todd L. Zimmerman, 2002. First record and habitat notes for the genus *Lightiella* (Crustacea, Cephalocarida, Hutchinsoniellidae) from the British Virgin Islands. Gulf and Caribbean Research Vol 14: 75-79.

Publications in Review

Kerr, Alexander M., Daniel Janies, and Junhyong Kim. Molecular Phylogeny of Coral-Reef Sea Cucumbers (Holothuriidae: Aspidochirotida) based on 16S mt rDNA sequence.

Finley, Rachel J. and Graham E. Forrester. Direct evidence for a strong impact of ectoparasites on the demography of small reef fish.

O'Connell-Rodwell, C.E., N. Rojek, T.C. Rodwell and P.W. Shannon. Artificially induced group display and nesting behaviors in the reintroduced population of Caribbean flamingos (*Phoenicopterus ruber ruber*) on Guana Island, BVI.

Running head: HOLOTHURIID SEA CUCUMBERS

Molecular Phylogeny of Coral-Reef Sea Cucumbers (Holothuriidae: Aspidochirotida) based on 16S mt rDNA sequence

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Members of the Holothuriidae are found globally at low latitudes and are often a dominant component of Indo-west Pacific coral reefs. We present the first phylogeny of the group, using eight species from all five genera and based on approximately 540 nt from a PCR-amplified conserved 3' section of 16S mt rDNA. Parsimony and likelihood analyses returned identical topologies, while minimum evolution differed in its placement of one species, *Holothuria* (*Platyperona*) *excellens*. Despite this difference, several robust conclusions could be drawn. First, some aspects corroborated the Linnean classification. *Actinopyga* and *Bohadschia* each appear monophyletic and *Pearsonothuria* is sister to *Bohadschia*. Other features of our phylogeny, however, were not in accord with the taxonomy of Holothuriidae or previous speculations about the group's evolutionary history. Most notably, the genus *Holothuria* appears paraphyletic. *Actinopyga* and *Bohadschia*, sometimes held to be closely related to one another because of their morphological similarities, are only distantly related. The morphologically distinct *Labidodemas*, even thought to warrant separation at the family level, is nested well within *Holothuria*. A maximum parsimony reconstruction of ancestral ossicle form on the phylogeny indicated that in addition to a probable bout of elaboration in ossicle form (the modification of rods or rosettes to holothuriid-type buttons), at least two rounds of ossicle simplification also transpired in which buttons reverted to rods or rosettes. Cuvierian tubules, defensive organs unique to numerous members of Holothuriidae, were probably present before the initial radiation of the family, but the reconstruction is ambiguous as to their ancestral function.

INTRODUCTION

Sea cucumbers, or holothurians, in the globally distributed family Holothuriidae are primarily inhabitants of the tropical eulittoral, usually coral reefs and adjacent sandy areas, though temperate shallows and deep water harbor a few species. Holothuriids are primarily deposit feeders, in contrast to the suspension-feeding dendrochirote holothurians that prevail at higher latitudes. Like nearly all other sea cucumbers, holothuriids have a reduced skeleton of isolated, microscopic ossicles embedded in a pliable body wall. And like other groups of soft-bodied organisms, their fossil history is threadbare, though button ossicles characteristic of holothuriids are known from the Upper Jurassic (Gilliland, 1993). The provenence of these few finds and the family's apparent Tethyan distribution, even at the level of subgenera (Clark and Rowe, 1971, Hendler et al., 1995), suggest the group's antiquity.

Holothuriidae is the second largest family of sea cucumbers with over 170 species, or about 11% of the diversity of living holothurians (Smiley, 1994). The family is composed of five genera, the largest of which is *Holothuria* with about 140 described species. This diverse genus has dominated comprehensive reviews of the family (Pearson, 1914, Panning, 1929-1935, Diechmann, 1958, Rowe, 1969), since the remaining genera are small, well delimited and easily diagnosed. Consequently, very little attention has been paid to the evolutionary relationships in the family as a whole. Pearson (1914) believed that two genera, *Actinopyga* and *Bohadschia* were closely related and primitive compared to *Holothuria*. Diechmann (1958) suggested that evolution in *Holothuria* had proceeded via progressive simplification of the ossicles from tables and buttons characteristic of many *Holothuria* to rods or rosettes like those found in *Actinopyga* and *Bohadschia*. In contrast, Rowe (1969) proposed that the genus had proceeded via the

opposite trend, from simple ossicles to complex ones and, based on this trend, he provided a tentative phylogeny of subgenera in *Holothuria* that would require the genus to be monophyletic. Most recently, Levin (1999), in partial agreement with Deichmann (1958), argued that the evolution of Holothuriidae has proceeded from a form similar to the burrowing *Labidodemas*, which has tables and sometimes buttons, to vagile exposed forms and to suspension-feeders, most of which have rods and rosettes.

In this study, we test the utility of a conserved 3' section of 16S mt rDNA to clarify relationships between the genera in Holothuriidae, as well as perform an initial test for monophyly of the of the largest genera. We then use the estimate of the relationships to make inferences about the evolution of morphology in the group.

METHODS

We sampled from one to two species in each of the five currently recognized genera of Holothuriidae and, as outgroups to root the holothuriid tree, two species from putatively divergent (morphologically disparate) genera within Stichopodidae. This latter family was selected largely because of its sister status to the study group Holothuriidae as inferred from a maximum parsimony analysis of morphological characters (Kerr and Kim, 2001).

To prepare DNA for sequencing, ca. 100 mg of ethanol- or DMSO-preserved gonad, muscle or tentacle tissue was air-dried and ground to a powder in liquid nitrogen, then whole genomic DNA was extracted using the CTAB method of Arndt et al. (1996) or the EZ-DNA genomic DNA isolation reagent (Morwell MD Biosciences, Montreal QE, Canada). We then amplified a

ca. 540 nt-long portion from the conserved 3' end of the mitochondrial gene coding for the 16S-like, large ribosomal RNA subunit using the echinoderm-specific universal primers 16Sar (5'-CGCCTGTTTATCAAAAACAT -3') and 16Sbr (5'- CTCCGGTTTGAACTCAGATCA -3'), then performed double-stranded PCR under standard conditions (an initial 30-s denaturation at 95°C, followed by 40 cycles of 95°C for 30 s, 50-55°C for 30 s, and 72°C for 1 min, then a final 4-min extension at 72°) on a Hybaid or Perkin-Elmer thermocycler. Amplified products were isolated by starch-gel electrophoresis, purified with QIAquick gel extraction kit (Qiagen, Inc., Valencia CA, USA) and directly sequenced both ways on an ABI 3700/3100 DNA sequencer (Applied Biosystems, Inc., Foster City CA, USA). Sequences were deposited in GenBank under accession numbers Uxxxxx – Uxxxxx.

Sequences were aligned using Clustal W 1.5 (Thompson et al., 1994) with default gap penalties, followed by a final check by eye. The resulting alignment, cropped of overhangs and primer sequence, was 544 sites in length, but homology in several stretches with numerous indels remained uncertain and so these sections were excluded. This left an unambiguously alignable sequence of 470 sites that was used in the phylogenetic analyses. A phylogeny was estimated using PAUP* 4.0b10 (Swofford, 2000) on a 68K Apple Macintosh without a floating point coprocessor as implemented on Executor, a MacOS emulator for PCs (Ardi, Inc., Albuquerque NM, USA). Three estimators were used: maximum parsimony, maximum likelihood and minimum evolution (Rzhetsky and Nei, 1992). The maximum parsimony analyses were done under the branch-and-bound setting, tree-bisection-reconnection, and addition sequence furthest. We used two character-weighting schemes, equal weighting and one where transitions were down-weighted relative to transversions based on a transition/transversion ratio estimated from

the data via maximum likelihood. To find the simplest model of sequence evolution for use in the maximum likelihood and distance analyses, we compared, using a likelihood ratio test, the likelihood scores of a branch and bound search using the shortest parsimony tree to that obtained from a general time-reversable model with invariant sites plus among-site rate heterogeneity (GTR+I+Γ) successively estimated from the data. The winning model and parameters were then used in an unconstrained likelihood search in a second and third round of parameter fitting and model selection. Likelihood options were set to branch-and-bound search, empirical base frequencies and ti/tv ratio used, starting trees obtained via stepwise addition, and addition sequence furthest. Model selection and parameters generating the distances in the minimum evolution analysis were estimated successively as with likelihood and the relevent PAUP* settings were identical as well, except that the exhaustive search setting was used. For all methods, data quality was assessed by bootstrapping using 500 replicates under the conditions used to attain the optimal trees. For the parsimony analyses, we also examined the "heirarchical signal" as skewness of the tree-length frequency distributions derived from an exhaustive search (Hillis and Huelsenbeck, 1992).

Ancestor reconstructions were performed on MacClade 4.03 (Maddison and Maddison, 2001) under both accelerated and delayed transformations of character change while allowing unordered multistate characters (Fitch parsimony). *Astichopus* is coded as tables present since the ossicles occur in very small specimens (Cutress, 1996). Though differing in several ways, rosettes in the outgroup family Stichopodidae are potentially homologous to those in Holothuriidae and are coded as such. Nevertheless, the coding along the branch leading to the ingroup is left uncertain as it is variably present in outgroup. This coding has no effect on

ingroup reconstructions. Similarly, *Pearsonothuria* sometimes displays tables with a reduced spire (Cherbonnier, 1988). The coding of *Labidodemas* as lacking Cuvierian tubules is based on the literature and is thus provisional, since recent assessment of other living holothuriids (A. M. Kerr, unpubl. data) indicates the presence of tubules in some species previously reported as lacking them. To test whether the shortest tree obtained by maximum parsimony was preferable to a longer tree with an alternative placement of a selected subtree, we compared them with a one-tailed, normal approximation of a Wilcoxon signed-rank ("Templeton's") test statistic *H*, as well as with a binomial sign test of winning sites as implemented in PAUP*.

RESULTS

Of the 470 sites in the analysed alignment, 281 were invariant, 56 variable characters were parsimony uninformative, leaving 133 parsimony informative sites. Pairwise distances as percent sequence dissimilarity between holothurian taxa ranged from a minimum of 4.12% between *Bohadschia argus* and *B. marmorata* and a high of 24.6% between *Pearsonothuria graeffei* and *Stichopus chloronotus*. Pairwise differences are given in Table 2. Ti/tv ratios were greater than two for six of 45 pairwise species comparisons, from a maximum of 6.50 between *Bohadschia argus* and *B. marmorata*, and one high value (4.43) between members of different genera, *Holothuria leucospilota* and *Labidodemas semperianum*, to a low of 0.67 between *Actinopyga agassizi* and *Stichopus chloronotus*. For the combined sequences, there was a slight mean excess of A residues (29.0%), while C residues were least frequent (23.1%) (Table 3). Across all species, GC content averaged 47.8%, from a low of 46.1% for *B. argus* to a high of 49.3% for *Labidodemas semperianum* (Table 3).

The equally weighted maximum parsimony analysis produced a shortest tree of length 407 and is shown in Fig. 1A. The consistency index (CI) for this tree was 0.700, the CI excluding uninformative characters was 0.641, the rescaled CI was 0.386 and the retention index was 0.552. The number of unambiguous changes on internal branches ranged from five to 24. The frequency distribution of tree lengths was highly left skewed, with a $g1$ score of -1.055, well beyond the $P<0.01$ significance level and indicating that at least part of the tree contains a considerable heirarchical signal, ostensibly due to genealogical structure (Hillis and Huelsenbeck, 1992). Bootstrap percentages for ingroup nodes were from a high of 99% to a low of 54%. The ti/tv ratio estimated via maximum likelihood was 1.515. Using this to weight the characters in a second parsimony analysis returned a tree with a topology identical to the previous tree that used equal weights and which had similar branch lengths (via a paired t test: $t = 0.876$, $P>0.10$). The likelihood ratio test indicated that the simplest model of sequence evolution fitting the data and given the tree topology was Kimura's two parameter form. After two further rounds of tree and parameter estimation, the maximum likelihood analysis returned a tree ($-\ln L = 2509.670$) whose topology was identical to that from the maximum parsimony analyses (Fig. 2B). This result appeared insensitive to the complexity of the substitution model as use of either a JC69 (equal base frequencies and one substitution rate) or a GTR+I+Γ model also returned the same tree. The minimum evolution tree (branch sum = 0.836) differed from the parsimony and likelihood trees in the placement of *Holothuria excellens* below the lineage ((*Bohadschia* spp., *Pearsonothuria*), (*Labidodemas* and *H. leucospilota*)) (Fig. 1C). This result was also insensitive to changes in the complexity of the model used to generate the distance measures.

In all analyses, *Actinopyga* and *Bohadschia* were each monophyletic with high bootstrap percentages. Additionally, *Pearsonothuria graeffei* was sister to *Bohadschia* spp. and *Labidodemas semperianum* was sister to *Holothuria leucospilota*, in each case with strong bootstrap support. The only difference between trees obtained by the different methods was in the placement of *H. excellens*. In all trees, regardless of reconstruction method, the genus *Holothuria* appeared paraphyletic. To further test this result, we compared the shortest parsimony tree (Fig. 1A) to the shortest tree obtained under the constraint that *Holothuria* is monophyletic (length = 425). The latter tree was significantly longer, and hence not preferred, by a Wilcoxon signed-rank test ($N = 30$, $H = 93$, $z = -3.268$, $P = 0.0010$) or a binomial test (winning sites = 24, $P = 0.0014$). Similarly, the placement of *Actinopyga* and *Bohadschia* together near the base of the tree (length = 418) was not preferred over their positions in the shortest tree ($N = 31$, $H = 160$, $z = -1.976$, $P = 0.0482$), though the marginal significance by the more conservative winning sites test in this instance (winning sites = 21, $P = 0.0725$) indicated that sites contributed somewhat unequally to the probability of the Wilcoxon statistic. The shortest tree with *Labidodemas* outside of *Holothuria* (length = 419) was also significantly longer than the shortest tree overall ($N = 14$, $H = 7.5$, $z = -3.207$, $P = 0.0013$; winning sites = 13, $P = 0.0018$).

DISCUSSION

Systematics

These results comprise the first phylogenetic test of the classification of the Holothuriidae. Parsimony and likelihood analyses returned identical topologies (Fig. 1A, B) with high bootstrap support, while the distance estimator, minimum evolution, differed in its

placement of one species, *Holothuria excellens* (Fig. 1C). The reason for this difference is uncertain, but may be due to a general shortcoming of distance methods, as compared to parsimony and likelihood, in accounting for multiple changes of character state while estimating branch lengths. In computing the least squares pairwise distances, the minimum evolution method requires repeated sampling of portions of the data, which can magnify the effect of any systematic error therein (Swofford et al., 1996). For this reason, we prefer the topology in Fig. 1A and B, but acknowledge the greater uncertainty in the placement of *H. excellens*. Hence, in the remainder of the discussion, we conservatively limit our inferences to those insensitive to the alternative placement of this species in the distance tree.

Our phylogeny corroborated some aspects of the currently used Linnean classification. Both *Actinopyga* and *Bohadschia* appear to be good genera. These taxa are relatively small (ca. 17 and 14 species, respectively), are morphologically well circumscribed and easily diagnosable by a set of characters from different organ systems that are unique to each genus. Within the limited sample of taxa used in this study, each genus is monophyletic and both are supported by high bootstrap percentages. Additionally, the finding of a close relationship between *Pearsonothuria graeffei* and *Bohadschia* echoes the previous placement of the former species in the latter genus (Rowe, 1969). The two species of *Pearsonothuria* have since been placed in their own genus in recognition of their distinctive spicules and secondary chemistry (Levin et al., 1984).

Other aspects of our phylogeny, however, were not in accord with the taxonomy of Holothuriidae or with previous speculations about its evolutionary history. Most notably, the

genus *Holothuria* appears paraphyletic. Rowe (1969) provides a tentative phylogeny which shows the group as monophyletic. The results presented here (Fig. 1), however, indicate that *Holothuria* is not a single lineage, but that it gives rise to *Pearsonothuria+Bohadschia* and *Labidodemas*. The paraphyly has strong bootstrap support regardless of the the reconstruction method used, though the exact arrangement of one species of *Holothuria*, *H. excellens*, on the tree is less robust to the optimality criterion. Additionally, the shortest parsimony tree in which *Holothuria* is monophyletic is significantly longer than, and hence not preferred over, the maximum parsimony tree pictured in Fig. 1A. The paraphyletic status of this genus is perhaps not unanticipated. *Holothuria* is a large (ca. 170 described species), morphologically diverse family with a complex nomenclatural history. While much of *Holothuria* can be diagnosed by distinctive ossicles (holothuriid tables and buttons), their absence in numerous other members could be well secondary. Moreover, there are no anatomical characters unique to the genus, rather only overlapping subsets of these traits are possessed by any one subgenus, and they are sometimes possessed by non-*Holothuria* holothuriids (e.g., shape and proportion of the ocsophageal calcareous ring elements, presence and shape of Cuvierian tubules, thickness of body wall, form and arrangement of dorsal papillae, gross shape, enlargement and calcification of anal papillae, arrangement of tube feet).

Another surprise is the evolutionary distance between *Actinopyga* and *Bohadschia*. While each of these genera is distinctive on several accounts, they also have similar ossicles, body walls, and calcareous rings as compared to other holothuriids and, as a result, have long been considered closely related (e.g., Pearson 1914). In this study, *Actinopyga* and *Bohadschia* were separated by 25 unambiguous changes on three branches, two with strong bootstrap support

(Fig. 1A). As well, uniting the genera or considering them as a paraphyletic unit at the base of the ingroup requires a significantly longer tree (418 vs. 407). Finally, we note that the unusual genus *Labidodemas* arises from within *Holothuria*. Among holothuriids, the three members of *Labidodemas* are unique in the arrangement of their dorsal papillae and tubefeet along the radii and possession of an undulating, ribbon-like calcareous ring. Because of these characters, Rowe (1969) considers *Labidodemas* distinct from the *Holothuria*, even suggesting that it may warrant separation at the family level. Levin (1999) also implies that the position of *Labidodemas* is near the base of the holothuriid tree, arguing that the genus displays the most primitive characters of the family. In this study, however, *Labidodemas* was nested well within *Holothuria* and was sister to *H. leucospilota* with very high bootstrap support (Fig. 1). Placing *Labidodemas* outside of *Holothuria* required a significantly longer tree (419 vs. 407). These two species' sequences were also undersaturated for transversions (ti/tv = 4.43), which further suggests a close relationship via a relatively recent divergence (or, less probably, a more distant relationship but a considerably arrested base substitution rate across a potentially large inclusive lineage).

Character evolution

The phylogeny also permited several tentative inferenees about the evolution of morphology and ecology within the Holothuriidae. Both Pearson (1914) and Rowe (1969) suggest that the family has evolved along the lines of increasing ossicle complexity, from those bearing "simple" rosette and rod ossicles in the body wall to those with more complex table and button elements. Conversely, Diechmann (1958) and Levin (1999) speculate that tables and buttons are ancestral, the group only later evolving rosettes and spiny rods. A parsimonious ancestral reconstruction on our phylogeny (Fig. 2A, B), though, indicated that in addition to an

elaboration of ossicle form, at least two rounds of simplification also transpired. The first occurred when *Actinopyga* diverged from the rest of Holothuriidae and apparently lost the table ossicles found in many other holothuriids and most non-holothuriids of the order Aspidochirotida. Then, along the branch leading to all holothuriids sans *Actinopyga*, buttons evolved, and later tables were again lost, perhaps by way of the intermediate state displayed by *Pearsonothuria* which possesses "racquet" ossicles that appear to be highly modified tables (Levin et al., 1984). Around this time or later, button ossicles were lost or reverted to rosettes and rods below *Bohadschia*. This picture of ossicle evolution within Holothuriidae indicates the minimum number of reversions that likely occurred and is less straightforward than previously speculated. We expect that the secondary loss of holothuriid tables and buttons is even more widespread, such that some members of groups joined by the absence of these features will prove to be only distantly related. This further predicts, as did Deichmann (1958) and Levin (1999), that suspension feeding will be a derived feature of the family, as it occurs only in subgenera lacking buttons or tables.

Finally, the phylogeny presented here permits tentative inferences about the evolution of an organ unique to the Holothuriidae. Cuvierian tubules are numerous, elongate evaginations at the base of the respiratory trees of numerous species of holothuriids (Smiley, 1994). Typically, the tubules, which become sticky when extruded, are ejected from the cloaca, extended and autotomized when the animal is disturbed (VandenSpiegel and Jangoux, 1987). However, in *Actinopyga* and *Holothuria* (*Microthele*) spp., the tubules are not extruded. An ancestral reconstruction (Fig. 2C) suggests that the organs were present before the initial radiation of the family and were subsequently lost in *Labidodemas*. Our consideration of tubule-bearing

holothuriids here includes *Pearsonothuria*, seldom reported as having the organs (but see Ferál and Cherbonnier, 1988), but which in fact possess well-developed tubules resembling those of *Bohadschia* that, however, dissolve rapidly when the animals are preserved in ethanol (A. M. Kerr, unpubl. obs.). Our reconstruction is ambiguous as to the ancestral function of Cuvierian tubules, since one lineage, *Actinopyga*, leading from the basal node of Holothuriidae has small, non-sticky tubules that are never discharged, while the other lineage exhibits long, adhesive and extrusable organs ostensibly used in defense. This raises the possibility that if the small tubules in *Actinopyga* represent the original state rather than a secondary condition, then Cuvierian tubules may have originally evolved in a role other than defense.

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TABLE 1

Taxa used in this study

Family Species	Voucher number	Locality
Holothuriidae		
Actinopyga agassizi (Selenka, 1867)	Dan	?Key Largo FL
A. mauritiana (Quoy and Gaimard, 1833)	E51760	Guam
Bohadschia argus Jaeger, 1833	Jenis freezer	Guam
B. marmorata Jaeger, 1833	E51759	Guam
Holothuria (*Platyperona*) *excellens* (Ludwig, 1874)	Jenis freezer	Guam
H. (*Mertensiothuria*) *leucospilota* (Brandt, 1835)	E51763	Guam
Labidodemas semperianum Semper, 1867	E53083	Guam
Pearsonothuria graeffei (Semper, 1868)	E51761	Guam
Stichopodidae		
Astichopus multifidus (Sluiter, 1910)	E47524	Guana Island, British Virgin Islands
Stichopus chloronotus Brandt, 1835	E47517	Guam

Note: voucher number abbreviations go here

TABLE 2

Uncorrected and K2P mean percent distances

	1	2	3	4	5	6	7	8	9	10
1. *Act agassizi*	-	9.79	25.0	20.6	20.1	21.4	19.4	20.6	19.7	23.7
2. *Act mauritiana*	9.09	-	23.4	16.2	17.5	23.2	19.0	20.8	21.5	23.5
3. *Ast multifidus*	21.2	20.0	-	25.2	26.0	25.9	23.2	27.1	26.8	17.2
4. *B argus*	17.7	14.3	21.3	-	4.35	15.9	16.4	14.5	12.9	25.6
5. *B marmorata*	17.3	15.3	21.9	4.12	-	17.5	16.5	14.8	13.8	26.5
6. *H excellens*	18.5	19.6	21.9	14.2	15.5	-	13.4	12.9	22.1	25.1
7. *H leucospilota*	16.9	16.6	19.9	14.6	14.7	12.1	-	9.55	19.7	25.8
8. *L semperianum*	17.7	17.8	22.6	13.0	13.3	11.7	8.81	-	19.8	25.3
9. *P graeffei*	17.1	18.3	22.5	11.7	12.5	18.8	17.0	17.1	-	30.1
10. *S chloronotus*	20.3	20.1	15.3	21.6	22.2	21.3	21.8	21.4	24.6	-

Note: uncorrected and K2P distances given below and above the diagonal, respectively.

TABLE 3

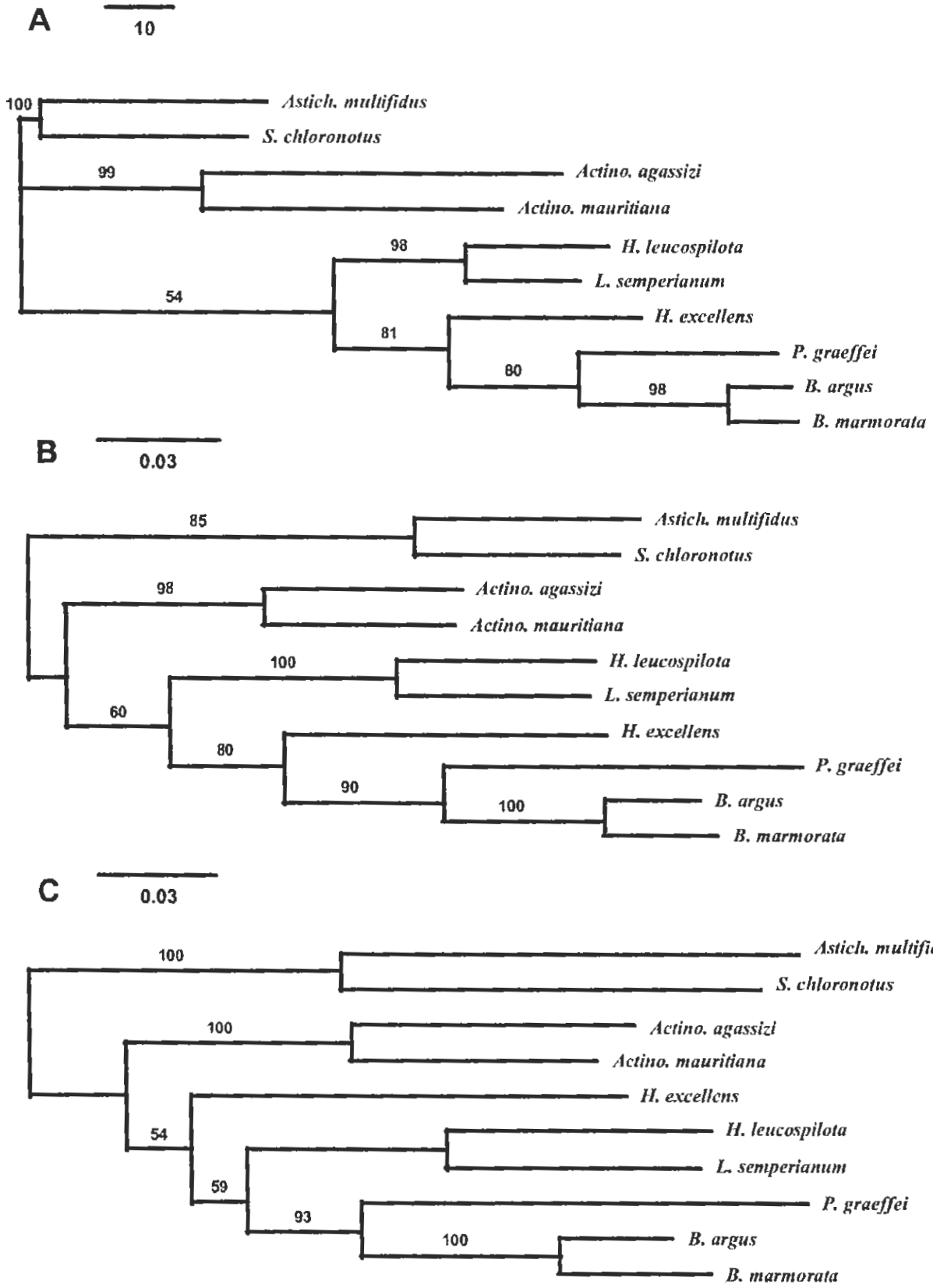
Percentage of bases in amplified partial 16S-like mt rDNA

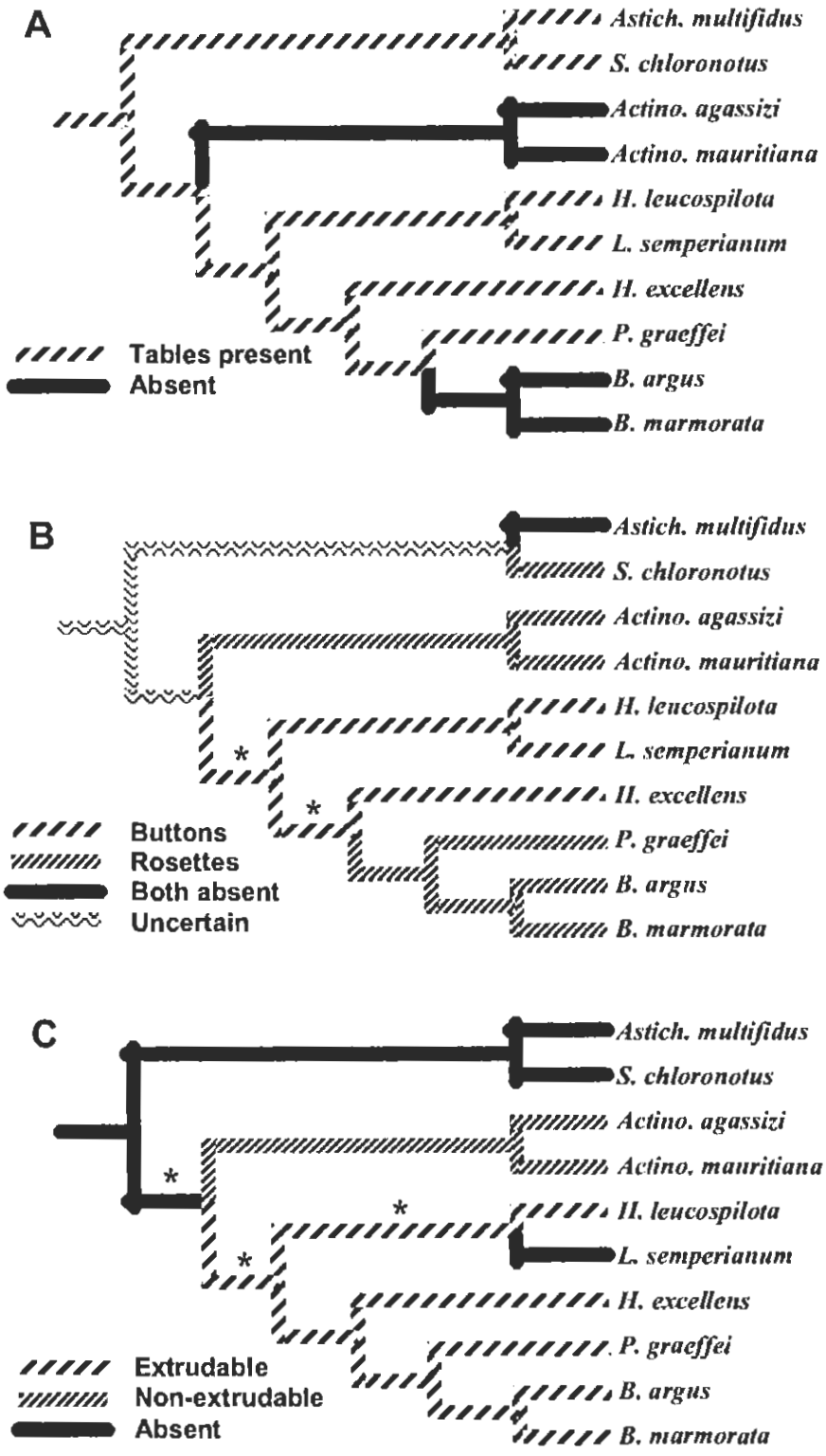
	A	C	G	T	GC content	Base count
1. *Act agassizi*	31.2	24.2	23.8	20.8	48.0	433
2. *Act mauritiana*	30.8	22.8	24.7	21.7	47.5	429
3. *Ast multifidus*	28.1	22.0	24.7	25.2	46.7	437
4. *B argus*	30.7	21.8	24.3	23.2	46.1	358
5. *B marmorata*	30.0	22.8	24.7	22.6	47.5	430
6. *H excellens*	28.9	21.9	24.2	24.9	46.1	429
7. *H leucospilota*	28.2	23.8	23.8	24.1	47.6	432
8. *L semperianum*	26.7	24.1	25.5	23.7	49.6	431
9. *P graeffei*	30.6	23.0	23.7	22.7	46.7	431
10. *S chloronotus*	25.5	24.5	24.8	25.2	49.3	440
Mean	29.0	23.1	24.4	23.4	47.8	445

Figure legends

FIG. 1. Phylogenetic trees. (A) Single most parsimonious tree using equal and transition-transversion weights (length = 407). (B) Maximum likelihood tree (-ln L = 2509.670). (C) Minimum evolution tree (sum = 0.836). Numbers above branches are bootstrap percentages of 500 replicates. Scale bars are number of changes for tree A and substitutions per nucleotide for trees B and C.

FIG.2. Maximum parsimony ancestral reconstructions of selected morphological traits. (A) Table ossicles in the body wall. (B) Button ossicles in the body wall. (C) Cuvierian tubules. See Methods for justification of state assignments. Ancestral states were resolved with an accelerated transformation of character change and asterisks on trees B and C indicate, respectively, that under a delayed transformation the underlying branch is unresolved or manifests the alternative state.


A
10
100
Astich. multifidus
S. chloronotus
99
Actino. agassizi
Actino. mauritiana
98
H. leucospilota
L. semperianum
54
H. excellens
81
P. graeffei
80
98
B. argus
B. marmorata
B
0.03
85
Astich. multifidus
S. chloronotus
98
Actino. agassizi
Actino. mauritiana
100
H. leucospilota
L. semperianum
60
H. excellens
80
P. graeffei
90
100
B. argus
B. marmorata
C
0.03
100
Astich. multifidus
S. chloronotus
100
Actino. agassizi
Actino. mauritiana
H. excellens
54
H. leucospilota
L. semperianum
59
P. graeffei
93
100
B. argus
B. marmorata


A
Astich. multifidus
S. chloronotus
Actino. agassizi
Actino. mauritiana
H. leucospilota
L. semperianum
H. excellens
P. graeffei
B. argus
B. marmorata
Tables present
Absent
B
Astich. multifidus
S. chloronotus
Actino. agassizi
Actino. mauritiana
H. leucospilota
L. semperianum
H. excellens
P. graeffei
B. argus
B. marmorata
*
*
Buttons
Rosettes
Both absent
Uncertain
C
Astich. multifidus
S. chloronotus
Actino. agassizi
Actino. mauritiana
H. leucospilota
L. semperianum
H. excellens
P. graeffei
B. argus
B. marmorata
*
*
*
Extrudable
Non-extrudable
Absent

Direct evidence for a strong impact of ectoparasites on the demography of a small reef fish

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Type of article: note

Running title: Ectoparasite effects on fish demography

Abstract

Empirical studies demonstrating effects of parasites on host population dynamics are relatively few, particularly for macroparasites and especially in marine systems. We tested the effects of a copepod macroparasite infecting the gills of a small coral reef fish. Fish that were naturally infected and uninfected were tagged as individuals and tracked in the field for 5 months. Parasitism was associated with an increase in gill ventilation rate, and a reduction in feeding. More importantly, parasitized fish showed significantly reduced growth (by 66%) and gonad mass (by 68%) compared to uninfected fish, and parasitism increased instantaneous mortality by a factor of 1.8. Since the prevalence of infection was higher in areas of high goby density, parasite-induced mortality is a possible cause of host density dependence. These results indicate a major effect of parasitism on host population dynamics and suggest that parasitism warrants closer attention by marine ecologists.

Key Words: *Pharodes tortugensis*, *Coryphopterus glaucofraenum*, host-parasite interaction, parasitic gill copepod

Introduction

Empirical studies demonstrating effects of parasites on host population dynamics are relatively few, and have lagged behind theoretical explorations of this subject (Scott & Dobson 1989, Grenfell & Dobson 1995). Of the empirical studies conducted, most have focused on microparasites and evidence for impacts of macroparasites on host dynamics is particularly sparse (Scott & Dobson 1989). Field research on both sorts of parasites has been conducted primarily in terrestrial habitats (Grenfell & Dobson 1995). In marine benthic systems, most explanations for population dynamics have focused on the effects of variable larval recruitment coupled with predator-prey and competitive interactions among adults (e.g. Caley et al. 1996, Bertness et al. 2001). Work on reef fishes reflects the general lack of attention to potential impacts of parasitism on host population dynamics in marine systems (Sale 1991, Caley et al. 1996). Despite lack of study, there is clear potential for parasites to affect fish populations because they support diverse parasite communities (Poulin 1995), many of which have pathogenic effects (Sindermann 1987). Theoretical studies (Dobson & May 1987) and studies of captive fish (Sindermann 1987) attest to potential impact of parasites on these host populations, though the applicability of these results to wild populations is uncertain.

The scarcity of direct evidence for impacts of parasites on wild fish populations may be partly because parasites are easily overlooked, and partly because we cannot routinely follow hosts of known infection status in the field (Scott & Dobson 1989, Grenfell & Dobson 1995, Dobson & May 1987). Here we test the effects of an external macroparasite on the demography of a small reef fish in a system where hosts can be tracked as individuals, and parasitized hosts can be

recognized visually. We were thus able to directly measure demographic rates of parasitized and unparasitized fishes in the field.

Materials and Methods

Study system

Host-parasite interactions were studied near Guana Island (64° 35'W, 18° 29'N), British Virgin Islands. The host species, the bridled goby (*Coryphopterus glaucofraenum* Gill), is a small benthic fish that occurs throughout the Caribbean. Larvae are planktonic and settle to reef habitats at ≈ 8 mm standard length (SL). Juveniles mature into females at ≈ 25 mm SL, and change sex to become males at ≈ 35 mm SL (Cole & Shapiro 1992). Gobies occupy small home ranges (< 4 m^2) in areas of interspersed sand and reef and are aggressive to neighbors. They feed on sand-dwelling invertebrates, and use crevices in the reef as shelter from predators.

A parasitic copepod (*Pharodes tortugensis* Wilson) infects the gill cavity of bridled gobies near Guana Island (R. Finley pers. obs.) and at least 4 other fish species elsewhere (Ho 1971). Bridled gobies infected with *P. tortugensis* have a swollen operculum. We used this criterion to visually diagnose parasitism underwater, and checked the accuracy of this method by subsequently capturing and dissecting fish (n = 106) to verify their infection status. Uninfected fish were reliably identified (46 of 50 correct), but identification of infected fish was less certain (47 of 56 correct). Most infected fish were large juveniles and females (16-30 mm SL). The mean intensity of copepod infection was 7.02 (range = 1-19) and the frequency distribution was negative binomial (χ^2_{16} = 15.69, n =118). Mean goby density at 9 sites around Guana Island varied from 2.15-14.55 m^{-2} and the prevalence of copepod infection ranged from 2-19% (based

on visual census of 5-10 randomly located 4 m^2 transects per site). Density and prevalence may be correlated ($r = 0.62$, $p = 0.06$).

Effects of parasites on host behavior and demography

Effects of *P. tortugensis* on gobies were tested at a site (560 m^2) where overall goby density was 1.83 fish m^{-2}, and 12.5% of gobies were parasitized (based on a census on 4-6 July 2001). Markers subdivided the site into a 2 x 2 m lattice, allowing us to repeatedly locate fish in space. Gobies ($n = 425$) were captured using hand nets, measured, tagged and immediately released at two times (30 June–14 July, and 4–7 August 2001). Sub-dermal tags allowed individual recognition of all gobies: larger fish (>25 mm SL) received numbered plastic tags, and smaller fish (15-25 mm SL) received colored spots of silicone. Both tag types are harmless and can be viewed without capturing the fish (Forrester 1995, Malone et al. 1999). The entire site, and surrounding habitat within 4 m, was searched for tagged fish every 3-5 days until 19 August, and finally on 27-28 October 2001. The infection status of tagged fish was visually assessed at every resighting.

Behavioral observations were made on tagged parasitized ($n = 35$) and uninfected fish ($n = 34$). Each fish was observed for 5 minutes, during which we recorded the feeding rate, the rate and outcome of aggressive interactions with neighbors, and the distance from the nearest shelter every 30 s. We then measured the gill ventilation rate over 30 s while the fish was not swimming or feeding.

While searching the site on 2-4 August 2001, we recaptured and remeasured haphazardly selected fish that had been infected ($n = 19$) or uninfected ($n = 49$) since tagging. We used analysis of covariance (ANCOVA) to test effects of parasitism on growth, with initial size as a

covariate to account for effects of size on growth (Sokal & Rohlf 1995). No tagged gobies emigrated from the study area, so disappearances were attributed to death. Some gobies apparently gained ($n = 48$) or lost parasites ($n = 33$) during the study. Survival curves thus were generated for fish never parasitized ($n = 214$), and fish parasitized on some or all dates on which they were observed ($n = 211$). We used a simple exponential survival model because it fit the data well (Lee 1992). Finally, we assessed the fecundity of parasitized ($n = 19$) and healthy ($n = 17$) female gobies (25.9-35.9 mm SL) captured just outside the study area by measuring gonad dry mass.

Results

Parasitized fish had a higher respiration rate and fed at lower rates than unparasitized fish (Table 1). Being parasitized did not, however, affect the mean distance to shelter, or outcome of aggressive interactions with conspecific neighbors (Table 1). Smaller bridled gobies grew faster than larger ones ($F_{1,64} = 13.8$ $p < 0.0004$), but the relationship between size and growth did not differ in slope for parasitized and unparasitized fish ($F_{1,64,} = 0.09$, $p = 0.76$). The ANCOVA with interaction removed was thus used to compare the mean growth parasitized and uninfected gobies (Sokal & Rohlf 1995), and revealed a significant reduction in growth associated with parasitism ($F_{1,65,} = 25.6$ $p < 0.0004$; Fig. 1). In addition, parasitized female gobies had gonads that were significantly smaller than those of comparable uninfected females (Table 1). Fish that were parasitized for all or part of the time they were observed also had significantly lower survival than fish that were parasite-free throughout (Fig. 2).

Discussion

Strictly speaking, we did not isolate the effect of parasitism because we simply correlated parasite presence with host responses, rather than experimentally infecting a random sample of hosts (Scott & Dobson 1989). Offsetting the limitations of our correlational study design is the ability to track individual gobies of known infection status. Effects of parasites on wild host populations are often assessed using indirect methods, whose assumptions are difficult to verify (Grenfell & Dobson 1995). Tracking individuals provided a simple direct measure of associations between ectoparasitism and host responses, and revealed striking impacts of *P. tortugensis* on bridled gobies. The one prior study of parasite impacts on reef fish, in this case of damselfish infected with ectoparasitic isopods, also reported demographic costs of parasitism (Adlard & Lester 1994). Researchers studying reef fishes have argued that predation and competition account for most mortality (Sale 1991). In fact, mortality due to parasitism was roughly equal to that attributable to predation in prior predator-removal experiments on bridled gobies (Forrester 2000, Forrester unpubl.). Agents of mortality need not be additive in their effect, however, and our findings raise the important possibility that parasitism in reef fishes may mediate vulnerability to predation (Dobson & Hudson 1994) or effectiveness in competition.

Also central to the importance of parasites for host dynamics is their potential to cause density dependent regulation (May & Anderson 1979). Mortality in bridled gobies is spatially density-dependent at scales from 8-64 m^2 (Forrester 1995, Forrester & Steele unpubl.). Goby density and *P. tortugensis* prevalence may also be positively related at this scale because a positive (though nonsignificant) correlation was revealed by the transect survey, and a stronger correlation was detected when the 560 m^2 monitoring area was subdivided into 16 m^2 sub-units ($r = 0.638$, $p < 0.001$). A higher prevalence of parasitism at high goby density may thus lead to

increased overall goby mortality in crowded areas and so regulate the goby population. This possibility is intriguing because cases of population regulation by macroparasites are quite rare, and macroparasites have been considered less likely to regulate host abundance than microparasites (Dobson & May 1987).

Acknowledgements

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Table 1. Behavior and fecundity of parasitized and unparasitized gobies. Presented are means (± SD) and results of *t*-tests comparing the two groups.

	Parasitized	Unparasitized	*t*-test
Feeding (bites 30 s^{-1})	2.8 ± 2.7	4.5 ± 3.6	$df = 67, t = 2.208, p = 0.031$
Respiration (gill ventilations 30 s^{-1})	48.7 ± 14.1	29.4 ± 8.2	$df = 67, t = 6.938, p < 0.001$
Aggression (losses 5 min^{-1})	1.4 ± 0.5	1.6 ± 0.8	$df = 12, t = 0.397, p = 0.675$
Aggression (wins 5 min^{-1})	1.8 ± 1.2	2.3 ± 1.9	$df = 8, t = 0.435, p = 0.675$
Distance to shelter (cm)	13. 1 ± 11.6	13.5 ± 9.3	$df = 67, t = 0.186, p = 0.853$
Fecundity (gonad dry weight mg)	0.63 ± 0.54	0.2 ± 0.1	$df = 28, t = 4.065, p < 0.001$

Figure legends

Fig. 1. Growth rates of tagged unparasitized and parasitized gobies recaptured on 2-4 August 2001. Growth rate was significantly faster in unparasitized than in parasitized gobies, and was dependent on the size of the fish at tagging; see text for details.

Fig. 2. Survival curves for gobies parasitized some or all of the time ($y = e^{-0.024x}$), and for gobies never parasitized ($y = e^{-0.013x}$).

Figure 1

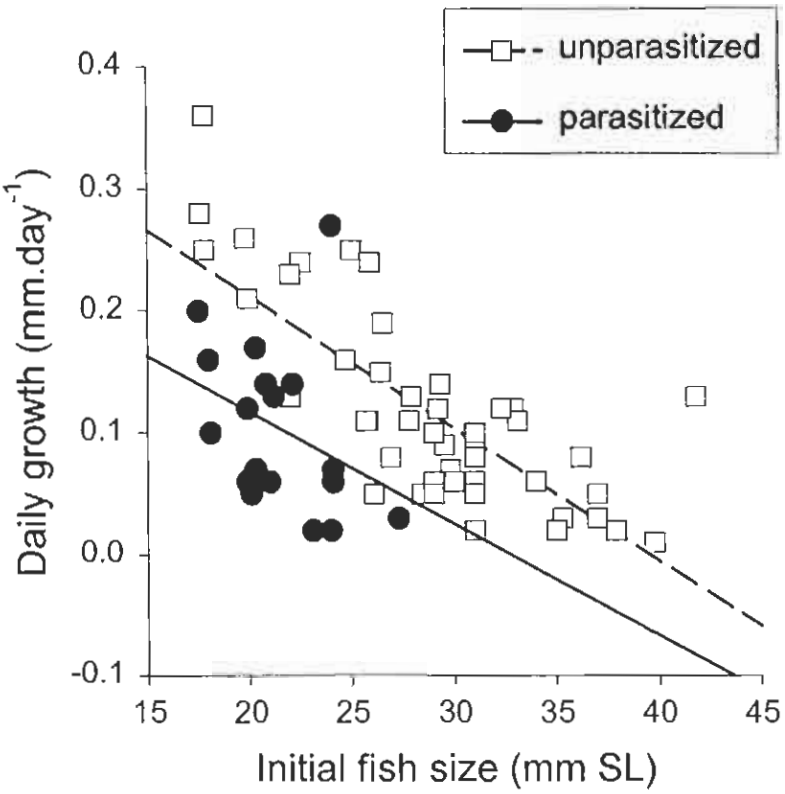
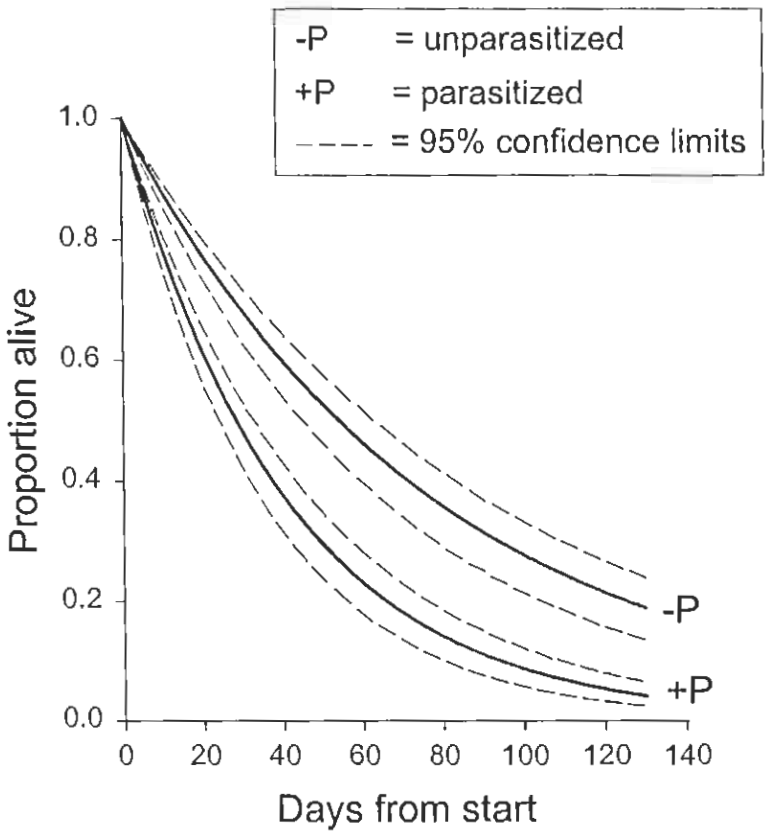

unparasitized
parasitized
Daily growth (mm.day^{-1})
0.4
0.3
0.2
0.1
0.0
-0.1
15
20
25
30
35
40
45
Initial fish size (mm SL)

Figure 2


-P = unparasitized
+P = parasitized
---- = 95% confidence limits
Proportion alive
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
140
Days from start
-P
+P

3 April 2002
PROCEEDINGS OF THE BIOLOGICAL SOCIETY OF WASHINGTON
115(1):108–117. 2002.

Microprosthema jareckii, a new species of stenopodidean shrimp (Crustacea: Decapoda: Stenopodidea: Spongicolidae) from Guana Island, British Virgin Islands

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Abstract.—A new species of the stenopodidean shrimp genus *Microprosthema* Stimpson, *M. jareckii*, is described from two specimens collected off the coast of Guana Island, British Virgin Islands. The species differs from known congeners by the shape and spination of the rostrum, spination of the carapace, shape of the third pereiopod, dentition of the mandibular palp, and coloration (the new species is completely white). The new species is compared to all other known species of *Microprosthema* in the Caribbean.

Relatively few species of stenopodidean shrimps have been reported from the Caribbean and western Atlantic. Published reports include only six genera and 11 species. In the genus *Stenopus* Latreille, 1819, only two species, *S. hispidus* (Olivier, 1811) and *S. scutellatus* Rankin, 1898, have been reported. In the genus *Richardina* A. Milne Edwards, 1881, *R. spinicincta* A. Milne-Edwards 1881, is known from a single specimen (Goy 1982). One species of *Spongiocaris* Bruce & Baba, 1973, *S. hexactinellicola* Berggren, 1993, is known from the Bahamas, Dry Tortugas, and Puerto Rico (Berggren 1993, and J. Goy, unpublished data). Two species of the genus *Odontozona* Holthuis, 1946 (*O. striata* Goy, 1981 and *O. libertae* Gore, 1981) have been described, as have four species of the genus *Microprosthema* Stimpson, 1860. The known species of *Microprosthema* are *M. semilaeve* (von Martens, 1872), *M. manningi* Goy & Felder, 1988, *M. looense* Goy & Felder, 1988, and *M. granatense* Criales, 1997 (see Criales 1997 for a review and key). Criales (1997: 538) lists *M. inornatum* Manning & Chace, 1990, among the known western Atlantic species of *Microprosthema*, although to my knowledge *M. inornatum* is known only from the type locality (Ascension Island, eastern South Atlantic; Manning & Chace 1990). Additionally, I am aware of at least two other undescribed species of *Microprosthema* and several unpublished records of *Richardina spinicincta* and *Stenopus spinosus* Risso, 1826 (J. Goy, unpublished data). Finally, the genus *Paraspongicola* de Saint Laurent & Cléva, 1981, is now known in the Atlantic from deep waters off Venezuela (J. Goy, pers. comm. regarding an unpublished finding by B. Rodriguez Q.). Below I describe a new species of *Microprosthema* from Guana Island, British Virgin Islands.

Materials and Methods

The specimens reported below were collected during the course of a biodiversity survey of the cryptic marine invertebrates of Guana Island, British Virgin Islands (18°28′33″N, 64°34′29″W) led by T. L. Zimmerman and J. W. Martin and funded by grants from the United States National Science Foundation and the Falconwood Corporation. Various collecting methods were employed during that survey, including light traps, hand collecting, and arrays of artificial reef matrices (ARMs). The

ARMs consisted of four slabs of concrete, each approximately 30 × 50 × 6 cm, containing holes of different sizes. The concrete slabs were set upon a basket filled with coral rubble, and the basket was set into the substrate so that the bottom concrete slab was roughly level with the sea floor. These arrays were deployed at a depth of 10 m at eight different locations around Guana Island, and at two locations in shallower water, in the summer of 1999 and were collected one year later. The two specimens of the new species of *Microprosthema* were collected from the ARM deployed off of Monkey Point, Guana Island, when that ARM was harvested on 20 July 2000. Other Caribbean material of the genus was examined during a visit to the National Museum of Natural History, Smithsonian Institution, Washington, D.C., in February of 2001, including the following specimens: holotype (USNM 233997) of *Microprosthema manningi* Goy & Felder, 1988; holotype (USNM 275993) of *Microprosthema granatense* Criales, 1997; non-type ovigerous female specimen (USNM 244439, Bahamas) of *Microprosthema semilaeve* von Martens. Both specimens of the new species have been deposited in the Crustacea collections of the Natural History Museum of Los Angeles County (LACM).

Results

Family Spongicolidae Schram, 1986
Genus *Microprosthema* Stimpson, 1860

Microprosthema jareckii, new species
Figs. 1–5

Material examined.—Holotype: male, LACM CR 2000 0081, photographic voucher number Vc1314, BVI Station 46C, 22 Jul 2000, ARM at Monkey Point, 10 m, SCUBA, morning dive, coll. T. Zimmerman, R. Ware, T. Haney, J. Martin. Allotype: female, LACM CR 2000 0082, photographic voucher number Vc1316, same collection data as for holotype.

Description.—Carapace (Figs. 1, 3a, b) with relatively few short dorsal and dorsolateral spines (as compared to other members of genus) directed anteriorly, and with numerous distally plumose setae giving overall "fuzzy" appearance. Cervical groove present but weak, with sharp spines just posterior to groove on dorsal and dorsolateral regions, and terminating at level of strong hepatic spine. Carapace spines more numerous and better developed on anterolateral (branchiostegal) regions. Antennal and orbital spines strong, acute, well developed. Rostrum large, extending to level of distal end of antennular peduncle, well developed, strongly curved downward, ventrally with 1 small subterminal tooth, dorsally with series of 5 teeth (excluding acute tip of rostrum), continuing posteriorly (with additional teeth) along well defined carina that extends back to cervical groove.

Eyes (Fig. 3a, b) each with cornea slightly smaller in diameter than eyestalk; eyestalk with small spines just proximal to, and extending slightly laterally over, cornea.

Abdomen (Figs. 1, 3c) smooth, lacking transverse ridges. Abdominal somites 1–3 with pleura terminating in rounded point bearing 3 setae (Fig. 3c), somites 4–6 more rounded than anterior ones. Pleura of somites 1–3 each bearing 2 or 3 teeth on anterior and posterior borders (those on anterior border more acute than those on posterior border).

Antenna 1 (antennule) (Fig. 4a, b) with large slightly curved stylocerite extending only to distal end of basal article, with smaller tooth on distal end of second article and pair of teeth on distalmost article of peduncle. Flagellar articles (Fig. 4b) heavily setose, with setae arising more or less circularly around each article, giving the flagella a bushy overall appearance (Fig. 2b).

Antenna 2 (Fig. 4c) with sharp teeth on all peduncular articles. Scaphocerite reaching well beyond tip of rostrum (tip of rostrum in dorsal view extending about 1/3 length of scaphocerite), strongly curved on medial border, nearly straight on lateral bor-

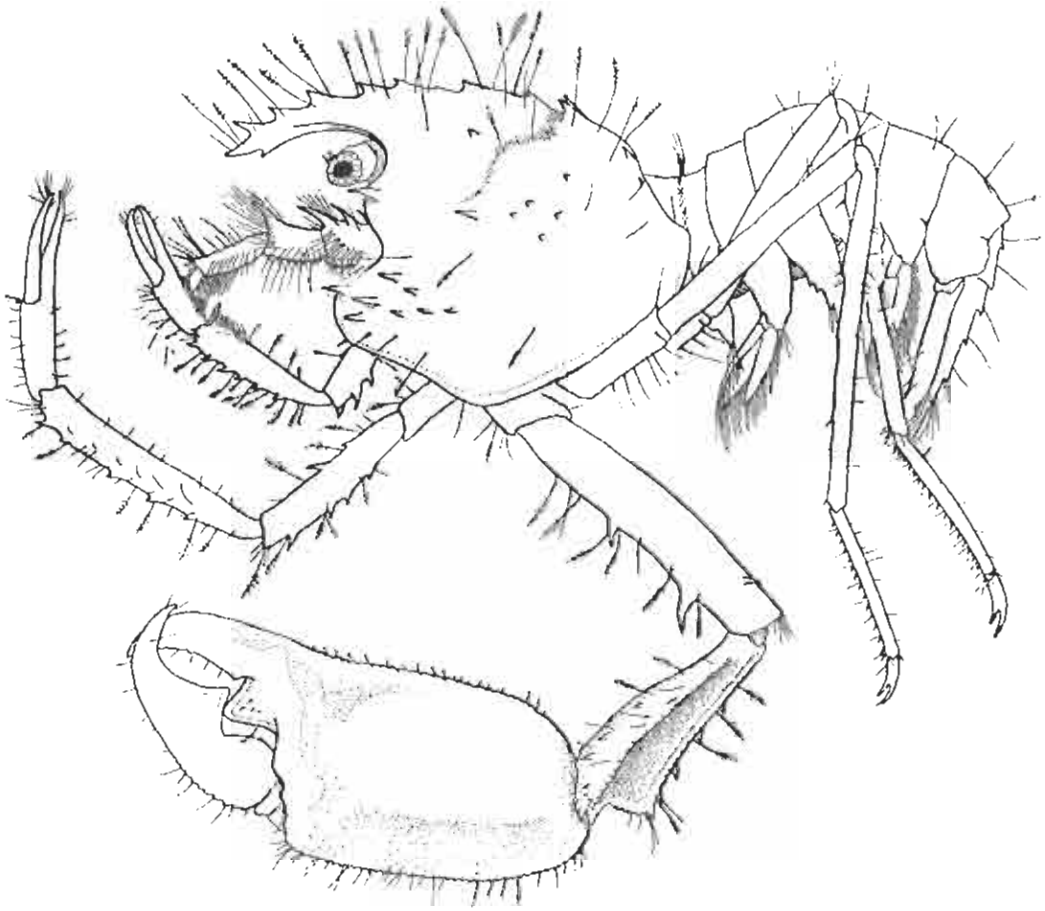

Fig. 1. *Microprosthema jareckii*, holotype male, LACM CR 2000 0082, composite view.

der. Lateral border with 4 sharp teeth in addition to sharp distolateral tooth at corner.

Mandible (Fig. 4d) on left side (right side not examined) with smooth, sharp, blade-like cutting edge, posterior side strongly concave (left side of Fig. 4d). Anterodistal corner of cutting edge marked by extremely long sharp tooth; posterodistal corner with shorter, subtriangular tooth. Mandibular palp composed of 3 articles, second of which bears 2 strong spines: 1 at approximate midlength and 1 near distal articulation with terminal article. Terminal article lanceolate, broadest at midlength, tapering to acute tip and bearing scattered setae as illustrated.

Maxilla 1 (Fig. 4e) with heavy, serrate spines on upper endite and scattered simple and plumose setae on lower endite. Palp with 2 articles, distalmost of which bears 2 short terminal setae. Maxilla 2 (Fig. 4f) endites strongly bilobed, with setation (proximal to distal) 10 + 5, 8 + 13. Blade of scaphognathite not examined.

Maxilliped 1 (Fig. 4g) with unsegmented endopod bearing 13 long plumose setae on

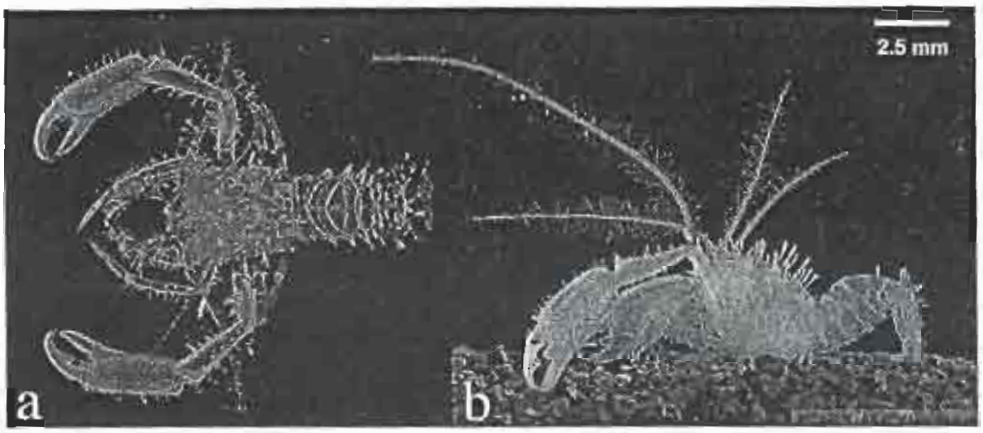

Fig. 2. *Microprosthema jareckii*, black and white photographs made from color 35 mm photographic slides (photographic voucher number Vc1314) of live holotype male (LACM CR 2000 0082), taken in small aquarium on Guana Island, BVI. a, dorsal view; b, lateral view. Photographs by T. L. Zimmerman.

curved anterolateral border. Basipodite long and wide, bearing numerous short setae, relatively straight along medial border and broadly curved on anterolateral border. Coxopodite small, approximately 1/5 length of basipodite and unsegmented. Exopod long and slender, with numerous plumose setae increasing in number distally and with minute crenulations beginning just distal to somewhat abrupt bend and continuing to tip. Epipod divided into equally sized distal and proximal lobes, neither with any setation.

Maxilliped 2 (Fig. 4h) with 4-segmented endopod. Dactylus lanceolate and densely setose on medial border. Propodus slightly shorter than dactylus and with dense medial setation similar to dactylus. Carpus approximately same length as propodus, triangular, with distal end broader, lacking dense setation but with 7–10 long, simple dorsodistal setae. Merus subrectangular, with 13 or 14 long simple setae spaced regularly on minute cuticular projections along medial border and with short simple setae on anterolateral border.

Maxilliped 3 (Fig. 4i, j) endopodite strongly developed, 5-segmented. Dactylus elongate-triangular. Propodus longer than dactylus, with dense setae along medial border and with distal dense setal brush ("setiferous organ" of Goy and Felder, 1988, and Criales, 1997); 4 long simple setae proximal to brush on medial border. Carpus approximately equal in length to propodus, with strong distolateral spine extending beyond distal border of segment, and 7 long, simple setae arising from slight cuticular protrusions along medial border. Merus slightly longer than carpus, with 4 strong spines along lateral border; distalmost such spine extending to midlength of carpus. Ischium longer than merus, with 8 spines along lateral border, increasing in size distally, and with single anterodistal spine on medial border. Exopod long, slender, reaching (excluding setation) just past midlength of endopodal merus, with numerous plumose setae beginning at approximate midlength of exopod, increasing in number toward tip.

Pereiopod 1 (Fig. 5a) short, stout, spinose. Dactylus approximately half length of propodus (including fixed finger). Propodus slightly swollen basally, with obvious cleaning brush on inner surface; setae of cleaning brush recessed centrally. Carpus with 6 heavy spines along outer (lateral) border and with cleaning brush on distomedial border (probably serving as opposing brush of propodus when pereiopod is flexed). Merus with 2 stout spines on me-

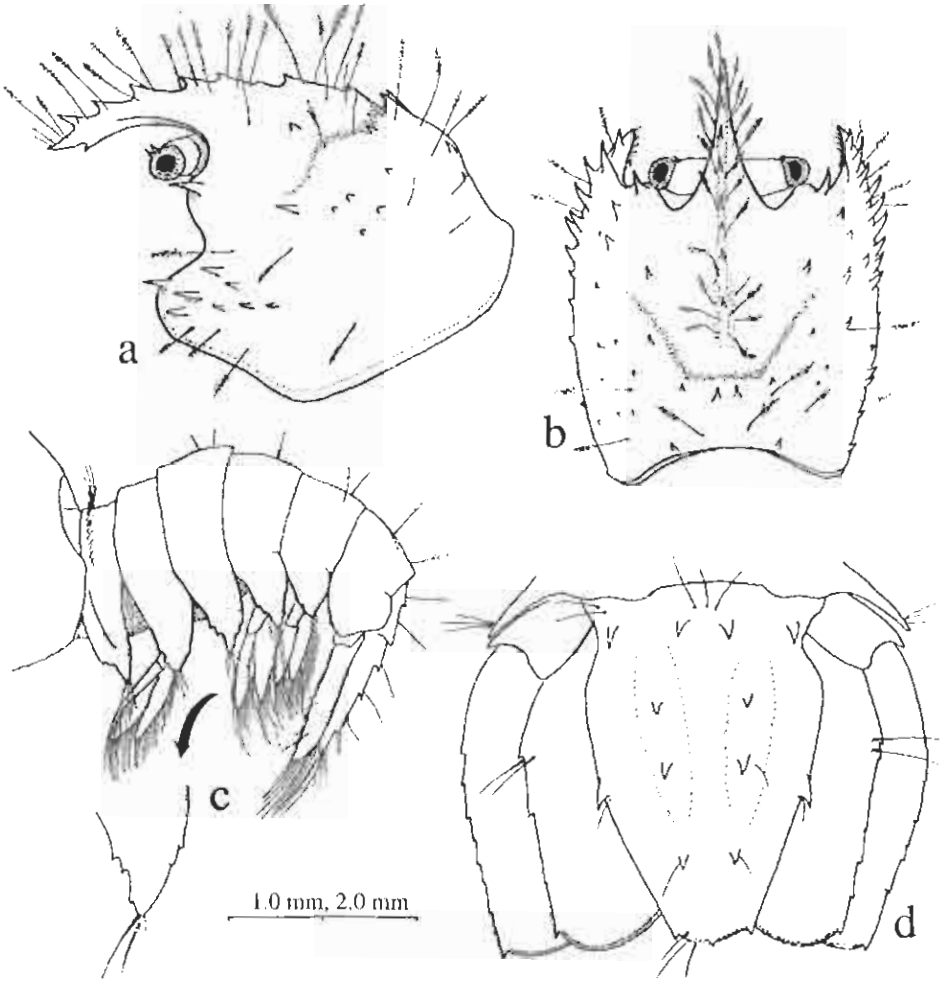

Fig. 3. *Microprosthema jareckii*, holotype male, LACM CR 2000 0082. a, carapace, lateral view; b, carapace, dorsal view; c, abdomen, lateral view, with tip of pleuron of abdominal somite 3 magnified to lower left (arrow); d, telson and uropods. Scale bar = 2.0 mm for a–c, 1.0 mm for d.

dial border and 4 spines, increasing in size distally, along lateral border, plus single sharp distolateral tooth. Entire appendage with scattered distally plumose setae on all articles except dactylus.

Pereiopod 2 (Fig. 5b) longer and more slender than pereiopod 1, lacking propodal-carpal cleaning brush. Cheliped fingers with small regularly spaced teeth along cutting edges; tips of fingers with clusters of simple setae. Carpus longer than other articles, with series of 5 heavy spines along outer (lateral) border. Merus with 2 stout spines on medial border and 4 stout spines along lateral border. Scattered, distally plumose setae on all articles except dactylus.

Pereiopod 3 (Fig. 5c) extremely large, heavy. Merus with 2 stout spines on inner (medial) border, 1 at approximate midlength and 1 at approximate ¾ length of merus. Carpus triangular in dorsal view and in cross section; dorsal surface slightly excavate, widening distally into shallow trough; lateral border of carpus with 4 stout spines and distolateral tooth; medial border with 3 small spines and cluster of short spines on distomedial border. Propodus deep, centrally thick but narrowing to bladelike carina

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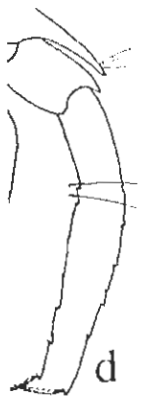

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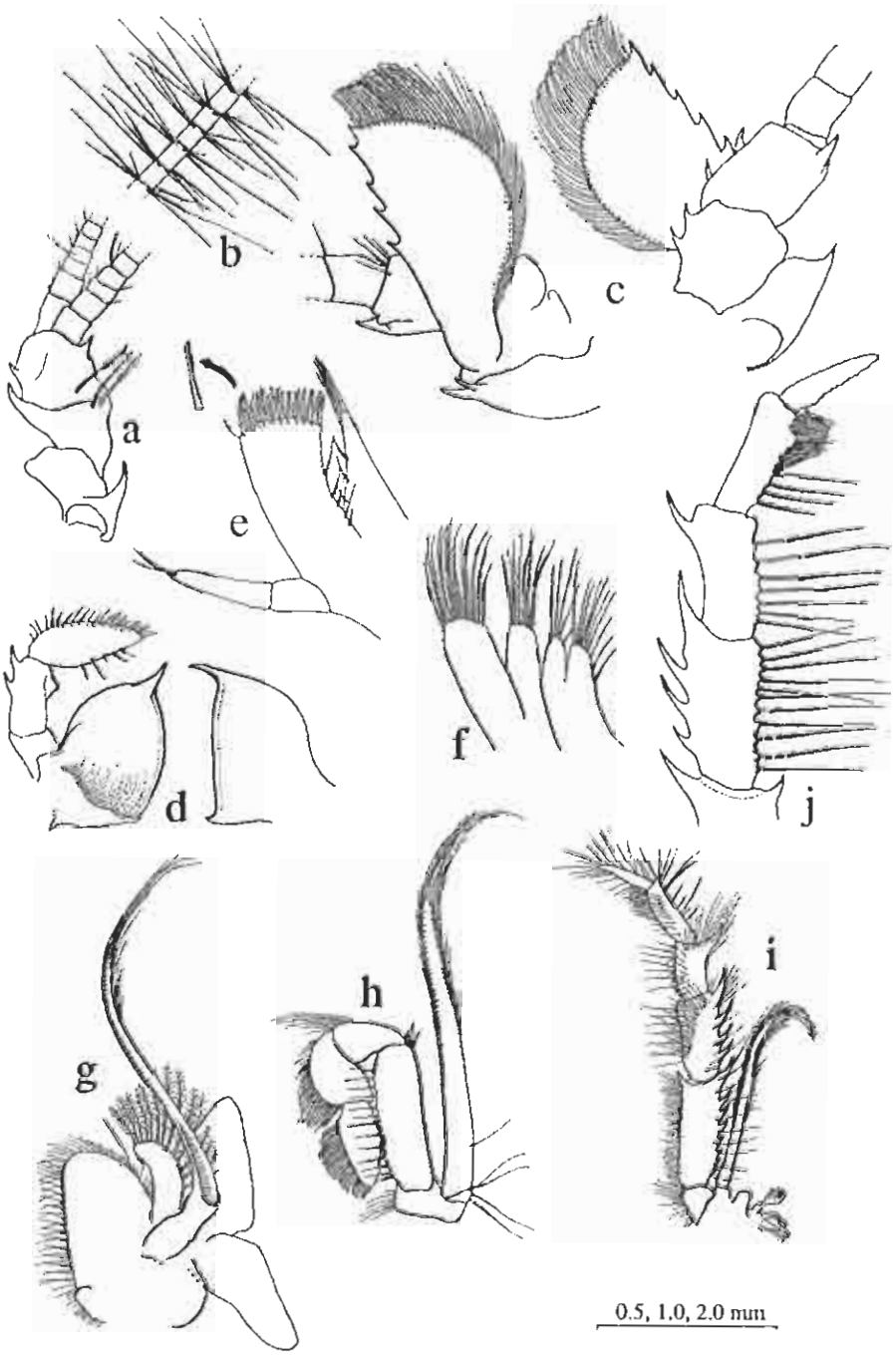

Fig. 4. *Microprosthema jareckii*, holotype male, LACM CR 2000 0082, antennae and mouthparts, left side. a, antenna 1 (antennule), ventral view; b, section of distal articles of antenna 1 flagellum showing setation; c, base of antenna 2 and scaphocerite, dorsal (left) and ventral (right) views; d, left mandible, inner (left) and outer (right) view; e, maxilla 1; f, endites of maxilla 2; g, first maxilliped; h, second maxilliped; i, third maxilliped (illustrated at different magnification from g and h); j, higher magnification of distal 4 articles of third maxilliped (same appendage as in i, but reversed to show other side) with only selected setae illustrated. Scale bar = 0.5 mm for e, f; 1.0 mm for a–d, g, h; 2.0 mm for i.

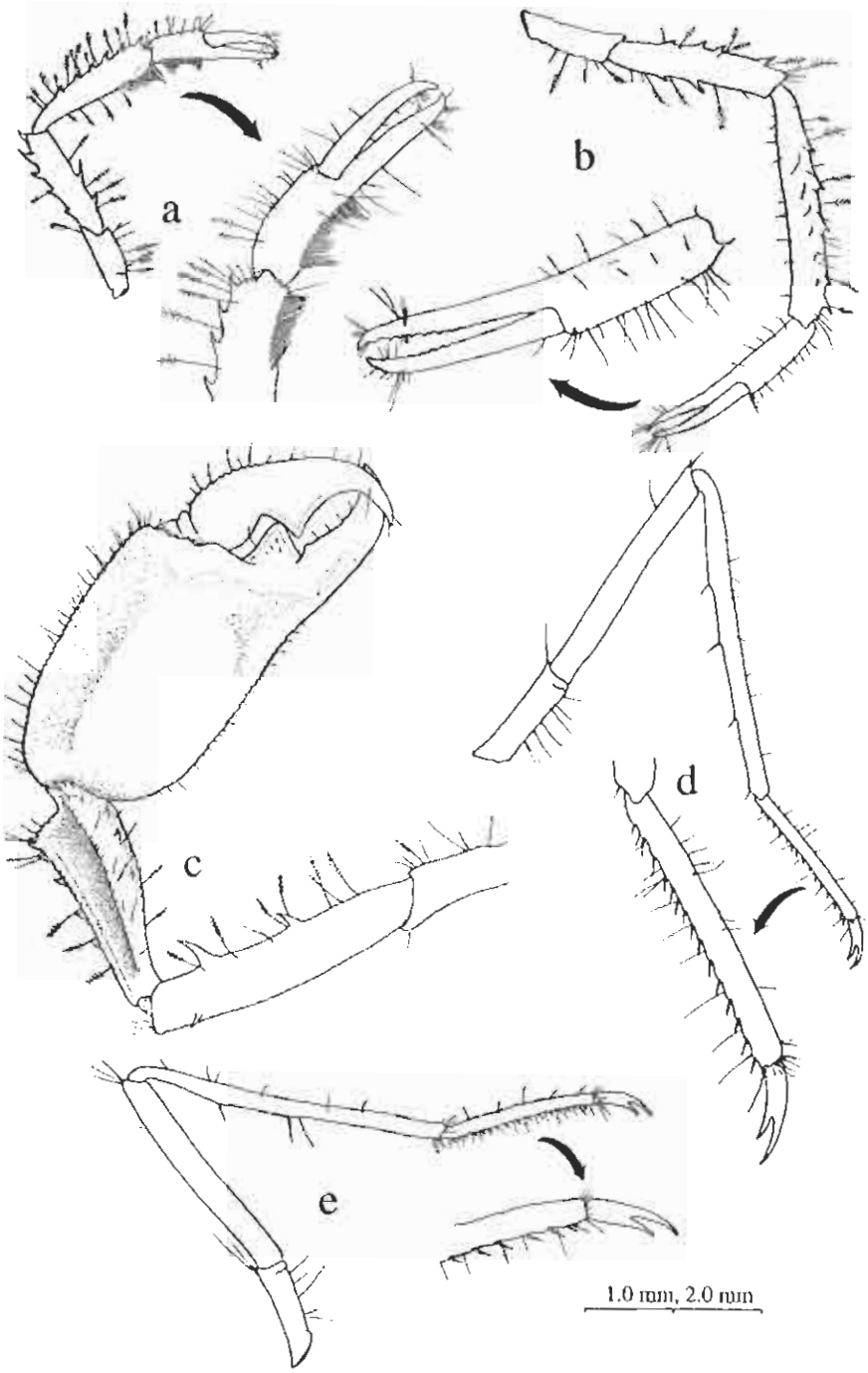

Fig. 5. *Microprosthema jareckii*, holotype male, LACM CR 2000 0082, left pereiopods. a, pereiopod 1, with higher magnification of chela and distal part of carpus (arrow); b, pereiopod 2, with higher magnification of chela (arrow); c, pereiopod 3, dorsolateral view; d, pereiopod 4, with dactylus and propodus magnified (arrow); e, pereiopod 5, with dactylus and part of propodus magnified (arrow). Scale bar = 1.0 mm for all figures except for close up views in a, b, d, and e (arrows), where scale bar = 2.0 mm.

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dorsally (dorsal crista), with minute serrulations on dorsal border and ventral border, fading to smooth along ventral border of fixed finger; cutting edge of finger with large triangular tooth at base. Dactylus with minute serrulations on proximal third of upper (dorsal) surface; cutting edge with large triangular tooth opposite and just distal to similar tooth on propodal finger. Dactylar and propodal fingers slightly overlapping when chela closed. Entire chela high (dorsal to ventral) but thin (medial to lateral); inner surface of chela slightly concave, with chela curved inward toward front of animal.

Pereiopods 4 and 5 (Fig. 5d, e) long, slender, similar to one another, with short, bifurcated dactylus; ventral branch of dactylus shorter, approximately half length of dorsal branch. Propodus undivided, with series of 15 (pereiopod 4) to 17 (pereiopod 5) short, sharp movable spines spaced regularly along ventral border. Carpus longer than merus, which is longer than propodus. Pereiopod 4 with 3 setae arising from slight ventral protrusion and with scattered simple setae dorsally; only 2 such setae (plus 1 seta not arising from protuberance).

Pleopods (not illustrated) as for genus (see Holthuis, 1946), with first pleopod uniramous and pleopods 2–5 biramous; all pleopods lacking appendices.

Telson and uropods (Fig. 3d) broad, strongly deflexed (Fig. 1, 2b, 3c), not visible or only partly visible in dorsal view in life (Fig. 2a); telson approximately equal in length to uropods. Exopod with 5–7 small teeth on lateral margin, terminating in acute tooth on distolateral corner; distal border smoothly rounded, with rounded border not exceeding length of distolateral tooth, continuing dorsally to form interior (medial) border. Endopod similar, with fewer teeth on lateral border and with rounded posterior border clearly extending beyond length of distolateral tooth. Both endopod and exopod heavily setose on posterior and medial borders. Telson subtriangular, with strong lateral teeth at approximate midlength. Dorsal surface with 4 teeth at anterior third and 2 longitudinal rows of 3 spines each. Lateral edges terminating distally in small acute tooth. Posterior border slightly curved, with small tooth at midpoint, and heavily setose.

Color (see Fig. 2).—In life, both specimens were completely white and slightly translucent (Fig. 2a, b). The only color discernable other than white was a yellowish central area under the carapace, caused by the hepatopancreas showing through the carapace. There were no other colors on any of the body parts.

Sexual dimorphism.—None apparent. The female allotype is similar in all regards to the male holotype, with the only distinguishing feature being the minute genital opening on the coxa of the third, as opposed to the fifth, pereopods. This difference is so slight, and the opening so difficult to detect, that it is even possible that both specimens belong to the same sex.

Etymology.—I am pleased to name this new species after Dr. Henry Jarecki, in appreciation for his concern for the preservation and conservation of our natural world, and especially for his vision in establishing a protected nature preserve on Guana Island, BVI.

Habitat.—Known only from Monkey Point, Guana Island, British Virgin islands, from an artificial reef matrix, 10 m depth. The surrounding seafloor was predominantly hard bottom with scattered coral heads, coral rubble, sea fans, and occasional pockets and channels of sand.

Remarks.—Of the previously described species of *Microprosthema* known from the Caribbean and western Atlantic, the new species is most similar in coloration to *M. manningi* and *M. looensis*, both of which were described by Goy & Felder (1988). Goy & Felder (1988: 1286) described coloration in *M. manningi* as being "whitish to pale tan; antennae, abdomen and appendages white, abdomen and pereiopods sometimes edged in tan or pale magenta." Coloration in *M. looensis* was described by them as "carapace and abdomen whitish

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tan; antennae, telson, uropods, and all appendages white." Specimens of *M. looensis* held in captivity later appeared completely white (J. Goy, pers. obs.). Thus, all three of these species are predominantly white or whitish. However, an abundance of morphological characters serve to distinguish *M. jareckii* from *M. manningi* and *M. looensis*. Spination of the carapace in *M. manningi* is much more uniform than in *M. jareckii*, and the cervical groove is indistinct. The carapace spines of *M. looensis* are numerons and mostly blunt, rather than acute as in *M. jareckii*, the chela of the third pereiopod lacks the dorsal crista, the rostrum is shorter and ventrally unarmed, and all of the pereiopods are unique in being covered with short setae (see Goy & Felder 1988: fig. 7).

The new species shares with *M. manningi* the unusual and strikingly similar character of stout spines on the middle article of the mandibular palp (not known for any other species in the genus), and the more commonly encountered dorsal crista on the third pereiopod. However, the cutting edge of the mandible of *M. jareckii* is more similar to that of *M. looensis* in possessing a long acute process on the dorsodistal angle.

Coloration of *M. granatense*, currently known only from the southern Caribbean, was not noted by Criales (1997). However, *M. jareckii* is easily distinguished from *M. granatense* by the complete absence of spines on pereiopods 1 and 2, but a more spinose pereiopod 3, in *M. granatense*, as well as by differences in the spination of the carapace, relative width of the scaphocerite, subdivision of the propodus of pereiopods 4 and 5, relative height of the propodus of pereiopod 3, and spination of the third maxilliped.

Comparison of the new species to the widespread and commonly reported species *Microprosthema semilaeve* proved to be more difficult than expected, as that species has not been illustrated other than by Rankin (1898, plate 29, fig. 2, side view of whole animal), Holthuis (1946, plate 3, fig. i, scaphocerite only), and Rodríguez (1980, fig. 51, partial views of carapace and abdomen). Although the color notes provided by Manning (1961) are quite detailed, I have not been able to locate the specimen on which that note was based (Manning did not mention a repository, and I did not see any specimens of *M. semilaeve* from his collection site among the specimens at the USNM). Thus, although commonly reported in the literature and given the common name "crimson coral shrimp" by Williams et al. (1989), *M. semilaeve* lacks a thorough modern description. For the purposes of this report I am assuming that the crimson and white coloration described by Manning (1961) is specific to this species, and thus color pattern is one obvious difference between *M. jareckii* (completely white) and *Microprosthema semilaeve* (mostly brilliant red). Additionally, Goy & Felder (1988) examined 78 specimens of *M. semilaeve* and noted, among other characters, that in all specimens examined the "carpi and propodi of the third maxillipeds lack spines" (*M. jareckii* bears one very heavy spine on the corpus; see Fig. 4i, j), the "merus of the first pereiopod lacks spines" (there are seven heavy spines present on the merus of pereiopod 1 in *M. jareckii;* see Fig. 5a), and the "second pereiopod bears only one or two meral spines" (six are present in *M. jareckii*, Fig. 5b). Therefore, even without considering coloration, these obvious morphological differences confirm that *Microprosthema jareckii* is distinct from *M. semilaeve*, at least as defined and understood by Goy & Felder (1988).

Acknowledgments

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the PEET program in Systematic Biology. I thank Doug Causey for his help and encouragement while at NSF, and the Jarecki family, and especially Lianna Jarecki, for hosting our working visit to Guana Island. I also thank the staff of Guana Island, British Virgin Islands, and the following persons for help while in the field: Todd Zimmerman, Rick Ware, Don Cadien, Leslie Harris, Gordon Hendler, Kirk Fitzhugh, Todd Haney, and Lianna Jarecki. I thank Dr. Regina Wetzer for help with locating needed literature and compiling and arranging the figures. Much of the manuscript was written during a visit to the National Museum of Natural History, Smithsonian Institution, Washington, D.C., in February of 2001, during which time I benefited greatly from the hospitality and kind assistance of R. Lemaitre, and also by late night discussions with T. Zimmerman, R. W. Heard, and D. Felder.

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FIRST RECORD AND HABITAT NOTES FOR THE GENUS *LIGHTIELLA* (CRUSTACEA, CEPHALOCARIDA, HUTCHINSONIELLIDAE) FROM THE BRITISH VIRGIN ISLANDS

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Introduction

The crustacean class Cephalocarida, as currently understood, is composed of five genera and ten species (Hessler and Elofsson 1996, Hessler and Wakabara 2000). Although in some instances numerous specimens have been collected in a single locale (e.g., the nearly 120 specimens of *Lightiella incisa* Gooding, 1963 from Puerto Rico studied by Sanders and Hessler (1964), and the numerous specimens of *Hutchinsoniella macracantha* Sanders, 1955 now known from Buzzards Bay, Massachusetts, see Hessler and Sanders 1973:193), most reports are based on very few specimens. For example, the original description of the genus *Hutchinsoniella* Sanders, 1955 was based on only eight specimens from Long Island Sound, New York (Sanders 1955); the genus *Sandersiella* was originally described by Shiino (1965) on the basis of only one specimen from Japan; the genus *Chiltoniella* Knox and Fenwick, 1977 was based on two specimens from New Zealand (Knox and Fenwick 1977), and the genus *Lightiella* Jones, 1961 was based on seven specimens from San Francisco Bay (Jones 1961). *Lightiella moniotae* was described for a single individual from New Caledonia (Cals and Delamare-Debouteville 1970); *Sandersiella calmani* for two specimens from Peru (Hessler and Sanders 1973); and *Sandersiella bathyalis* for two specimens from the deep ocean off southwest Africa (Hessler and Sanders 1973). The single eastern Caribbean record (Barbados) of a cephalocarid also was based on two specimens (Gooding 1963), although Gooding also discussed two specimens from Puerto Rico in that account.

Cephalocarids are of such interest morphologically and phylogenetically, and are found so infrequently, that their presence anywhere is noteworthy. As part of an ongoing survey of the cryptic marine invertebrates of certain Caribbean islands, we obtained a single specimen of a cephalocarid from Guana Island, British Virgin Islands, that matches most closely the description by Gooding (1963) of *L. incisa*. The find is of interest not only because it is the first record for the far eastern Caribbean other than Gooding's (1963) two type specimens from Barbados, but also because of the unusual habitat in which it was found.

There are three additional records of cephalocarids in the Caribbean other than Gooding's (1963) original description of *L. incisa* from Barbados and southwestern Puerto Rico. Sanders and Hessler (1964) reported *L. incisa* from the Puerto Rican site. This same species also is known from the Yucatan Peninsula (De Troch et al. 2000) and Carrie Bow Cay, Belize (Schiemer and Ott 2001).

Several records of cephalocarids are known for waters just outside the Caribbean. Wakabara (1970) recorded *Hutchinsoniella* from Brazilian waters, and the genus *Sandersiella* was also reported from Brazil by Wakabara and Mizoguchi (1976). The latter record was corrected by Hessler and Wakabara (2000), who described the species in question as new, making it the type of their newly erected genus *Hampsonellus*. There are several reports of *Lightiella* from the east and west coasts of Florida (Hessler and Sanders 1973, McLaughlin 1976, Saloman 1978, Stoner 1981) and a single record from the coast of Alabama (Heard and Goeke 1982).

Materials and Methods

The single specimen was collected during a biodiversity survey of the cryptic marine invertebrates of Guana Island, British Virgin Islands (18°28'33"N, 64°34'29"W), led by T.L. Zimmerman and J.W. Martin. Various collecting methods were employed during that survey, including light traps, hand collecting, yabby pumps, and arrays of artificial reef matrices (ARMs). The cephalocarid was found by sorting through a collection of sand (a mixture of siliceous and calcium carbonate) and gravel collected by hand using SCUBA on July 8, 2001. The sand and gravel were from an area of large

boulders among scattered coral heads, sponges, and soft corals at a depth of approximately 5 m immediately south of Long Point, Muskmellon Bay, Guana Island (2001: Station 12 of the Zimmerman and Martin survey, individual specimen number Vd 0054). Observations and illustrations of the preserved specimen were made with a Wild M5APO dissecting stereoscope and a Nikon Labophot, both with drawing tubes. The specimen has been catalogued in the Crustacea collection of the Natural History Museum of Los Angeles County as LACM CR 2001-005.1.

DESCRIPTION

The single specimen (Figure 1) measures 2.1 mm from the tip of the cephalic shield to the tip of the telson. The body (Figure 1A, B) consists of a cephalic shield followed by 9 thoracic and 12 post-thoracic somites including the telson; the first thoracic somite is covered to some extent by the cephalic shield, possibly an artifact of preservation. All of the post-thoracic somites except the telson bear acute lateral spines, increasing in size toward the posterior somites. The telson bears a ventral comb row of spine-like teeth across its full width (Figure 1D); no other somites bear such a row. The dorsal medial surface of the telson (Figure 1C) is extended posteriorly as a pair of triangular teeth that project beyond the posterior margin of the ventral comb row. The caudal rami are relatively short and thick, and each is shorter than the combined length of the last abdominal segment and the telson. The tip of each ramus is strongly indented, with an acute medial spine-like tooth and a shorter and less acute lateral tooth. Each ramus bears one long and two short setae (one of the short setae is broken on the left side); a much longer seta on each ramus was present in life but has been broken and is not figured. The single egg is attached to the tip of the modified 8th limb on the ventral surface of the animal's right side. In dorsal view (Figure 1A), the egg protrudes to the right of the body; in ventral view (Figure 1B) it appears directed slightly to the posterior.

REMARKS

Characters visible to us without dissection are in general agreement with those described for *L. incisa* by Gooding (1963) and Sanders and Hessler (1964). In particular, the low number of thoracic limbs (7 total, excluding the modified egg-bearing limb), the relatively short and stout caudal rami, the single comb row on the ventral border of the telson, and the single extruded egg are together indicative of the genus *Lightiella* and serve to separate members of this genus from other cephalocarids (e.g., see Hessler and Sanders 1973, McLaughlin 1976, Hessler et al. 1995). Mouthpart morphology was not examined because of our reluctance to dissect the single specimen.

Hessler et al. (1995) noted that the large extruded egg of *H. macracantha* is "cemented to the knob which forms the tip of the small ninth thoracic limb." The situation is similar in *Lightiella*, except that a single egg is attached to what appears to be a modified "eighth" limb (which is located on the ninth thoracic segment but is numerically the eighth because of the missing thoracopod in *Lightiella*; see Sanders and Hessler 1964). Sanders and Hessler (1964) examined 17 ovigerous adults of *L. incisa*, and 16 of them carried a single egg sac (the other individual carried paired egg sacs as in *Hutchinsoniella*). Although to our knowledge the present paper contains the first illustrations of the extruded egg of *L. incisa*, its occurrence has been noted previously (Gooding 1963, Hessler and Sanders 1964, De Troch et al. 2000). Hessler et al. (1995: Figure 1) illustrated the paired egg sacs in *H. macracantha*.

Notes on Movement

The specimen was sorted from the sample while it was still alive. In fact, what brought the small animal to our attention, and distinguished it from the surrounding copepods that it resembled, was its movement pattern. The animal moved in a very graceful and smooth way reminiscent of a branchiopod notostracan. It would often make very tight reversals upon itself when changing direction. This type of movement was noted also by Sanders (1963:9–13, Figures 12, 13) in his classic work on functional morphology and anatomy of *H. macracantha*.

Habitat Notes

The habitat is of interest because it is atypical for cephalocarids. Station/Sample 12, 2001, of the Zimmerman and Martin survey is an apparently well oxygenated shallow (5–10 cm) layer of sand and pea gravel overlying a more or less solid rock base at the bottom of a fissure (1–2 m wide at the base) in the bedrock that slopes away from the base of the island. The fissure runs perpendicular to the shore and slopes slightly upward; the depth where the sand and gravel were collected was approximately 5 m. This was in an area characterized by large boulders calved from the cliff face above. At the base of the boulder field, at a depth of about 7 m, the bottom consisted of coarse gravel, sand, and

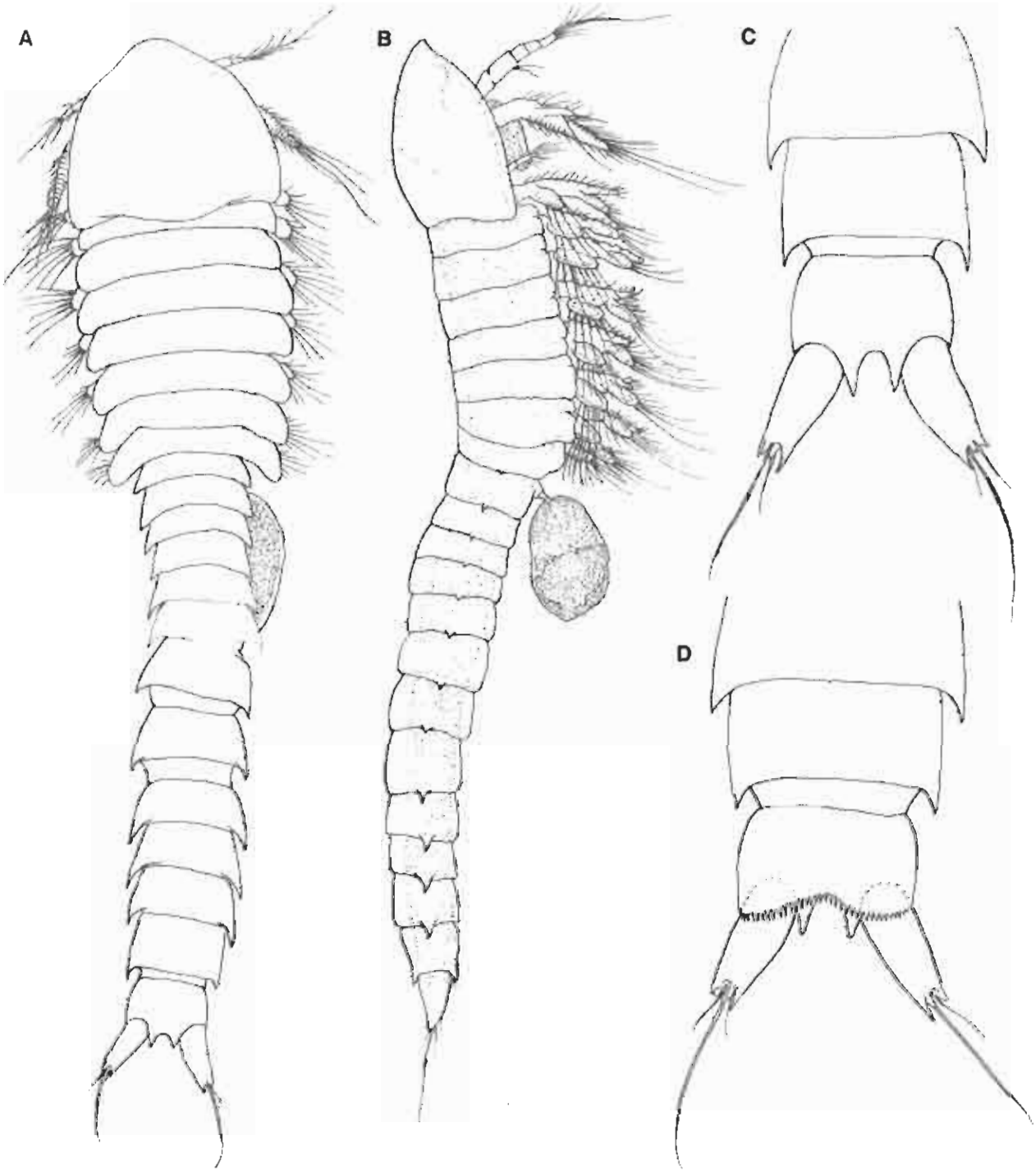

Figure 1. *Lightiella incisa* Gooding, 2.1 mm specimen from Long Point, Guana Island, British Virgin Islands, Caribbean (LACM CR 2001-005.1). A, entire animal, dorsal view. B, same, lateral view. C, posterior two somites, telson, and caudal rami, dorsal view. D, same, ventral view showing comb row of spine-like teeth on posteroventral border of telson.

cobble with scattered small coral heads, sponges, and soft corals. Further seaward the coral heads coalesce into reef. The bottom sediment is often covered with a thin layer of fine calcareous algae and flocculent matter, although this material was less prevalent in 2001 than in the two previous years of our survey. Other organisms sorted from the sand and gravel in the rock fissure (Station 12) included lancelets, polychaete worms, molluscs (chitons, bivalves, and gastropods), pycnogonids, and varied crustaceans (amphipods [including some that appear to be ingolfiellids], decapods, tanaidaceans, and ostracods).

Gooding's (1963) original four specimens of *L. incisa* (two from Barbados and two from Puerto Rico) were all from flocculent sediment within a *Thalassia* grass bed; three of the four were aspirated from decapod burrows. Similarly, De Troch et al. (2000) found large numbers of specimens "between the roots of sea grasses." Hessler and Sanders (1973:195) noted that "the single common feature of all cephalocarid habitats is the flocculent nature of the superficial sediment to which these animals are intimately bound by their basic mode of life." Schiemer and Ott (2001) recently shed additional light on the microhabitat of *L. incisa*, documenting its occurrence only below the redox potential discontinuity layer and with a maximum density at 12–15 cm below the surface at a shallow sand bar on Carrie Bow Cay, Belize. Schiemer and Ott (2001) suggested that *L. incisa* inhabits "oxygen-rich microzones" in deeper sediments. This was also suggested by De Troch et al. (2000), in their study of *L. incisa* from the Yucatan Peninsula. De Troch et al. (2000) concluded that *L. incisa* was "an endobenthic species occupying anoxic sediments oxygenated by bioturbation (e.g., Polychaeta) rather than being an animal living in the oxygenated top layers." Thus, its occurrence in anoxic flocculent sediments may be tied to the occurrence in these same sediments of polychaete worms or other burrowing organisms that provide limited oxygenation via their burrowing and ventilatory activities. Although we found our specimen among sand and gravel, it is possible that the gravel acts in a manner similar to turtle grass beds as a "sediment trap," collecting the flocculent material that in turn supports cephalocarids (see Sanders and Hessler 1964).

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Artificially induced group display and nesting behaviors in the reintroduced population of Caribbean flamingos *(Phoenicopterus ruber ruber)* on Guana Island, BVI

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Running title: Artificially induced group displays in Caribbean flamingos

ABSTRACT:

We used artificial social stimulation (decoys, vocalization playbacks, and artificial nests) to encourage breeding in a population of six Caribbean flamingos *(Phoenicopterus ruber ruber)* that had not successfully bred since their introduction to Guana Island in 1992. During a control period prior to the introduction of stimuli, flamingos exhibited no social displays or nest building activities. All flamingos were observed approaching the decoy area as a flock within a few hours after introduction of stimuli, and social displays were exhibited at a low rate by a few birds within the first 24 hours. In a twelve-hour watch conducted two-weeks post introduction of artificial stimuli there was a significantly greater number of group display behaviors, as well as nest-building behaviors, as compared with the control period and immediately after the introduction. The majority of groups displays were performed by two individuals (although at least one social display posture was observed for each bird) and three birds exhibited nest-building behaviors. Overall, individuals spent most of their time feeding and resting/sleeping during all observation period. We suggest that social attraction techniques may be a useful tool to stimulate breeding in captive and wild small populations of flamingos.

INTRODUCTION:

While their pre-Columbian distribution is not well known, Carribean flamingos *(Phoenicopterus ruber ruber)* historically occurred widely on islands and mainland shores in the Carribean (Sprunt, 1975). Flamingos were known to breed in the British Virgin Islands, particularly on Anegada Island where large numbers were recorded by European travelers in the 1800s (Lazell, in press). But the population quickly declined as the birds were hunted for food and by the 1950s no resident flamingos were observed (Colli 1996). Although flamingos are not historically documented at Guana Island, a salt pond does exist that could have provided foraging and nesting habitat.

In an attempt to reestablish Caribbean flamingos in the British Virgin Islands, eight birds from the Bermuda Zoo were brought to Guana Island, a wildlife sanctuary, in 1987 (Lazell, in press). By 1992, four of these birds (all pinioned) had died and the remaining four free-flying birds had flown off the island. In 1992, eight more birds were released on Guana Island and 18 birds were reintroduced to Anegada Island (Lazell, in press). Courtship behavior and nest building was observed in the Anegada population, but no chicks were observed until 1995 after four wild birds (possibly the four from original Guana Island introduction) had joined the Anegada flock. Although both populations appear to not be limited by food supply or excessive predation (Colli, 1996), only the Anegada population has successfully bred and has grown from 18 to 63 individuals (Lazell, in press).

The Guana population currently consists of six individuals, four males and two females ranging

from 9 to 21 years old. In the year following the 1992 release of birds, nest building activity was observed in the center of the salt pond, where an artificial island had been created. No egg laying occurred and no social displays or breeding activity has been observed in subsequent years. The island was subsequently removed.

Successful reproduction in flamingos may require a minimum flock size (Stevens & Pickett, 1994). The lack of breeding activity in the Guana population may be due to an inadequate colony size to stimulate breeding behavior. In captivity, a relationship has been found between behavioral stimulation from group displays and breeding success. Increasing the flock size at Zoo Atlanta from 17 birds to 21 birds played a role in increasing the frequency of display activity by 48% and synchronous group displays by 100%, which resulted in a doubling in the frequency of mounts and copulation events (Stevens, 1991). In colonial waterbirds, vocalizations are also important for attracting individuals to a breeding site (Kress, 1997). In captive flamingos, it has been shown that increases in group displays (which includes a vocalization component) stimulates breeding behavior and increases reproductive success (Stevens, 1991).

The success of restoration programs for colonially breeding birds depends on several factors, including food abundance, predation pressure, and reproductive success that in some species is dependent on social stimulation and a threshold population size. Methods to reestablish colonial waterbird colonies and artificially stimulate breeding and nesting activities using "social attraction techniques" were developed in the 1970's by Stephen Kress of the National Aubudon Society of the United States. Social attraction techniques involve the use of decoys and vocalization playbacks to artificially simulate a large breeding colony. This technique is

becoming an effective management tool for encouraging the recolonization of extirpated breeding colonies. The combination of decoys, mirrors, tape recordings of vocalizations, and in some cases, predator control, has led to led to restoration of Arctic (*Sterna paradisaea*), Common (*S. hirundo*) and Roseate (*S. dougallii*), Sandwich (S. sandvicensis), and Least Terns *(S. albifrons)*; Black Skimmers (*Rynchops niger*); Atlantic Puffins (*Fratercula arctica*); Leach's Storm-Petrels (*Oceanodroma leucorhoa*); Dark-rumped Petrels (*Pterodroma phaeopygia*); Laysan Albatross (*Diomedea immutabilis*); and Common Murre (*Uria aalge*) (Parker et al. 2000; Kress, 1997; Watanuki and Terasawa 1995; Schubel 1993; Podolsky and Kress 1991; Podolsky and Kress 1989; Podolsky 1985).

In the case of the terns, sightings doubled within the first year following introduction of stimuli and nest building behaviors were exhibited in the first and second years following the stimuli introduction. It wasn't until the third year that breeding occurred, but by the fifth year breeding occurred without the assistance of social attraction tools with a total of 424 nests from three species of terns (Kress, 1983). The common murre project had dramatic results with birds observed amongst the decoys a day after the introduction of stimuli and successful breeding occurred in the months that followed at a colony that had been inactive for 10 years (Parker et al., 2000). These active seabird restoration techniques are new tools that have the potential of supplementing traditional management techniques such as site acquisition and the protection of existing colonies (Kress and Strilich, 1993).

Some indication that artificial stimuli may enhance flamingo breeding comes from studies of both captive and wild flamingos. Large mirrors placed in the enclosure of captive Lesser

Flamingos *(Pheniconais minor)* resulted in an elevated rate of "marching displays" (a social group display) (Pickering and Duverge, 1992). In France, the construction of an island and artificial nest mounds attracted wild flamingos which had lost their nesting habitat nearby (Johnson, 1976). Four years after construction of the island, successful mating occurred and was initiated in the area with the artificial mounds.

In this study, we tested whether the introduction of artificial stimuli would induce group displays or any other reproductive behavior in the Guana Island population of Caribbean flamingos *(Phoenicopterus ruber ruber)*. The stimuli included the addition of decoys to simulate a larger population, broadcasting group display calls (Head-Flagging calls), and the addition of artificial nests and eggs. Behaviors of each bird were monitored prior to and after the introduction of the stimuli and analyzed to determine whether there was a measurable change in overall activities towards social displays and/or reproductive behavior.

METHODS:

This study was conducted at the salt pond (approximately 300 m by 150 m) on Guana Island, in the British Virgin Islands, over a three-week period during the month of July, 2001 when the island is primarily turned over to scientists to conduct various conservation related marine projects during Marine Science Month. The six flamingos were monitored for a 12-hour period over three days, so that all dawn to dusk hours were covered (from 7 a.m. to 7 p.m.), prior to the introduction of the artificial stimuli. All birds had numbered plastic leg bands allowing recognition of individuals. The behavior of each individual bird was documented, and assigned a behavioral code, every five minutes over the 12 hours by visual observations, using binoculars

and a 15-45x spotting scope, and by video recording, using a Sony Digital 8. Observations were conducted from the west end of the pond, the furthest distance from the north-east end of the pond, where the birds were known to spend the majority of their time.

Normal, everyday or "comfort movement" behaviors were categorized as feeding, preening, wing-flapping, wing-stretching, walking, resting, or sleeping (Kahl, 1975). Group or "ritualized" displays associated with breeding were categorized using terms and descriptions by Kahl (1975) and Studer-Thiersch (1975), as Marching, Head-Flagging, Wing-Salute, Twist-Preen, Wing-Leg Stretch and Inverted Wing-Salute, False-Feeding, and Broken-Neck. Vocalizations associated with behaviors were also documented. Courtship and nest building behaviors were categorized as outlined in Shannon, 2000. Courtship behavior is not as discrete as group display behaviors and involves a pairing off of a male and female, a female initiating copulation by stepping away from the group, the male following, and the female lowering her head into the water (False-Feeding) and spreading her wings. Nest building behaviors were noted when a bird either stood on a nest, used it's beak to fix a nest, or made contact with an artificial egg.

After the 12-hour baseline behavioral data were collected, 10 wooden flamingo decoys were placed near the shoreline of the south-east end of the pond to artificially increase the perceived population size, including seven decoys in Head-Flagging postures. Head-Flagging is the first in a series of group display postures that initiates subsequent group display postures (Kahl, 1975; Studer-Thiersch, 1975). A cluster of 8 artificially-constructed mud nests was built at the edge of the salt pond and 3 decoys in incubating postures and 5 artificial eggs were placed on various nests, an egg in each nest with the incubating decoys and 2 eggs on nests without decoy birds.

Head-Flagging calls were obtained from the Dallas Zoo and spliced together into a 30-minute recording that was burned onto a CD. The CD was broadcast in a loop for 12 hours a day after the introduction of the decoys using a Sony water resistant CD player, charged by a 12 V marine battery that was recharged once a week as needed.

Two additional observation periods were conducted: within 24 hours after the introduction of the artificial stimuli and two weeks later. For each observation period we calculated the percentage of scans devoted to social/ reproductive behaviors (groups display or "ritualized" movements as listed above, plus courtship and nesting) for each individual bird. A univariate repeated measures ANOVA and Tukey's multiple comparison procedure were applied to compare the rate of social/reproductive behaviors displayed by the birds between the three observation periods. This test was calculated in SAS, version 8.02 with observation period as a fixed factor and individual bird as a random factor and $\mu = 0.05$.

RESULTS:

The occurrence of social/ reproductive behaviors increased significantly following the introduction of artificial stimuli ($F_{2,10} = 5.80$, $P = 0.0212$). Tukey multiple comparisons indicated no difference between the observation periods before and immediately following the introduction of stimuli but a significant difference between both of these observation periods and the observation period two weeks later (Figure 1).

No social displays or reproductive behaviors were exhibited during the observation period prior

to the introduction of artificial stimuli. During the observation period immediately after stimuli introduction, three Head-flagging social displays were recorded for two birds. Two weeks after the stimuli introduction, a total of 31 social/reproductive behaviors were observed. During this last observation period, all but one individual exhibited social displays (including Head-Flagging, Wing-Salute, Twist-Preen, Wing-Leg Stretch and Inverted Wing-Salute) and three individuals engaged in nest-building activities. Two individuals (one male and one female) displayed more and investigated the nests more than the others (13 and 10 recorded social/ reproductive behaviors for these two birds, respectively).

During all observation periods, the birds spent the majority of their time feeding (between 55-68% of time), followed by sleeping or resting (between 15-27% of time), and preening (between 8-11% of time). Two weeks after stimuli introduction, 3.6% of time was spent in social/reproductive behaviors and were observed between the hours of 0700-1000 and 1600-1900.

DISCUSSION:

Sampling Rates and Relative Observations:

Although we chose a sampling regime of 5 minute blocks over 12 hour periods and the data were significant two weeks after the introduction of artificial stimuli, there were many more behaviors observed outside of the 5 minute blocks, particularly in the 12 hour period just after the introduction of the artificial stimuli. There were 17 group display behaviors observed overall in this period, 3 of which fell within the 5 minute blocks. In addition, upon analysis of video data, more group displays were observed in the dusk period just after our 12 hour watch ended,

indicating that there was a larger change in behavior in this period then represented by the way in which the data were prepared for analysis.

The Adaptive Role of Group Displays and Associated Vocalizations:

Key studies have demonstrated the importance of male vocalizations in priming female hormones for reproduction. Lehrman and Freidman (1969) demonstrated that vocal stimulation done without visual cues caused a doubling in size of ovarian follicles in ring doves. This phenomenon is also thought to be the case for parakeets (Ficken et al., 1960) and canaries (Warren & Hinde, 1961). In the little blue penguin (Waas, 1988) it was further demonstrated that crested penguin calls had no effect on their reproductive status, while male calls from their own species did. In royal penguins, the vocalizations from the colony as a whole facilitates sexual activities (Waas et al., 2000).

Flamingos perform mass, mixed-sex group displays thought to play a role in ensuring synchronous nesting and/or facilitating pair formation (Pickering & Duverge, 1992). The frequency of displays varies widely between individuals, unrelated to sex (Pickering & Duverge, 1992), a phenomenon we also observed in our population, which may be an indication that certain members of the flock play a key role in instigating group displays. One male in particular played a key role in initiating displays. This has also been observed in other Caribbean flamingo flocks (Shannon, 2000).

In flamingos, both sexes call during particular group displays, the Caribbean flamingo having two distinct vocalizations associated with group displays, Head-Flagging and Wind-Salute calls (Kahl,

1975). These vocalizations are most likely important to prime both sexes for reproduction. It is unclear what the relative role of the group display vocalizations and visual stimuli play in priming hormones in flamingos. It is interesting to note that the flamingos in this experiment orientated towards the decoys when preparing to display. The decoys were investigated on many occasions, and outside of display periods, at least three of the flamingos spent time amongst the decoys during nest building, resting and sleeping. The source of the vocalizations, on the other hand, was never investigated. It would be interesting to have a site devoted to vocalization playbacks, a site devoted to decoys and a third site containing both sources of stimulation to determine which stimuli was more important, if not both.

Field experimentation with social attraction techniques demonstrate the probable importance of the presence of decoys as a visual cue to land from a distance and in creating the appearance of a larger flock or colony. The relative importance of decoys versus vocalization playbacks is not completely understood yet.

Timing of Artificial Stimuli:

Although the Anegada population has bred between April and June (Jarecki, pers. comm., 2000), the typical breeding season for Carribean flamingos in captivity in North America occurs between May-August (Reo and O'Gara, 2001; Shannon, 1996), some clutches occurring in April and September, but rarely in other months (Shannon, 1996). Others report that breeding can occur at any time throughout the year in captivity, and may also breed twice in a year (Sedenko, 2001). The Anegada population has had two clutches in the past, one in April and one in July (Jarecki, pers. comm.) It was reasonable to expect, then, that the flamingo population on Guana Island

may respond to breeding queues during the month of July. Even though flamingos may not breed every year and breeding and nest building may depend on rainfall and its effect on food supply, we still expected that at least social group displays may be induced artificially at that time, regardless of whether any breeding behaviors. Although we observed nest building behavior in July, egg laying may not have been possible due to the other environmental factors necessary for the flamingos to breed later than normal.

Having demonstrated that artificial stimuli induce group displays and subsequent reproductive behavior (nest building) in Caribbean flamingos and cause a significant increase in these behaviors over time, in the future, we plan to conduct these experiments prior to the breeding period (March/April) in order to maximize the potential of breeding. Typically, group displays are initiated one month prior to breeding, where the displays escalate throughout the month, culminating in pairing and mating in the following month (Shannon, 2000). Follow up studies will then be conducted over the subsequent months to determine nesting success, clutch size, and population growth.

CONCLUSIONS:

Social attraction techniques have played an important role in the recstablishment of colonial nesting birds in the wild. Current population estimates of wild Carribean flamingos is in order of a few 100,000 but no simultaneous censuses have been conducted throughout its range and we do know that their distribution and numbers were historically larger (Johnson, 2000). While flamingos are no longer hunted in huge numbers as they were in the last century, loss of habitat and pollution is a major concern for a species that relies on the unique environment of pristine salt

ponds for foraging and breeding. Worldwide there are probably fewer than 30 major breeding sites for all 6 flamingo species (Conway, 2000). A recent conference on the conservation biology of flamingos indicated the importance of salt ponds: "flamingos are individually numerous, but colonially and reproductively, endangered in a world of changing landscapes and vanishing feeding and breeding sites." (Conway, 2000).

Our study demonstrates that the use of artificial stimuli could play an important role in flamingo reintroduction programs, and perhaps even stimulate reproduction in wild populations of flamingos whose numbers have been drastically reduced. This technique could also be useful in captive breeding programs where other measures have failed to help stimulate breeding.

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Figure legend:

Figure 1.) % Occurrence of group displays/reproductive behavior 12 hrs prior to, 12 hrs after, and two weeks after introduction of artificial stimuli. Tukey multiple comparisons test indicates a significant change in behavior two weeks after the introduction of artificial stimulation. Error bars represent standard error.

INTRODUCTION

The West Indian topshell *Cittarium pica* is a well-known littoral gastropod found along rocky shores in the Caribbean and north to Bermuda. Individual *C. pica* can attain sizes of >100 mm and are found primarily in the surf and pink zones on wave-splashed shores, including the eastern shores of Barbados (Lewis 1960), the Bahamas (Debrot 1990b), the Yucatan Peninsula in Mexico (Britton and Morton 1989), Columbia (Brattstrom 1980) and wave-exposed cliffs on Bimini (Voss and Voss 1960). Once abundant throughout the Caribbean and the southeastern Atlantic Ocean, it is present only as fossils in Florida. In Bermuda, the species became locally extinct in the early 1800s (Wingate 1989); however, topshells are reliably found after reintroduction efforts initiated in the 1980s (Wingate 1995).

Topshells are an intensively fished intertidal marine snail in the West Indies, second only to the queen conch *Strombus gigas* in economic importance among Caribbean gastropods (Randall 1964). However, little information exists on local or regional patterns of topshell distribution and abundance. Harvested by humans since prehistoric times (Debrot 1990b), locals in the British and U. S. Virgin Islands collect *C. pica* for use in "whelk" stew. In an attempt to prevent over-exploitation of the species, the harvest of topshells in the U. S. Virgin Islands (but not the British Virgin Islands), has been restricted to the months from October through March and limited to 1 gallon person^{-1} day^{-1} of shells at least 2.5 inches in maximum width. Despite these limitations, poaching of *C. pica* in the U.S.V.I. is extensive, and larger individuals are less abundant than they once were (R. Boulon, pers. comm.). This pattern of both fewer and smaller individuals is also true of the eastern Yucatan coast (Britton and Morton 1989).

Topshell populations unexploited by humans can differ in abundance and size distribution patterns between wave exposures. In the Exuma Cays, Bahamas, topshells were in greater densities but were generally smaller at wave-exposed sites than at wave-protected sites (Debrot 1990a). These patterns were attributed to more intense predation pressure on exposed shores from carnivorous gastropods that were themselves in greater densities at wave-exposed sites.

I initiated research at Guana Island to quantify patterns of size distribution and abundance of *C. pica* in an area of unregulated harvesting by humans, the British Virgin Islands. Specifically, I have sought to test hypotheses generated by the findings of Debrot (1987, 1990a, b) that topshell 1) densities and size distribution differ between wave exposures, 2) predator densities differ between wave exposures, and 3) shell morphology (*i. e.*, the ratio of shell width to height) differs between wave exposures.

METHODS

Guana Island is a small (340 ha), privately owned island and wildlife sanctuary located at the northeast end of Tortola Island in the central part of the British Virgin Islands (Fig. 1). The island is of ancient volcanic origin, with present-day shorelines that are mixtures of igneous extrusives, hardened lava and ash. Intertidal areas around the island vary from gradually sloping ledges bordering boulder fields and sand beaches to vertical walls descending steeply into the subtidal. Areas selected for transect sampling were generally continuous sloping ledges on either side of nearby sand beaches or, in the case of Long Point, on a relatively steep sloping ledge.

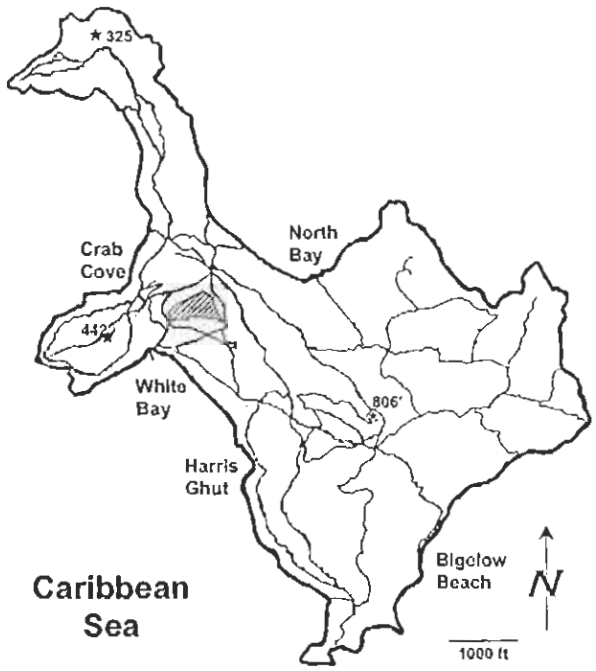

Fig. 1

Six sites were selected around the island to capture the range of conditions experienced in the intertidal zone. Long Point, North Bay, and Bigelow Beach are located on the northern and eastern shores of the island and are exposed to the prevailing easterly winds, while Harris Ghut, White Bay, and Crab Cove are located on the southern shores of the island and are largely protected from these winds (Fig. 1). Maximum wave-force dynamometers (Bell and Denny 1994) deployed in August 1999 verified these qualitative categorizations (Good, in review).

The tidal range in this part of the Caribbean is quite small; daily tidal amplitudes in White Bay were less than one meter (Good, unpubl. data). Topshells and the algae upon which they feed are thus confined to a narrow band around the perimeter of the island. The upper zone is typified by the barnacle *Chthamalus fragilis,* with some ribbed barnacles *Tetraclita squamosa stalactifera*. Erect green algae, mainly *Cladophora prolifera* and *Chaetomorpha aerea*, occur as tufts in this zone at the more exposed sites. The lower zone is typified by algal crusts, primarily *Lithothamnia* spp., at protected sites, while algal turfs of *Bryopsis plumosa* and *Caulerpa racemosa*, *Sargassum polyceratium*, *Padina gymnospora* and *Turbinaria turbinata and T. tricostata* , *Laurencia papillosa*, *Dictyota mertensii*, *Acanthophora spicifera* and *Amphiroa brasiliana* occur at the more exposed sites. Dense patches of large barnacles (*Balanus* sp.) are prevalent in the lower zone at Long Point, while crusts and corals dominate space in tidepools.

I quantified patterns of topshell abundance and distribution using three 10x1 m band transects at six sites on Guana Island in June/July, 2000. In all transects, I counted all topshells and predatory gastropods, including the wide-mouthed rock snail *Purpura patula*, Florida rock snail *Thais haemostoma floridana*, deltoid rock snail *T. deltoidea*, and rustic rock snail *T. rustica*. To determine topshell shape, I measured maximum shell width and height as defined by Debrot (1987) as well as spire height (as defined by the distance from the tip of the spire to the top of the aperture) of 25 individuals at all six sites on Guana Island. I also quantified the number of commensal limpets (*A. leucopleura*) on the underside of all topshells in transects at all sites to determine if a size-dependent degree of association of topshells with their commensal limpets varied with wave exposure.

For comparison, data on size distribution, abundance, and shell morphology were collected at two relatively protected sites on St. John, U.S.V.I. The first site was located on the southern shore in the Virgin Islands National Park. The site was on the western shore of Little Lameshur Bay, the bay just east of that studied by Randall (1964). The second site was located on the north shore, in a relatively inaccessible area but one situated just outside the Virgin Islands National Park. Both sites were around 25 km from Guana Island and possessed similar intertidal flora and

fauna to the sites studied there.

RESULTS

Topshell abundance varied with wave exposure on Guana Island. Mean density (# snails/10 m^2 transect ± 1 se) was significantly greater at protected sites (51.56 ± 6.55) than at exposed sites (31.67 ± 5.28) ($t_{15} = 2.13$, $p < 0.05$). Mean density at the south shore site on St. John, U.S.V.I. (55.67 ± 3.76) was similar to those found at protected sites on Guana Island; mean density at the north shore site on St. John, U.S.V.I. (88.67 ± 36.25) was greater than found at any sites on Guana Island.

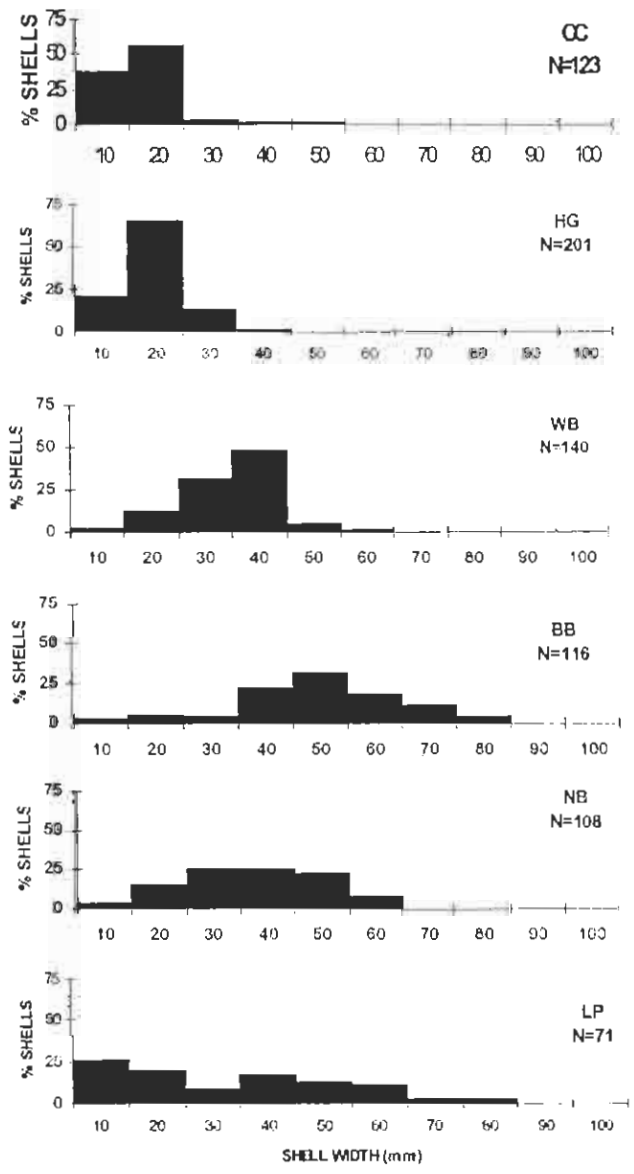

Fig. 2

Topshell size distributions varied among sites (Fig. 2), with the larger shells (>60 mm shell width) found exclusively at the three more exposed sites. Size distributions at the three exposed sites combined were skewed toward larger topshells as compared to the protected sites (Kolmogorov-Smirnov two-sample test, $p<0.001$). Mean shell width (mm ± se) was also greater at exposed sites (35.12 ± 0.80, n=411) than at protected sites (18.47 ± 0.45, n = 464) ($t_{873} = 18.71$, $p < 0.0001$). On St. John, U.S.V.I., mean shell width at the south shore site (13.58 ± 1.02; n=167) was generally similar to that at protected sites on Guana Island, while mean shell width at the north shore site (29.18 ± 1.01; n = 266) was generally similar to that found at exposed sites on Guana Island.

Predatory thaid gastropod densities were associated with wave exposure. Thaid densities (mean ± se /m^2) were greater at exposed sites (0.74 ± 0.13) than at protected sites (0.22 ± 0.12). Of these thaids, the *Purpura* rock snail had greater densities at exposed sites (0.6 ± 0.11) than at protected sites (0.03 ± 0.10) ($t_{25} = 3.8$, $p < 0.001$), while the three *Thais* rock snail species had similar densities at exposed sites (0.15 ± 0.06) and protected sites (0.19 ± 0.05) ($t_{25} = 0.45$, n.s.).

The ratio of width to height shell measurements did not vary between wave exposures (Fig. 3). The slope of regressions of shell height on shell width for exposed and protected sites were virtually 1.0, suggested equal growth in the height and width dimension. Spire height also varied allometrically with width and did not differ between exposed and protected sites.

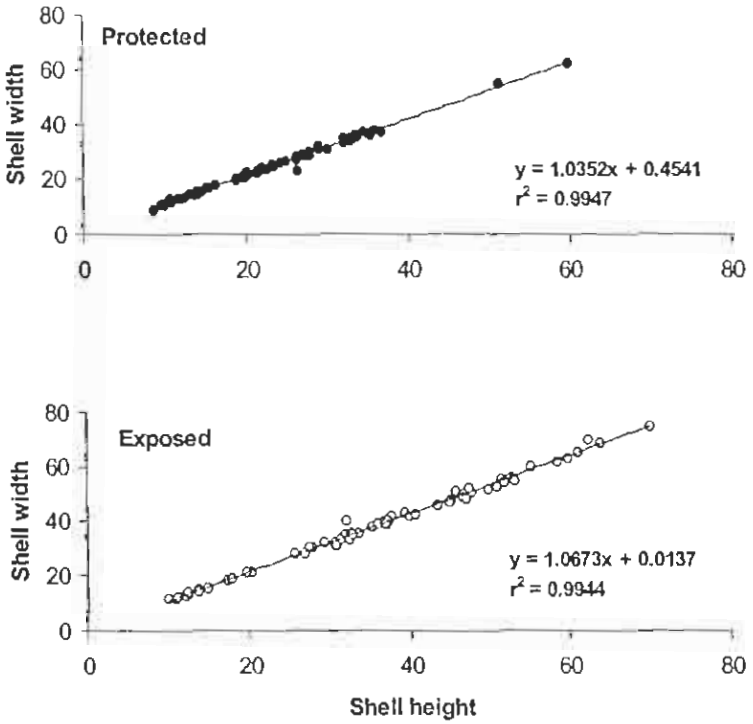

Fig. 3

The relationship of topshells with commensal limpets was variable across wave exposures (Fig. 4). The number of dwarf suck-on limpets on the underside of topshells generally increased with topshell width ($r^2 = 0.45$; $p < 0.0001$). The extent to which numbers of limpet commensals increased also differed between exposed and protected sites, with topshells at exposed sites having more limpet commensals than equally sized topshells at protected sites.

DISCUSSION

Topshell distribution and abundance patterns at Guana Island, BVI are markedly different from those found in the Exuma Cays, Bahamas, possibly reflecting differences in the extent of human predation in the two locations. In the Exuma Cays, where topshells are not harvested, topshells are more abundant but smaller at *exposed* sites, and this is attributed to pressure from predatory gastropods at exposed sites (Debrot 1990a,b). At Guana Island, topshells are more abundant but smaller at *protected* sites, even though predatory gastropods—*P. patula* and several *Thais* spp.--are more abundant at exposed sites, and larger individuals are almost exclusively at exposed sites. Topshell densities at protected sites on Guana Island were 50 times those at protected sites in the Exuma Cays, while densities at exposed sites were half of those at exposed sites in the Exuma Cays. Sampling at sites on St. John, U.S.V.I. revealed patterns supportive of this hypothesis. Topshells were abundant and large at the relatively exposed, inaccessible site on the north side and rare at the south shore site that was protected and had sandy beaches and calmer bays.

These data suggest that, in parts of the Caribbean where harvesting occurs, topshell density and size distribution can be influenced by accessibility and ease of human harvesting. Even on Guana Island, which is a wildlife refuge, there is evidence of collecting around the island, and day-workers have been known to collect bags of topshells to take back to Tortola. The impact of human predation of topshells should not be underestimated. Surveys of natural resources in the mid-1800s in Bermuda documented a lack of live West Indian Topshells, and they are thought to have gone locally extinct in the early 1800s. While speculation continues as to whether the

extinction was natural, climatic or human harvest-induced (Wingate 1995), fossil and historical records suggest topshells were resident and common on Bermuda in pre-colonial and colonial times. Recent re-introduction has boosted the local populations (despite renewed poaching), as well as renewed shell resources for the hermit crab *Coenobita clypeatus* that has become rare and localized due to the paucity of large shells on Bermuda (Wingate 1995). Human predation has influenced other intertidal gastropods in Panama (Ortega 1986) and Chile (Castilla 1999), and the exclusion of human predators had profound impacts on the entire intertidal community. Intensive poaching of topshells in the USVI (Boulon, 1987) suggests seasonal and take limits are not effective deterrent.

PROPOSED RESEARCH FOR 2002

For the 2002 field season, I plan to investigate more fully the ecology of West Indian Topshells at Guana Island, as well as to investigate patterns at nearby islands, where human impacts are ***more*** likely (Tortola, Virgin Gorda) and ***less*** likely (Salt I., Peter I., Norman I.). I plan to do this via 1) continued sampling of topshell distribution and abundance patterns at established monitoring sites on Guana Island, 2) initiating mark-recapture studies to determine growth and mortality patterns at established monitoring sites on Guana Island, 3) establishing long-term monitoring sites on Tortola, and 4) sampling topshell distribution and abundance on nearby islands.

The first two objectives will enhance my previous work on the ecology of West Indian Topshells on Guana Island by expanding the scope of the study from establishing patterns of distribution and abundance at sites varying in wave exposure. Determining patterns of growth and mortality of topshells *in situ* is important for determining the processes behind the patterns detected. On Guana Island, human impact may contribute to the observed patterns, however it is difficult to quantify. Tortola provides the closest likely sites for comparing sites likely to experience impact from human collection. My hypothesis is that topshell densities and size distribution will reflect human intervention, especially at protected sites, where land and sea access make collection easy. Discussion with local biologists will help focus the selection of study sites, and I have hopes that I will enlist those biologists to incorporate my studies into their curriculum or into a study project for an interested student. For example, sites with a) no road access, b) recent access, and c) extensive road access might be compared to determine the impact of human collection on the ecology of topshells. With no regulations concerning the taking of topshells in the BVI and few marine protected areas, a natural experiment of the kind I propose is the logical route to take. More quantitative studies with some thought to management of the topshells as a resource are rare, given the importance of this gastropod to local peoples.

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Research Report: Rachel Finley, URI

A Comprehensive Experiment of Ectoparasite Transmission in Reef Fish

Infectious diseases are a dominant force impacting the fitness and survival of individuals, in regulating host populations, and structuring communities. The primary focus of infectious disease studies has been on microparasites (viruses and bacteria, *sensu* Anderson & May 1979) and their impact on human health, or on crops and livestock essential to our livelihood (Scott & Dobson 1989); but more recently the focus has broadened to include the ecological impact of macroparasites (i.e. helminthes) on wildlife populations (Grenfell & Gulland 1995; Grenfell and Dobson 1995). Despite the recent interest in studying macroparasites in natural populations, surprisingly few studies look at parasites regulating abundance and distribution of host populations compared to parasitoids and predators (Crawley 1992). Parasites may not be addressed in ecological studies of natural populations because of difficulties in identifying infected hosts in the population, tracking individual hosts spatially and temporally, and manipulating parasite presence and burden. Specifically, techniques that manipulate parasite intensity and prevalence at the population level are confined to lab, or small-scale field studies, and may be restricting the scope of investigations of populations under natural conditions.

In many ecological studies, the impact of predation or competition can be observed by altering the presence, abundance or type of predator or competitor (e.g. Hixon & Carr 1997). In field host-parasite studies, it is often difficult to manipulate the presence or abundance of the parasite. Disinfection of ectoparasites can often be accomplished by hand removal of parasites on individuals (see Arendt 1985; Grutter & Pankhurst 2000 for examples of individual treatment), or using general disinfectants for larger groups. Fumigation of nests and colonies have successfully controlled parasites in bird studies (Brown & Brown 1996; Moss & Camin 1970); and it is common practice to use insecticides in aquaculture and fish farm operations to treat and control the spread of parasites (although these techniques are not applicable to wild fish). Treating endoparasites in wild animals often involves capturing infected hosts and administering anti-helminth drugs individually. This technique has been successfully performed in wild Red grouse populations (Hudson *et al.* 1998), and in free ranging Soay sheep (Gulland *et al.* 1993); however, in both cases the treatment only depressed parasite numbers and did not completely eliminate parasite burden.

Experimental transmission of parasites to healthy individuals is more difficult and rare. Often individual hosts must be anaesthetized and individually infected to establish the infection in a population (in mice Scott 1987, in fish Scott & Anderson 1984). Larger-scale infections (of tens to possibly hundreds of individuals) may be possible in certain systems. For example, Khan (1988) was able to infect groups of cod held in raceways with an ectoparasitic copepod by exposing the cod to lumpfish harboring the larval copepods. And Tompkins *et al.* (2000) were able to control and monitor introduced nematode infections in Grey partridge individuals raised in captivity then released in the wild. An advantage of experimental transmission is that the intensity of infection may be manipulated by varying the exposure time of the host to the infective stage of the parasite. Lemly and Esch (1984) controlled the intensity of trematode infection in bluegill sunfish by varying the time the fish were exposed to snails releasing infective cercaria. The above examples from lab studies provide evidence that it is possible to establish an infection in captive individuals; however, controlled experimental transmission *in situ* has, to our knowledge, yet to be tested

Experimental transmission and disinfection in previous field studies have not tried, or been able to, maintain controlled infections in wild populations in a manner that allows complex ecological questions about host-parasites relationships. Reef fish are often locally abundant, are sedentary or site specific, and do not migrate after settlement into the adult habitat (Sale 1991). Complex and novel ecological questions have been addressed using reef fish; however, parasites are often overlooked or avoided in field studies (Sale 1991; Caley *et al.* 1996). Reef fish and their parasites may offer a system that is easier to manipulate than terrestrial wildlife studies, and questions on how parasites influence host dynamics can be posed. The results of a pilot study conducted this summer offer promise that an ectoparasitic copepod–benthic reef fish relationship is ideal to study the impact parasites have in population dynamics; however, details in of the system need to be worked out before complex interactions can be studied.

A novel system to examine host-parasite relationships

The host-parasite relationship was studied near Guana Island (64° 35'W, 18° 29'N), British Virgin Islands. The bridled goby (*Coryphopterus glaucofraenum* Gill), is a small benthic fish that occurs throughout the Caribbean. A parasitic copepod, *Pharodes tortugensis* Wilson, infects the gill cavity of bridled gobies near Guana Island (R. Finley pers. obs.) and at least 4 other fish species elsewhere (Ho 1971). Gobies infected by *P. tortugensis* have a slower growth rate, reduced fecundity, and suffer from higher mortality than their unparasitized counterparts (Table 1; Figs. 1 & 2; Finley & Forrester *in review*). These results, however, are correlative and do not isolate parasitism as the causal factor creating differences between infected and healthy fish. Some fish may be more susceptible to parasitic infection because of poor health, genetic predisposition, or compromised resistance and would naturally suffer lower fitness regardless of the parasites (Gulland *et al.* 1993; Hudson & Dobson 1995). Isolating parasitism by *P. tortugensis* as the primary factor affecting growth, mortality and fecundity in *C. glaucofraenum* requires a controlled experimental infection of healthy gobies, with comparisons between the control and treatment group.

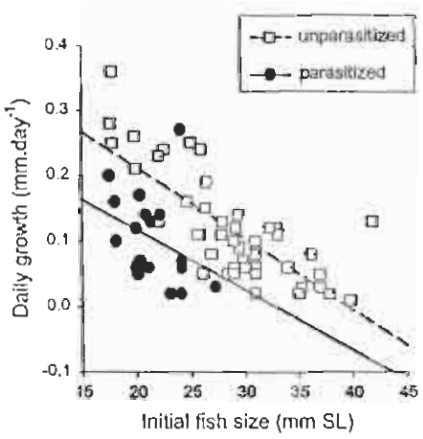

Fig. 1. Growth rates of tagged unparasitized and parasitized gobies recaptured on 2-4 August 2001. Growth rate was significantly faster in unparasitized than in parasitized gobies, and was dependent on the size of the fish at tagging.

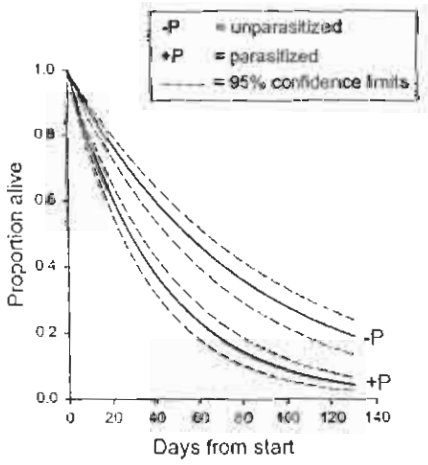

Fig. 2. Survival curves for gobies parasitized some or all of the time ($y = e^{-0.024x}$), and for gobies never parasitized ($y = e^{-0.013x}$).

The goby-copepod relationship may be ideal to study host parasite interactions and answer ecological questions on the impact of parasites to host populations for several reasons. *C. glaucofraenum* are benthic, sedentary fish that have a relatively short life span, allowing individual fish to be monitored for a large portion of their lives. Gobies infected with *P. tortugensis* have a swollen operculum and are easily recognized in the field. Furthermore, the fish and can be individually tagged so that the fate of parasitized and healthy fish can be followed (Malone *et al.* 1999). These fish have been used successfully in many other manipulative studies (e.g. {Forrester 1995,1999; Steele et al. 1998; Malone et al. 1999; Forrester & Steele 2000; Steele & Forrester in press) and are an excellent model organism to address questions that are of interest to ecologists.

Although the complete lifecycle of *P. tortugensis* is unknown, there is a general trend in parasitic copepods for a reduction in the number of naupliar stages, and to infect a fish host in the first copepodid stage {Kabata 1981}. No naupliar stages of *P. tortugensis* have been observed on the gills of infected fish (pers. obs.), and there is a range of sizes in the copepods found on the fish gills. The size of copepods (not including transformed females) ranged from 0.29 mm wide (cephalathorax width) by 0.34 mm long (cephalathorax and genital segment), to 0.91 mm wide by 1.04 mm long. It is very likely that *P. tortugensis* becomes infective after metamorphosis from the naupliar to the copepodid stage, then progresses through the rest of the copepodid molts and into adulthood as a resident on the fish (see Kabata 1981 for examples of other parasitic copepods). Parasitized *C. glaucofraenum* are aggregated in distribution within the population (R = 0.90; z = 2.65; Clark and Evans 1954 nearest neighbor method (Krebs 1999)). Therefore, there is a strong possibility that parasites are transmitted directly from parasitized gobies to uninfected gobies by a brief, dispersive, naupliar stage.

P. tortugensis, negatively affects the fitness of *C. glaucofraenum* and is found at a relatively high prevalence in the population (ranging from 2-19%): density and prevalence of *P. tortugensis* in the population of *C. glaucofraenum* around Guana may be correlated ($r = 0.62$, $p = 0.06$). Most infected fish were large juveniles and females (16-30 mm SL) with mean infection intensity of 7.02 copepods (range = 1-19). The frequency of infection intensity with host size was negative binomial distributed ($\chi^2 = 15.69$, df =16, n =118). Similar to other parasitic manipulation studies, we can remove parasites from infected fish by applying a general disinfectant to fish held in aquaria; but more interestingly, we may be able to infect healthy fish.

Table 1. Behavior and fecundity of parasitized and unparasitized gobies. Presented are means (± SD) and results of *t*-tests comparing the two groups.

	Parasitized	Unparasitized	*t*-test
Feeding (bites 30 s^{-1})	2.8 ± 2.7	4.5 ± 3.6	$df = 67$, $t = 2.208$, $p = 0.031$
Respiration (gill ventilations 30 s^{-1})	48.7 ± 14.1	29.4 ± 8.2	$df = 67$, $t = 6.938$, $p < 0.001$
Fecundity (gonad dry weight mg)	0.2 ± 0.1	0.63 ± 0.5	$df = 28$, $t = 4.065$, $p < 0.001$

***Experimental transmission of* P. tortugensis *to* C. glaucofraenum**

Identifying the transmission dynamics of the parasite, and how hosts become infected, is central to understanding the epidemiology of the parasite and the first step discovering how the parasite may be able to regulate host population dynamics. Aside from the well known Red grouse - nematode system studied by Hudson and colleagues (Hudson *et al.* 1992; Dobson & Hudson 1992; Hudson *et al.* 1998), field studies investigating the population level impacts of parasites to their hosts are rare: leading to a lacuna in the field of epidemiology for macroparasites in natural populations. A possible reason for the paucity of manipulative experiments in host-parasite studies may be due to the difficulty in establishing and maintaining infections in controlled conditions. The goals of this proposal are to:

Goal 1: Determine if a parasitic infection can be experimentally established in healthy fish.

Goal 2: Determine if experimental populations of healthy and parasitized *C. glaucofraenum* can be established and maintained in the field.

Goal 3: Compare experimentally and naturally parasitized fish.

Parasite Transmission Experiment

Experimental transmission of *P. tortugensis* to *C. glaucofraenum* will initially be tested in the laboratory. By performing the experiment under controlled conditions, we can monitor the individuals and look for changes in infection status on a finer time scale (observations made multiple times a day) than can be achieved in a field experiment alone (at best, observations made daily).

Parasitized and healthy fish 16-30 mm SL (the size range of fish commonly infected – Finley & Forrester *in review*) will be collected from reefs near Guana Island using hand nets and SCUBA, and housed in running seawater tanks on the island. Fish that appear to be healthy will be treated with CopperSafe® aquarium disinfectant for 5 days prior to the experiment to ensure that they are not harboring a low parasite burden or small, undetected copepods. Disinfection of *C. glaucofraenum* parasitized by *P. tortugensis* was successful with CopperSafe® in a pilot study. Only parasitized fish carrying gravid female copepod will be used in the treatment; parasitized fish carrying gravid copepods can easily be identified without harming the host. All fish will be tagged individually (using a visual implant tag, or a unique combination of paint injections – see Malone *et al.* 1999 for techniques) to track parasite acquisition in the healthy fish, and the status of copepod eggs on the parasitized fish (i.e. whether the copepods have hatched or are still in ova). While the fish are held in aquaria they will be fed *ad libum* daily either a commercial fish food or, when possible, microcrustaceans collected from the field.

Experimental treatment will be randomly assigned to 20 tanks: infection tanks will pair healthy fish with infected fish to facilitate parasite transmission, while control tanks will contain only healthy fish. There will be equal numbers of fish in each treatment tank. Each tank will receive its own water source, with seawater continuously flowing through the tank. Parasitized fish will be examined daily to determine when copepods are released and potentially infective; healthy fish will be examined daily for signs of infection. If a parasitized fish dies during the experiment it will be replaced by a similarly sized parasitized fish. Alternatively, if a healthy fish dies it will immediately be examined for signs of infection and will be replaced only if it *did not* contain parasites (e.g. parasite-induced death was not apparent). The experiment will be

terminated when half of the healthy fish (in the infection tanks) show signs of parasitic infection. Upon termination of the treatments, all fish will be dissected and inspected for parasites and the size and status (transformed female, or untransformed male or female) of copepods determined.

The results of the lab experiment will be interpreted as follows: If healthy fish become infected in the parasite treatment tanks, and no fish in the control treatment become infected, then direct transmission from parasitized to unparasitized fish is presumed. Alternatively, if uninfected fish in both the treatment and control tanks become infected then direct transmission and indirect transmission from a planktonic source could be possible. If healthy fish in either treatment fail to become infected, then the transmission route is unclear.

In addition to incorporating the results of the lab experimental transmission, the field experiment will assess the feasibility of establishing parasitized and control groups in the field for larger scale studies. The field experiment will have the same treatment arrangement as in the lab (treatment = healthy and parasitized fish; control = only healthy fish), but the fish will be stocked on artificial reefs constructed in sandy areas near Guana Island. Using a technique similar to Forrester (1995, 1999) we will create multiple patch reefs separated from each other and nearby reefs by at least 10 m of sand. The gobies will be stocked at densities higher than the average, but within the range normally found on the nearby reefs. As in the lab experiment, healthy fish will be disinfected with CopperSafe® and all fish will be tagged as individuals. Each patch reef will be inspected daily for mortality or disappearance of individuals, but fish will only be captured on a weekly basis to inspect for parasites. As fish can only be hand netted, and all work must be performed on SCUBA, it will be impossible to assess the infection status of individuals on a time scale shorter than weekly. The relative density of fish between treatments will be maintained: e.g. healthy fish will be added to control or treatment reefs if a substantial number disappear from a reef, and parasitized fish will be added if all disappear from a treatment reef.

The results of the field experiment will be interpreted as follows: the experiment will be ranked *successful for establishment and maintenance of parasitized and healthy goby populations* if the majority of fish on the control reefs remain parasite free and the majority of healthy fish on the treatment reefs contract parasites. The experiment will be ranked *successful for maintenance of healthy goby populations* if the majority of fish on control reefs remain parasite free even if healthy fish on treatment reefs remain uninfected. The experiment will be *ranked unsuccessful* if the majority of fish on control reefs become infected and fish on treatment reefs remain uninfected.

The final goal of this proposal is to compare "experimentally" infected fish with fish that have been "naturally" parasitized. Our previous study (Finley and Forrester in review) demonstrated that parasitized fish suffer a lower growth rate and reduced gonad mass than healthy fish; however, these results were only correlative and parasitism could not be isolated as the causal factor creating the observed differences. Groups of experimentally infected and naturally infected gobies will be tagged and followed for several weeks in the field and lab to determine if differences in the two groups are inherent characters of the fish or are explicitly a result of the parasitic infection. We may also be able to determine if some fish are more susceptible to parasitic infection or more likely to die as a consequence of the infection (e.g. test for a size/parasitic status interaction).

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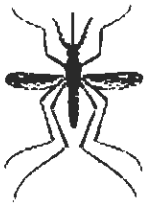
Student Participation in MSM 2001 – 2002

In our first-ever full-time program for students at MSM, four students from the H. Lavity Stoutt Community College joined the scientists on Guana for MSM 2001. These students helped with ongoing research projects and also conducted their own studies. Funding for student participation came from two "Research Experiences for Undergraduates" scholarships from the U.S. National Science Foundation; these grants were attached to the NSF-funded survey of small invertebrates headed by Jody Martin and Todd Zimmerman of the Los Angeles County Museum of Natural History. The BVI Government's Environmental Health Department provided an additional scholarship for one student to investigate salinity tolerance in mosquito larvae.

These students also received credit for their work on Guana as they were enrolled in the College's "Research in Biology" course, and two were given scuba diving certification courses prior to their work on Guana. The students' final reports follow; these were presented as posters in the BVI's 2002 National Science Fair.

In 2002, A Carib Indian student from a high school in Dominica assisted with all projects during the whole field season. Additionally, one of the scholarship students from the 2001 program returned briefly to assist with underwater research.

Salinity tolerance of mosquito larvae inhabiting mangrove wetlands in the British Virgin Islands: Can salinity-tolerant larvae be controlled using elevated salinity treatments?



Prepared for the

Environmental Health Department,

British Virgin Islands

By the

H. Lavity Stoutt Community College

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Shanie Dasrath and Lianna Jarecki

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INTRODUCTION

Mosquitoes are well-known pests of humans and other animals. In the British Virgin Islands (B.V.I.), mosquitoes are a major nuisance, and some species carry diseases like dengue, malaria, and yellow fever. Although most species of mosquitoes breed in fresh water, there are some whose larvae tolerate saline water. *Aedes taeniorhynchus*, a salt marsh mosquito, breeds in tidal estuaries and temporary saline pools. Although this species does not carry notorious diseases, it can become relentlessly irritating to people living near mangrove wetlands during rainy periods (see Appendix B). Each year, the B.V.I.'s Environmental Health Department spends considerable effort and funds to control populations of *A. taeniorhynchus*. Presently, the Environmental Health Department uses Malaria Oil to control the *A. taeniorhynchus* larvae. The oil sits on the surface and cuts off the air supply of the larvae. Malaria Oil is currently the best option for treating natural water bodies because it is very light and evaporates quickly, which minimizes it's effect on other wetland species. However, because of this evaporation, it has to be applied frequently. Several non-pest species require the same habitat as the mosquito larvae, and Malaria Oil tends to kill air-breathing invertebrates indiscriminately. Confounding these effects, Malaria Oil is often mixed with Diesel before treatment (to minimize expense), which causes far greater environmental harm than Malaria Oil alone. Diesel is extremely toxic. It kills aquatic animals and plants, including mangrove trees near the treated pools. Overall, the methods used to control *A. taeniorhynchus* in the B.V.I have been damaging to mangrove wetlands, which are valued for the numerous environmental functions they provide.

Because the BVI does not have tidal estuaries, salt marsh mosquitoes tend to use mangrove wetlands for breeding. Unfortunately, many people in the BVI view mangrove wetlands merely as mosquito-breeding habitats. Few understand the absolutely critical role that mangrove wetlands play in maintaining the BVI's coastal environment. Mangrove wetlands and their associated salt ponds are known

to protect coastal areas from storm damage, to mitigate flooding during rainstorms, to stabilize shorelines, to control erosion, and to retain nutrients, sediments, and pollutants that are damaging to marine habitats. Maintaining these functions requires that the ecological character of wetlands remains intact. Furthermore, most people do not distinguish between the shaded, temporary pools that develop within mangrove forests and the large, exposed salt ponds that are fringed by mangrove trees. Salt ponds, in general, do not host mosquito larvae. With only a couple exceptions (e.g. Josiah's Bay after heavy rains), salt ponds are far too saline and their sun exposure is too intense to support mosquito larvae. Instead, they host a productive community of salinity tolerant crustaceans, insects, plants, and microorganisms, none of which exist outside of the salt pond habitat or cause problems to humans (Jarecki, 1999). Today, mangrove wetlands and their associated salt ponds are the most threatened ecosystem in the BVI. Thus, a mosquito-control program directed at mangrove wetlands must be balanced against the environmentally damaging effects of the control program.

This study was commissioned by the Environmental Health Department to investigate whether controlling mosquitoes by elevating salinity of their habitats would a) be feasible and b) be potentially less damaging to the wetland environments than current control techniques are. Because the BVI has a long dry season, natural pools associated with mangroves become hypersaline (saltier than seawater) and often dry completely. Mosquito breeding therefore is a problem only during the rainy season. If we assume that the organisms associated with mangrove wetlands are adapted to naturally occurring increases in salinity during dry periods, then a mosquito control program that focused on increasing salinity beyond the tolerance limit of salinity of mosquitoes may allow other species to survive the treatment without harm. The exact limit of mosquito tolerance to hypersalinity in the BVI was heretofore unknown. *Aedes taeniorhynchus* is the only species in the Caribbean reported to have substantial mosquito tolerance, and it has been reported to survive salinities up to three times the salinity of seawater (up to 105 parts per thousand) in Florida (Nayar, 1985).

MATERIALS AND METHODS

This study took place between June 20th and July 31st, 2001. An initial survey of five known mangrove habitats for mosquitoes was conducted. Sampling on Tortola included Sleepy Hill/Sophie Bay (June 30th), Kingston/Brandywine Bay (June 30th), Cane Garden Bay (June 28th), and Josiahs Bay (July 4th); on Beef Island, Trellis Bay mangrove areas were sampled. Mosquito larvae from the Josiahs Bay pool were collected on July 4th and preserved in 70% ethanol. They were identified as *Culex bahamensis* using microscopic analysis and a key to Culicidae (Walker and Newson, 1996). Entomologist Caitlin O'Connell-Rodwell, Ph.D., who was visiting the BVI from Stanford University, verified identifications.

A source of *Aedes taeniorhynchus* was later found in a man-made ditch of 31ppt, on Guana Island. The ditch was dug recently to replace a water pipe, and it was later buried. It was located in a mangrove area between the beach and the salt pond on Guana. Mosquitoes collected in this ditch were identified and verified in the same manner as described above. Preserved samples of both species were given to Mr. Minche Israel, of the Environmental Health Department, who came to Guana to view the project during July.

A. taeniorhynchus is known to lay its eggs on the sand/mud shore of saline pools rather than directly into the water (Nayar, 1985). Since mosquito larvae were found only in small, shaded mangrove pools and not in their associated salt ponds, it was hypothesized that either the mosquitoes were not laying eggs along the shores of salt ponds or the larvae were dying in the salt ponds shortly after hatching. A test was designed to distinguish between these alternative hypotheses. If mosquitoes were laying eggs both at the shaded saline pools, where the larvae were found, and at the shores of the main salt ponds of Josiahs Bay and Guana, then surface samples taken from near the pools and the salt ponds should yield larvae under conditions that initiate hatching. Hatching can be induced by immersion of sediments containing mosquito eggs in fresh or low-salinity water. Ten replicate surface scrapings were taken from Guana's pond

and from Josiahs Bay pond. Two replicate control scrapings were taken from the mosquito ditch on Guana and the mosquito pool at Josiahs. Scrapings were put in 300ml deli-tubs filled halfway with tap water, covered and left in a shaded area for one week. They were checked daily for the presence of hatched larvae.

A second experiment was designed to test the salinity tolerance of both *Culex bahamensis* and *Aedes taeniorhynchus*. Experimental salinities were 31 (control) 50, 75, and 100ppt. These were prepared by filtering several buckets-full of water taken from the mosquito pool at Josiahs Bay (for *C. bahamensis* tests) and from Guana's mosquito ditch (for *A. taeniorhynchus* tests). A 5µm funnel-filter was used. Filtered water was evaporated by boiling to create 5 liters of a 200ppt salinity stock solution. This stock solution was then fractionally diluted with tap water to achieve the experimental salinities. Ten replicate deli-tubs (300ml) were set up with 200ml of test water for each experimental salinity, totaling 40 tubs in all.

Mosquito larvae were captured with a fine hand-net and transported in a large bucket (to prevent overcrowding) to a nearby building, when using Guana's ditch larvae, and to the College, when using Josiahs Bay pool larvae, where the experiment was set up. Ten healthy larvae were transferred arbitrarily to each experimental tub using an extra-wide mouthed eyedropper. Tubs were covered and left in the shade for 24 hours. No salinity acclimation was performed because the experimental design was intended to simulate treatment with super-saline water in a mosquito-control program. After 24 hours, each tub was opened and the live and dead larvae were counted. The LD_{50} criteria were used to determine toxic effects of salinity (in Kitchell, 1998, pg. 191). Tubs in which 50% or more of the larvae were dead were considered to contain lethal concentrations of salt. This study was repeated using the pupae of *C. bahamensis*.

In the third part of this study we sought to estimate some factors related to the feasibility of controlling mosquitoes by adding supersaline water to their breeding pools. A formula was developed to determine how much supersaline water would be needed to achieve salinities lethal to both *C. bahamensis* and *A. taeniorhynchus* based on the size, depth, and salinity of a naturally-occurring pool. The final

experiment was designed to determine how long a small pool might maintain hypersalinity after treatment. Three holes 1m in diameter by 1m in depth were dug at the shore of Guana's salt pond near the mangrove area. Two were filled ¾ full with 80ppt water from the salt pond. The last hole was filled ¾ full with 35ppt water from the sea. These artificial pools were each about 1/3 the size of the mosquito pool found at Josiahs Bay. The salinity of each artificial pool was measured once per day with a hand-held salinity refractometer. During this time, the area experienced several rain showers, particularly on the second day of the experiment. It was predicted that, over time, the artificial pools containing hypersaline water would be diluted by groundwater.

RESULTS AND DISCUSSION

Survey of mosquito breeding habitats

Natural saline pools supporting mosquito larvae were difficult to find as the study took place in the middle of the BVI's dry season. A survey of four mangrove wetlands on Tortola, one on Beef Island, and one on Guana Island, turned up only two populations of mosquito larvae. These were both found in small shaded pools within the mangrove forests, not in the main salt ponds associated with the mangrove wetlands. One population was found in a small pool at 27ppt salinity among a patch of red mangroves at the seaward end of Josiahs Bay wetland. This population consisted solely of *Culex bahamensis*. The main salt pond of Josiahs Bay was completely dry at this time, but a second pool, lying just behind the mosquito pool and looking, for all intents and purposes, exactly the same (shaded, small, shallow), had a salinity over 100 ppt! In this pond, of course, no mosquito larvae were detected. Perhaps the pool closest to the sea is fed by an underground spring that maintains low salinity and supports mosquito larvae. The second population was found in a man-made ditch among white mangroves between the beach and the salt pond at Guana Island, and this population consisted solely of *Aedes taeniorhynchus*. The salinity in this ditch

was 31ppt. No mosquito larvae were found in Guana's nearby salt pond, which had a salinity of 80ppt at the time of sampling.

Scrapings of surface sediments at the edge of Josiahs Bay salt pond and Guana's salt pond contained no mosquito eggs, as determined by a hatching experiment. Control sites—the edge of the mosquito pool at Josiahs and the ditch at Guana were also tested, but only scrapings from the pool at Josiahs hatched mosquito larvae. This experiment was designed to explain the lack of mosquito larvae in salt ponds near the mangrove pools in which mosquitoes were found. The result supported the hypothesis that adult females actively avoid laying eggs at the edges of salt ponds. However, because the scrapings at Guana's ditch were also negative for hatched larvae, the result is not entirely conclusive. The negative result at this site could have been due to the sampling method in that scrapings were taken at the top of the ditch rather than directly adjacent to the water. The artificial nature of this ditch and its steep sides were very different from the Josiahs Bay pool, which did yield hatched mosquito larvae when scrapings were immersed in low-salinity water.

Nevertheless, this result is supported by the findings of a salt pond ecosystem study undertaken by the first author (Jarecki). In 150 samples, taken from 20 different BVI salt ponds since 1995, only three samples had mosquito larvae. These larvae were present only when pond salinities were 40ppt or below, which occurs only in a few ponds after very heavy rains.

Together, these results indicate that mosquitoes generally do not use salt ponds as a breeding habitat. Instead, they show a great preference for the smaller, shaded, and lower salinity pools that form in depressions among mangrove trees during the rainy season.

Salinity tolerance of *Culex bahamensis* and *Aedes taeniorhynchus*

Culex bahamensis larvae were thriving in a natural pool of 27ppt. When exposed to higher salinities, however, they quickly died. Not a single larva survived the lowest test salinity of 50ppt though all survived the control salinity of 27ppt. Absolute salinity tolerance of this species in Florida was shown to be

42ppt (REF!!), but it probably doesn't occur naturally above 35ppt. Prior to this study, this species was not recorded to occur in saline wetlands of the BVI (Israel, personal communication). Thus, that *C. bahamensis* not only colonizes natural saline pools but can also survive at salinity near that of seawater is a significant finding. An expanded survey of wetlands in the BVI is needed to determine the extent to which *C. bahamensis* utilizes naturally occurring saline pools for its reproduction.

A test using a smaller number of *C. bahamensis* pupae showed that pupae were far more tolerant of increased salinity than were larvae. Unlike larvae, mosquito pupae are covered by a hard, impervious exoskeleton, which protects them from some environmental hazards. The pupae in this experiment survived 24-hour exposure to 50ppt salinity treatment, and a few of them even survived 75ppt treatment. The conclusion that mosquito pupae are far more resistant to salinity increases was assumed to hold for all mosquito species, and, therefore, this experiment was not repeated for *A. taeniorhynchus*. In light of this result, mosquito control using elevated salinity treatments is likely to be ineffective against pupae.

Aedes taeniorhynchus, on the other hand survived when exposed to increased salinities (see Table 1 through Table 4.) All individuals in the control group survived the 24-hour experiment. However, increasing numbers of larvae died with increasing salinity. Means and standard deviations of survival in each treatment are shown in figure 1. Using LD_{50} criteria, the limit of salinity tolerance for *A. taeniorhynchus* was 50ppt, because more than 50% of the larvae in most replicates were dead after 24 hours. At 75ppt, there was even greater mortality, but even at this salinity, three of the ten replicates showed more than 50% survival. In the 100ppt test, none of the replicates showed more than 50% survival. However, a few individuals remained alive in all of the highest salinity treatments. Interestingly, at 100ppt, several of the larvae pupated over the 24-hour experimental period. As discussed previously, pupae were found to be more resistant than larvae to elevated salinity. Perhaps the intense salinity in the highest treatment induced the oldest larvae to pupate in order to avoid the lethal effects of high salinity. It is unlikely that there were more larvae near the age of pupation in this treatment than in the others because

all of the larvae came from the same population, were collected at the same time, and were distributed arbitrarily into experimental tubs. Pupation of *A. taeniorhynchus* occurred at a rate of 6% ± 7% in the 100ppt treatments and never occurred at the lower salinity treatments. Half of the 100ppt replicate treatments showed pupation; no pupae were recorded from the other half. The increased incidence of pupation at high salinities has not been previously reported and warrants further investigation with larger sample sizes.

Although this study reported a generally lower salinity tolerance for *A. taeniorhynchus* than previous studies, it also showed that a small number of individuals might tolerate 100ppt, the upper limit reported by Nayar (1985). However, the fact that this species can survive moderate salinities does not mean that it will successfully grow through all of its larval and pupal stages to reach adulthood. Often when a species lives near the limit of its survival tolerance it fails to grow normally and may not reach adulthood. For mosquito control, elevating mangrove pool salinity to 50ppt would kill more than half of *A. taeniorhynchus* larvae and all of *C. bahamensis* larvae.

Tables 1 – 4: Results of the *Aedes taeniorhynchus* tests of survival at ambient salinity (31ppt) and elevated salinities of 50, 75, and 100ppt.

Table 1: CONTROL AT 31 PPT

control	Alive	Dead
1	10	0
2	10	0
3	10	0
4	10	0
5	10	0
6	10	0
7	10	0
8	10	0
9	10	0
10	10	0

Table 2: 50 PPT TREATMENT

Treatment 1	Alive	Dead
1	3	7
2	3	7
3	4	6
4	3	7
5	3	5
6	5	5
7	1	9
8	3	7
9	1	9
10	3	7

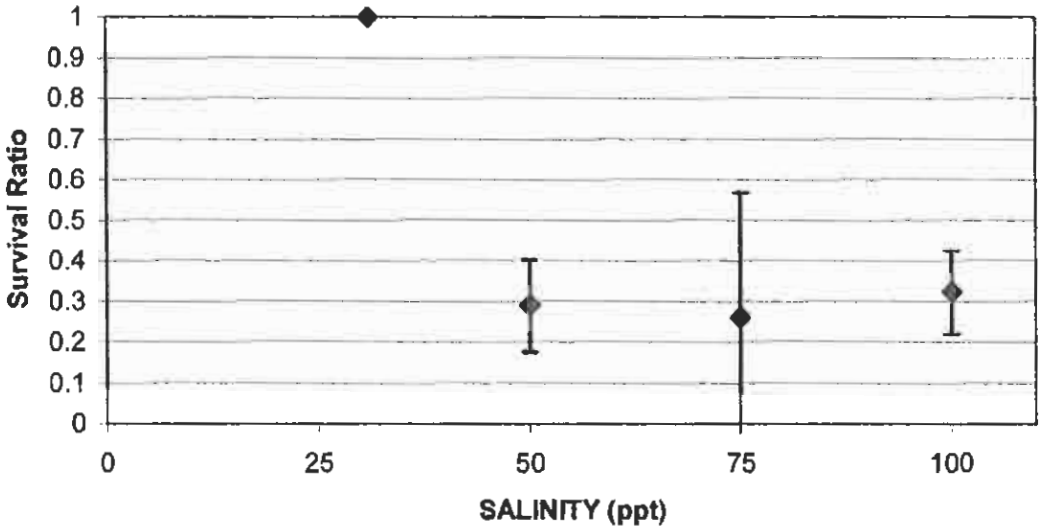
Table 3: 75 PPT TREATMENT

Treatment 2	Alive	Dead
1	1	9
2	1	9
3	0	10
4	0	10
5	1	9
6	2	8
7	9	1
8	0	10
9	7	3
10	5	5

Table 4: 100 PPT TREATMENT

Replicate	Alive	Dead	Pupae hatched
1	5	4	1
2	3	7	0
3	3	6	1
4	3	6	1
5	2	7	1
6	2	8	0
7	5	3	2
8	3	7	0
9	0	10	0
10	3	7	0

Firgure 1: Survival of *Aedes taeniorhynchus* in elevated salinity treatments



Maintaining elevated salinity in small pools

This part of the study was aimed at answering two questions: 1) how much hypersaline water, of what concentration, is needed to treat a small (potentially mosquito-infested) saline pool in order to achieve a salinity that will be lethal to more than half of the mosquito larvae (as determined by the LD_{50} criteria reported above), and 2) how long will the resulting hypersalinity of treated water be maintained without further treatment?

The first question required a mathematical approach to calculate the volume of a typical mangrove pool, take into account its salinity, and predict the volume of hypersaline water needed to elevate its salinity to 50ppt. 50ppt was considered toxic to mosquito larvae as discussed previously. The salinity of treatment water was designated as 200ppt. This level of salinity was chosen because it is well below the level of

NaCl crystallization (300ppt) and therefore can be produced reliably by boiling or other means of evaporation, and it is high enough to minimize the amount of hypersaline water needed to treat a small pool.

As an example, we used the mosquito pool at Josiahs Bay to represent a typical small pool that may be treated to control mosquito larvae. The size and volume of this pool was assumed to be three times that of our experimental treatment holes, which are described later. These holes had a known diameter (1m) and depth (1m). Their volume was calculated using integral mathematics, which is shown in Appendix A. Through these calculations, the volume of the mosquito pool at Josiahs Bay was estimated to be 117.780 liters. The salinity of that pool at the time of sampling in early July was 27ppt. Further calculations showed that, in order to raise the pool's salinity from 27ppt to 50ppt, it would be necessary to add to the pool a total of 29.4 liters of 200ppt hypersaline treatment water. These calculations are also shown in Appendix A.

For fear of environmental damage exceeding the effects on mosquitoes, this experiment was not carried to its next logical next step--to actually treat the natural pool with 29 liters of 200ppt water, confirm that 50ppt salinity was achieved, monitor how long hypersalinity was maintained, and measure the rate of survival of mosquito larvae. It is important to note here and observation that was made earlier in this report—that the Josiahs Bay pool may be fed by an underground spring. If this is indeed the case, then hypersalinity after treatment may be diluted within a few days. Furthermore, the size of the pool at Josiahs Bay was quite small and it was observed to fluctuate, getting smaller during dry periods and bigger after rains. During the rainy season, then, it is likely to be substantially larger than it was during July, and thus a greater amount of treatment water—perhaps 2 or 3 times as much—would be required to elevate salinity to the desired level.

The results of the experiment to test the second question, regarding the longevity of the effect of a single treatment, are shown in Table 4. Salinity of all three experimental 1m x 1m holes dug near Guana's

salt pond increased in salinity over the four-day experiment. The two experimental holes, which were originally filled with hypersaline water of 80ppt, increased to 100ppt and 85ppt, despite several rain showers that decreased salinity on day 2. The control, which was originally filled with seawater, increased to 57ppt by the end of the experiment. It was expected that hypersaline water in pools would be diluted by groundwater flow through the pores in the bottom sediments. However, this did not appear to be the case during July. Evaporation of water in the pools was high and tended to concentrate the elevated-salinity water faster than seawater. In a period of 29.5-hours, one of the experimental pools increased by 40ppt, a 140% increase in salinity. This pattern of evaporation and salinity concentration is typical of salt ponds as well. Salt ponds typically increase their salinity by about 4x concentration in the dry season as compared to the wet season.

Because the artificial pools were placed near the salt pond edge, the observed rate of evaporation and concentration is probably more typical of an open, unshaded pond than it is of the mosquito pools that occur under the mangrove canopy. Because of the difficulty digging holes among the mangrove roots, it was, unfortunately, not possible to conduct this experiment under the mangrove canopy. The observed results would vary by several factors, including the degree of shading, which would limit solar evaporation, the porosity of the soil, which would limit the retention of the hypersaline water put into the pool, proximity to the sea, which would allow seawater to percolate through the ground and perhaps dilute the hypersaline treatment water, and by rainfall, which can not only dilute the pools directly but can also percolate through the ground and dilute the pools indirectly. In particular, we expect these results to be quite different during the rainy season, which is the most important time for mosquito control. Nevertheless, this experiment shows that hypersaline conditions can be maintained after initial treatment in a typical salt pond/mangrove habitat as long as little rain falls.

Table 5: Daily salinities of experimental pools

Date (2001)	*Time*	*Pool 1*	*Pool 2*	*Pool 3 (control)*
23 July	11:00 am	80 ppt	80 ppt	35 ppt
24 July	11:10 am	86 ppt	76 ppt	41 ppt
25 July	8:55 am	60 ppt	74 ppt	42 ppt
27 July	2:20 pm	100 ppt	85 ppt	57 ppt

Environmental effects of elevated salinity treatments in mangrove pools

As described in the introduction, mangrove wetlands are critically important ecosystems in the BVI, particularly in their capacity to buffer storm damage, which protects real estate, and in their capacity to filter of runoff water from land, which protects water quality in coastal marine environments such as seagrass beds and coral reefs. It is, therefore, important to understand the environmental implication of mosquito control methods to be applied in mangrove wetlands. Effects of such treatment are extremely difficult to quantify as they may affect many parts of the complex mangrove ecosystem in indirect ways. However, what we know of mangrove biology in general and of BVI salt pond ecosystems in particular can be applied to make some general predictions about the potentially negative effects of elevated salinity treatment in mangrove wetlands. Environmental managers, then, must weigh these effects against those of alternative treatments, such as malaria oil, and they must balance environmental protection with the feasibility and cost of alternative treatments.

A few aquatic organisms are uniquely adapted to high salinity. These species are typically found living in the natural hypersaline ponds living in the BVI (see Jarecki 1991). Some of them also occur in saline pools within the mangrove forest. However, the mangrove forest hosts a great variety of species that are not tolerant of salinities much higher than seawater. Nevertheless, saline mangrove pools in BVI mangrove forests probably do experience salinities in excess 50ppt fairly regularly during dry periods.

Because these pools tend to be small, salinity-intolerant species may move elsewhere in the forest or may enter a dormant stage in their life cycle until rains dilute the pool waters.

Mangrove trees, however, are particularly sensitive to salinity changes. They can neither move nor enter dormancy. Under extended periods of hypersalinity, mangroves will certainly suffer, and some may die. Because the mangrove trees form the foundation of the wetland ecosystem, any damage to mangroves will affect other organisms in the mangrove community, including insects, crabs, and birds.

There are three species of mangroves commonly found in mangrove wetlands. Of these, the red mangrove is least tolerant of hypersalinity. Red mangrove grows well in waterlogged soil, with its roots immersed in seawater. Where salinities are generally higher than seawater, the black mangrove replaces red mangrove. The black mangrove's tolerance for hypersalinity is higher than that of red mangrove, and it too will thrive in waterlogged soil. White mangrove, on the other hand, may be tolerant of saline soil but it will not survive if soil is water-saturated for extended periods (more than a few weeks).

The sensitivity of white mangrove to 'drowning' in waterlogged soil illustrates the need to minimize the amount of hypersaline water used to treat mosquito pools. Thus, a small amount of highly concentrated brine is preferable to a large amount of moderately concentrated brine, as long as it is added directly to the pool water and not spilled on soil or mangrove roots.

Depending on the type of mangrove found around a mosquito pool, elevated salinity will have varying effects. It will effect red-mangrove the most severely. However, even red mangrove will tolerate short –term exposure to salinities just above that of seawater. However, if the same pool were treated several times, salts would accumulate in the soil by evaporation. This accumulation could severely damage mangroves from the indirect effects of salt accumulation. This points to the need for moderation when treating mangrove areas—unlike malaria oil, salts will not evaporate or go away. They will stay in the system until very heavy rains (which are infrequent and unpredictable) wash them into the sea.

The potential for environmental damage of elevated salinity treatment, then, is really a matter of scale, just as the person who smokes two cigarettes a month is likely to remain fairly healthy compared to the one who smokes two cigarettes a day. If elevated salinity treatment is directed at a few small pools within mangrove wetlands, and treatment is limited to three or four times per year, environmental effects will be minimal. In comparison to the current technique of introducing a malaria oil/diesel fuel mix to mangrove wetlands, this would be a grand improvement. As with any new technique, however, a detailed monitoring study should be conducted in concert if elevated salinity control is to be tested in the field. This should include parameters such as mangrove health (i.e. leaf abscission, yellowing, insect attack, or any other sign of weakness), size of the treated pool over time, salinity of the pool, salinity and water content of surrounding mangrove soil, and the species complement of aquatic biota in the treated pools.

CONCLUSIONS

The results of this study give some important insights for forming a strategy for and evaluating the feasibility of a novel approach to controlling mosquitoes in that breed in mangrove wetlands in the BVI. This approach, if pursued, involves treating natural saline pools in which mosquito larvae occur with concentrated seawater in order to elevate salinity beyond the tolerance of the mosquito larvae.

Only small, shaded low-salinity pools containing mosquito larvae should be targeted for treatment. These pools are rare during the BVI's long dry season since they only form as the result of rain collecting in depressions, usually in mangrove wetlands. Salt ponds, which are larger and more saline, generally do not support mosquito larvae and should not be treated.

Two species of mosquitoes—*Aedes taeniorhynchus* and *Culex bahamensis*— use mangrove pools during the larval stages of their life cycle. Both species live at salinities lower than seawater. We showed that an elevated salinity concentration of 50ppt was toxic to more than 50% of *A. taeniorhynchus* larvae and to 100% of *C. bahamensis* larvae within 24 hours of continuous exposure. Our results did not, however

refute published accounts that *A. taeniorhynchus* could tolerate salinities up to 100ppt (Nayar 1985), as a very few individuals did survive this extreme salinity for 24 hours. Moreover, 6% of the larvae exposed to 100ppt salinity pupated within 24 hours. Pupae were found to be more tolerant of high salinity treatments than were larvae, and the pupation of A. taeniorhynchus at extreme salinity may be a survival mechanism. Despite their documented survival in hypersaline water (above seawater salinity), neither species has been recorded to occur naturally in the BVI at salinities above 40ppt. Adult females probably show a preference for laying eggs near pools of low salinity, and moderate salinities, while not killing the larvae outright, may stunt their growth and prevent further development.

We suggest that 50ppt is an adequate salinity to control both species of salinity-tolerant mosquitoes. However, too achieve this, a large amount of supersaline water needs to be added to natural mosquito pools. For example, a very small pool of about 3m in diameter, at 27ppt salinity would require nearly 30 liters of a 200ppt concentration of brine in order to bring its salinity to 50ppt. As long as rains or underground springs do not dilute the treated water, hypersaline conditions would remain or even increase by evaporation. In the rainy season, when mosquitoes are most abundant, rain and runoff water would dilute the treated pools.

Introducing supersaline water into mangrove wetlands has potentially devastating effects on mangrove trees, which form the base of the wetland ecosystems. Hypersalinity can stress or, in the worst case, kill mangroves directly; it can drown mangrove roots; and it can lead to salt accumulation in the wetland soil. To minimize the effect on mangroves and consequently the indirect effects on the entire wetland, mosquito pools should be treated infrequently—not more than twice per year. Extreme care should be taken so that supersaline water is only applied to standing water and not onto mangrove roots or soil. Finally, salinity should only be elevated to 50ppt and not beyond. This will require careful calculation, taking into account the initial size of each mangrove pool to be treated and the salinity of its water at the time of treatment. Salinities in mangrove pools change naturally with changes in rainfall and thus must be

determined just before to each treatment. Finally, a detailed long-term monitoring study should be conducted alongside any application of hypersaline water to a natural habitat for the purpose of controlling mosquito populations

Controlling mosquito larvae by elevating the salinity of natural pools could be both effective and feasible for infrequent use on small pools. But it is unlikely to be feasible or safe for use on large saline pools. Great care must be taken to minimize environmental damage to the BVI's crucially important mangrove wetlands, and therefore long intervals of several months must be allowed between treatments to allow natural recovery of the soils.

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GUANA ISLAND PROJECT

Defining the Bottom Types of White Bay as well as Sampling the Invertebrate Organisms Existing in these Regions

Introduction- Intermittently over the past decade, scientists have visited Guana Island in search of knowledge and answers, all in the name of scientific study. During that period of time, White Beach has been a common place at which the varying invertebrate organisms have been collected and identified. The species collected range from rare to quite familiar organisms. However, one might ask, why is it that only certain types of species are found within a particular area of a beach? A beach, as defined by the *Collins Concise Dictionary*, is an area of sand or shingle, sloping down to a sea or lake, especially the area between the high to low-water marks on the seacoast. Due to the inevitable incline of the sand, certain organisms are forced to remain within an area that enables them to both avoid predators as well as position themselves in such a way that food is not hard to attain. This area is known as their habitat.

The study being done in this project will be conducted in a manner quite different to most beach studies because it is being done on a small scale. Most beach studies are conducted on a large-scale basis, which prevents the characterization and identification of individual bottom types (a bottom type is characterized by the shape of the sea floor caused by different types of wave action). These studies are generally done using 100m intervals as opposed to the 10m intervals that will be used in this project. The use of smaller intervals will allow for a more detailed study of the beach, which decreases the likelihood of important changes in the topography of the beach being overlooked. The study will also allow the identification and sampling of the several microhabitats,

including the organisms found in burrows, which are generally overlooked. In some cases, two or more species exist within the same bottom type, which is generally uncommon due to competition for resources. However, several sand samples will be taken in hopes that a reason for this cohabitation may be identified. White Beach on Guana Island will be separated into the varying habitats and bottom types existing there. In addition to the sand samples being taken, samples of the species existing in the sand will also be taken via various sampling methods.

This project will serve as a template for future studies done at this beach in the years to come. It will be used to show the changes in the bottom types over the years, which may be attributed to varying sea patterns. Also, the varying populations of the invertebrate organisms existing in this environment may be monitored.

Methods- Before any sampling occurred, the site of study was mapped (the distances of the varying sub-area of study from the vegetation) out to 50m from the shoreline. The 100m measuring tape was lined up along the shoreline at the point that the vegetation begins. This is known as the baseline. Starting at 0m, the 50m measuring tape was run perpendicular to the original measuring tape out towards the sea. Distances of the various bottom types (swash zone, ripple zone etc.) were then measured and recorded. The two previous steps were repeated for every 10m along the beaches and out a perpendicular 50m. As the beach areas

were being mapped, sand samples were taken at various intervals; this was generally when there was a noticeable change in sand types or at least every 100m.

The sampling of areas was done for each of the habitats and bottom types with each of the sampling methods (sieve, yabby pump, box net and chase method). The method of sampling used for each type of sampling apparatus is listed below:

1. Yabby pump- 15 plunges within a specific habitat.
2. Box net- straight sampling mainly along the swash zone and ripple zone for about 5m.
3. Sieve- 5 times within each habitat area.
4. Chase method- Catch as many invertebrate species as possible within each habitat area with a 20-minute interval.

NB: For the sampling of the reef habitat, the sand pockets around this area will be sampled. The organisms caught will be taken back the lab for identification, analysis and in some cases, photographing. In the case of the discovery of a possibly unidentified species, it will be preserved in alcohol for further analysis.

Results- During the study, it was noted that White Beach consists of about 7 bottom types:

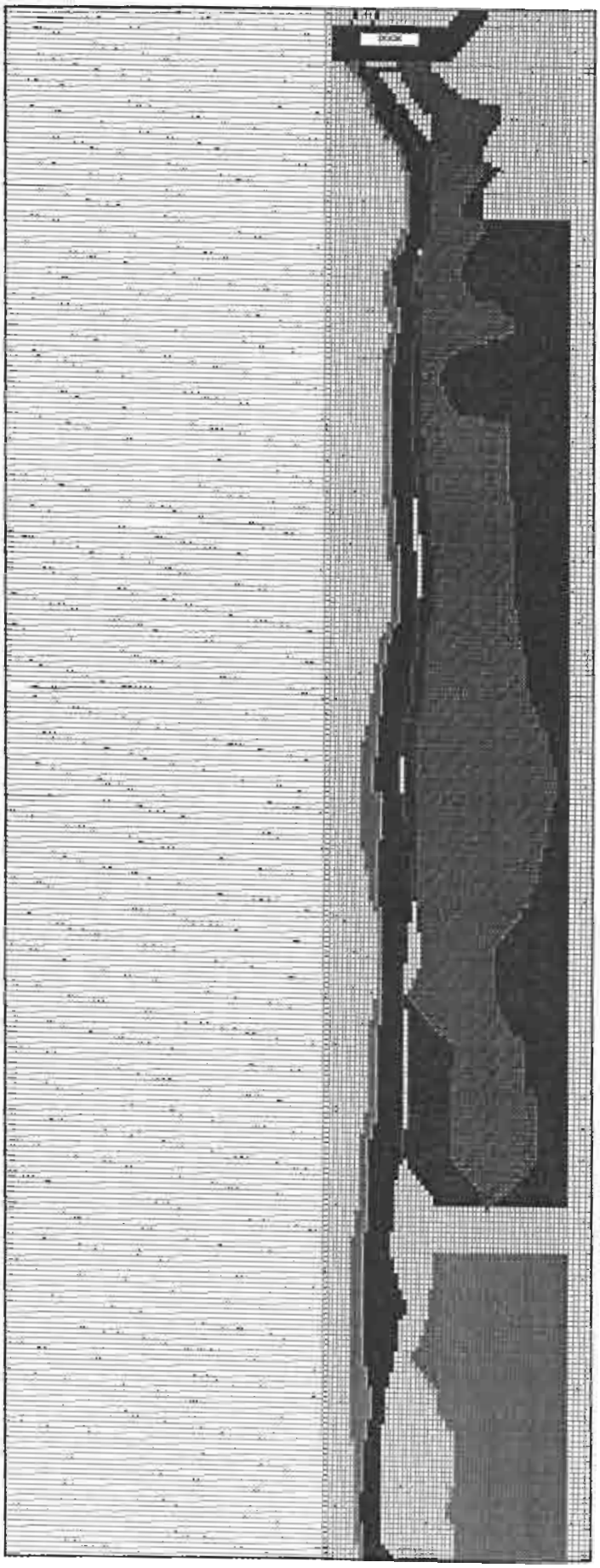
1. Swash Zone – the region between the limit of run-down and run-up; i.e. the portion of the beach that is both wet and dry under the action of waves (Swash Zone Research).
2. Ripple Zone – region on which ripples are formed on the sea floor due to the oscillatory flow of water back and forth.
3. Rubble Area – region at which dead coral and other small particles are found on the sea floor. This area occurred mainly in the swash and ripple zones.
4. Green Algal Area – area over sand that is covered by green algal growth.
5. Brown Algal Area – region usually around the ripple zone where there is a brown covering over the sand.
6. Reef Area – the region where live coral is present.
7. Rock – region where rocks are present.

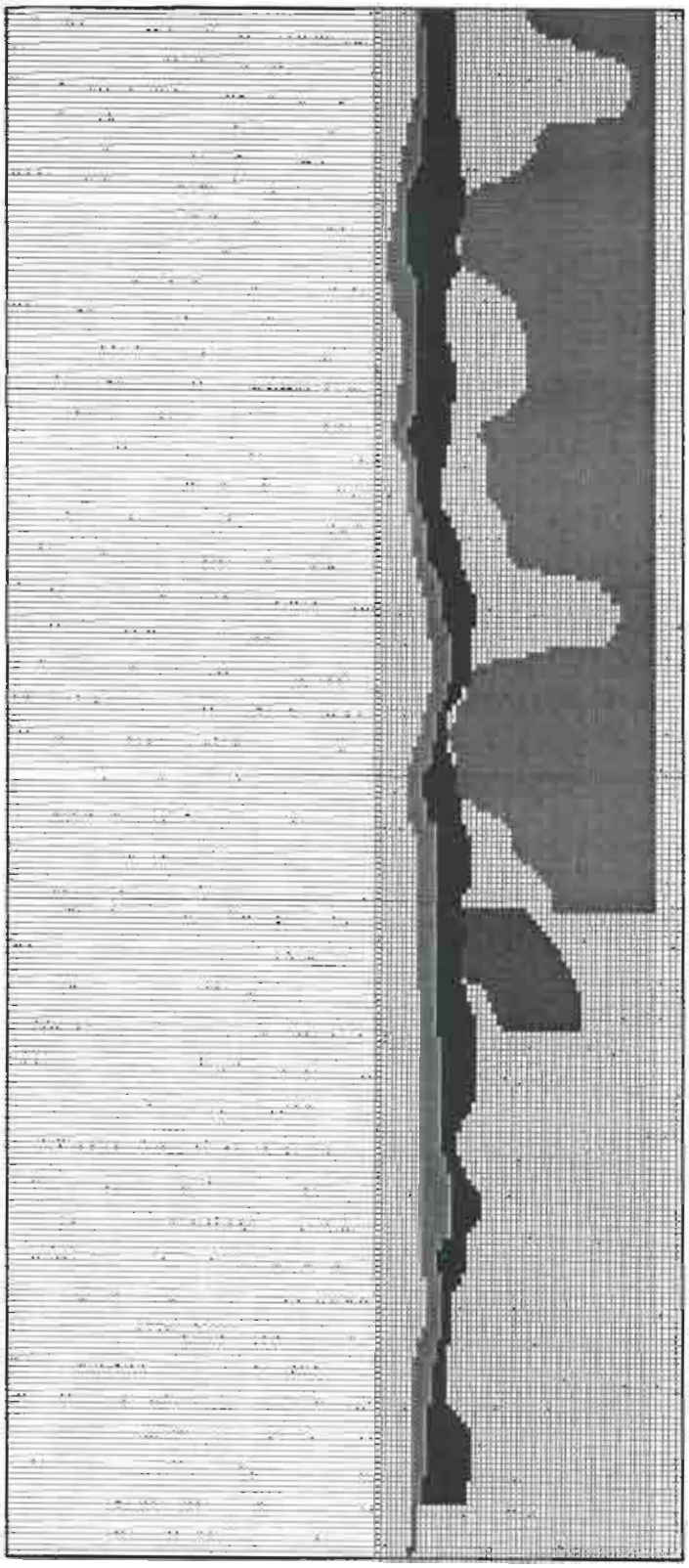
The other information gathered is found on subsequent data sheets including a representative graph of White Bay – the area of study.

Beach Data Sheet

Distance	Swash	Ripple	Rubble	Green Algae	Brown Covering	Reef	Rock	Comments
-20	1.8	-	-	-	7.6 -	-	0 -0.4 (s&r)	berm at 7.6m
-10	6.4	-	-	-	11.4 -	-	0 -	berm at 9.9m
0	4.4	6.5 - 7.9	-	-	8.9 -	-	-	dock from 0 - (-7)m
10	9.8	11.2 - 14.6	-	16.3 - 22.6	14.6 - 16.3	-	-	
20	17.2	18.9 - 22.1	41.4 -	23.6 - 38.7	21.1 - 38.7	-	-	
30	17.8	18.7 - 22.6	42.3 -	24.1 - 35.2	22.6 -	-	-	
40	13.3	15 - 18	45.4 -	19.9 - 30	18.5 -	-	-	
50	14.8	16.5 - 19.8	35.2 -	22.4 - 41.8	19.8 -	-	-	
60	13.4	15.3 - 19.2	40 -	21.8 - 25	19.2 -	-	-	Rubble ends at 67m
70	13.3	15.3 - 18.2	-	22.4 - 41	18.8 -	-	-	43m from the base line
80	13.3	15 - 19.5	-	23.2 - 41	19.5 -	-	-	
90	14	15.5 - 19	-	24.2 - 42	21 -	-	-	
100	14.8	16.6 - 20	-	24.8 - 42	22 -	-	-	
110	13	14.5 - 21.5	-	23.2 - 43	20.4 -	-	-	
120	11	12.6 - 19.1	-	22.1 - 45	19.1 -	-	-	
130	10.4	11.7 - 16.5	-	21.1 - 50	16.5 -	-	-	
140	8.6	10.5 - 16	-	21 - 52	17.8 -	-	-	
150	9	11.7 - 19.9	-	20.1 - 50	17.8 -	-	-	
160	9.9	12 - 19.2	-	21 - 46	19.2 -	-	-	
170	11.5	12.5 - 18	-	22 - 38	22 -	-	-	
180	11.5	13.3 - 16.5	-	19.3 - 38	18 -	-	-	
190	9.9	11.9 - 17	-	26 - 50	19.3 -	-	-	
200	10	11.7 - 16.7	-	30 - 45	19.4 -	-	-	
210	8.1	9.8 - 17	-	29 - 48	18 -	-	-	
220	7.7	10.3 - 15.3	-	35.6 - 37.6	25.2 -	-	-	227m green algae stops
230	7.4	9.1 - 14.3	-	-	21 -	24.6 -	-	
240	7.9	9.3 - 18.1	-	-	21 -	23.6 -	-	
250	7.6	9.4 - 14.6	11.3 -19.8	-	-	19.8 -	-	8m of rock on the shore
260	7.5	10.8 - 13.3	18.1 - 26	-	-	26 -	-	1m wide from 266m-274m
270	7	8.7 - 11.7	21 - 30.4	-	-	30.4 -	-	
280	6.6	7.8 - 11.7	-	-	-	28.2 -	-	
290	7.8	9.3 - 14.5	-	-	-	29.5 -	-	
Distance	Swash	Ripple	Rubble	Green Algae	Brown Covering	Reef	Rock	Comments

300	7.8	9.8 - 14.9	3.8 - 6.9	-	19.7 - 51.2	51.2 -		
310	5.5	9.4 - 16.2	1.9 - 3.8	-	13 - 31.7	31.7 -		
320	3.9	6.3 - 16.6	2.3 - 3.5	-	10.2 -16.6	16.6 -		
330	2.2	5.9 - 15.5	-	-	9.9 - 13.5	15.5 -		
340	4.2	7.5 - 12.7	-	-	12.7 - 28	28 -		
350	4.6	7.7 - 14.8	7.4 - 14.8	-	10.3 - 30	30 -		
360	2.8	5.6 - 13.5	5 - 20	-	10.3 - 20	20 -		
370	2.5	8 - 12	8 - 22.2	-	12 - 22.2	22.2 -		
380	7.3	10 - 15.5	4.3 - 25.8	-	15 - 25.8	25.8 -		
390	9.7	12.6 - 18.8	4.3 - 18.8	-	18.3 - 48.9	48.9 -		
400	10.8	14.5 - 18.7	2.6 - 18.7	-	-	18.7 -		
410	8.5	12 - 13	2.7 - 13	-	-	13 -		
420	5.5	9.2 - 14.9	1.8 - 16.3	-	-	16.3 -		
430	8.3	12 - 18	5.5 - 27	-	-	27 -		green algae begins at 437m
440	8.3	11.7 - 17.2	4.5 - 16.1	14.4 - 28.9	-	35 -		13m from base line
450	8	12 - 16.4	5.5 - 7.8	17 - 38.4	-	39.3 -		
460	9.3	12.1 - 18.6	6.1 - 17.6	25 - 40	-	44.9 -		
470	8.9	12.5 - 18.9	6.8 - 30.3	-	-	45.3 -		
480	9.1	12.8 - 15.1	6.1 - 8.6	-	-	38.9 -		
490	9	14.7 - 20.1	6.3 - 10.4	-	-	22.8 -		
500	9.4	12.4 - 17.1	5.2 - 16.1	-	-	16.1 -		
510	10	12.5 - 14	5.7 - 18.7	-	-	18.7 -		
520	7.1	10.9 - 12.8	13.3 - 19.4	-	-	19.4 -		
530	7.1	9.5 - 17.6	17.6 - 18.2	-	-	18.2 -		
540	6.8	9 - 16.8	3.9 - 8	-	-	16.8 -		
550	6.4	-	2' - 15	-	-	20 -		





Sand Sampling Results					
Filter Paper /g	Sieve Level	Bottom Type	Water and Sand /g	24 hrs after Drying	Sand after Drying
1.90	1	Ripple Surface	5.10	4.72	2.82
1.84	2	Ripple Surface	5.64	3.30	1.46
3.72	3	Ripple Surface	151.42	116.34	112.62
1.88	4	Ripple Surface	6.12	3.42	1.54
1.86	1	Ripple Bottom	5.22	2.82	0.96
1.84	2	Ripple Bottom	6.76	3.58	1.74
1.82	3	Ripple Bottom	84.74	81.76	79.94
1.88	4	Ripple Bottom	6.48	3.44	1.56
1.86	1	Splash Bottom	4.50	2.40	0.54
1.86	2	Splash Bottom	5.68	2.60	0.74
1.84	3	Splash Bottom	105.40	84.72	82.88
1.86	4	Splash Bottom	19.24	12.82	10.96
1.86	2	Green Surface	5.80	2.40	0.54
1.88	3	Green Surface	56.82	35.34	33.46
1.88	4	Green Surface	80.42	65.48	63.60
1.76	5	Green Surface	11.60	5.56	3.80
1.88	1	Splash Surface	0.82	2.02	0.14
1.84	2	Splash Surface	5.02	2.38	0.54
1.86	3	Splash Surface	107.64	79.40	77.54
1.88	4	Splash Surface	52.56	38.62	36.74
1.88	2	Brown Bottom	6.68	2.10	0.22
1.88	3	Brown Bottom	55.50	37.88	36.00
3.76	4	Brown Bottom	58.22	41.80	38.04
1.86	5	Brown Bottom	6.86	2.30	0.44
1.88	1	Green Bottom	0.08	1.94	0.06
1.88	2	Green Bottom	6.22	1.90	0.02
1.88	3	Green Bottom	30.14	18.76	16.88
1.88	4	Green Bottom	0.75	52.78	50.90

Sand Sampling Results					
Filter Paper /g	Sieve Level	Bottom Type	Water and Sand /g	24 hrs after Drying	Sand after Drying
1.88	5	Green Bottom	4.30	2.02	0.14
1.86	2	Brown Surface	4.95	2.24	0.38
1.82	3	Brown Surface	120.82	93.40	91.58
1.86	4	Brown Surface	5.08	2.00	0.14
1.82	5	Brown Surface	4.36	0.94	-0.88
1.86	1	Reef 1 Surface	3.52	1.38	-0.48
1.84	2	Reef 1 Surface	5.64	2.72	0.88
1.86	3	Reef 1 Surface	50.84	32.88	31.02
1.84	4	Reef 1 Surface	101.02	73.90	72.06
1.84	5	Reef 1 Surface	5.14	2.20	0.36
1.82	1	Reef 1 Bottom	1.14	1.88	0.06
1.86	2	Reef 1 Bottom	3.74	2.00	0.14
1.82	3	Reef 1 Bottom	56.24	37.26	35.44
1.84	4	Reef 1 Bottom	85.68	66.78	64.94
1.86	5	Reef 1 Bottom	5.16	2.20	0.34
1.84	1	Green 2 Bottom	6.38	5.42	3.58
1.84	2	Green 2 Bottom	13.66	8.94	7.10
1.86	3	Green 2 Bottom	51.44	47.04	45.18
1.84	4	Green 2 Bottom	101.60	73.42	71.58
1.84	5	Green 2 Bottom	4.20	1.86	0.02
1.84	1	Green 2 Surface	8.22	6.66	4.82
1.86	2	Green 2 Surface	11.16	7.16	5.30
1.82	3	Green 2 Surface	128.18	103.28	101.46
1.82	4	Green 2 Surface	7.66	3.70	1.88
1.84	5	Green 2 Surface	4.68	1.90	0.06
1.84	1	Reef 2 Surface	47.56	41.84	40.00
1.86	2	Reef 2 Surface	44.66	36.54	34.68
1.84	3	Reef 2 Surface	58.64	40.60	38.76
1.84	4	Reef 2 Surface	3.18	1.94	0.10

Sand Sampling Results

er Paper /g	Sieve Level	Bottom Type	Water and Sand /g	24 hrs after Drying	Sand after Drying
1.84	1	Reef 2 Bottom	16.72	14.56	12.72
1.84	2	Reef 2 Bottom	39.36	29.42	27.58
1.82	3	Reef 2 Bottom	79.02	55.10	53.28
1.84	4	Reef 2 Bottom	4.30	2.02	0.18
1.86	5	Reef 2 Bottom	5.12	1.92	0.06
1.84	1	Rubble in Swash Surface	6.82	6.04	4.20
1.84	2	Rubble in Swash Surface	31.12	24.02	22.18
1.86	3	Rubble in Swash Surface	130.48	92.48	90.62
1.86	1	Rubble in Swash Bottom	51.46	36.48	34.62
1.84	2	Rubble in Swash Bottom	45.92	35.48	33.64
1.84	3	Rubble in Swash Bottom	77.44	57.88	56.04
0.90	1	Finger Reef Surface	2.50	1.56	0.66
0.90	2	Finger Reef Surface	6.76	3.66	2.76
0.90	3	Finger Reef Surface	71.86	43.10	42.20
0.90	4	Finger Reef Surface	65.14	45.54	44.64
0.90	5	Finger Reef Surface	3.90	1.42	0.52
0.80	1	Finger Reef Bottom	1.12	1.08	0.28
0.80	2	Finger Reef Bottom	1.40	1.20	0.40
0.88	3	Finger Reef Bottom	57.36	37.66	36.78
0.90	4	Finger Reef Bottom	82.88	57.26	56.36
0.80	5	Finger Reef Bottom	43.20	1.32	0.52
1.90	1	Brown 2 Surface	21.10	17.56	15.66
1.94	2	Brown 2 Surface	15.46	9.60	7.66
1.90	3	Brown 2 Surface	115.46	84.60	82.70
1.92	4	Brown 2 Surface	4.42	2.08	0.16
1.94	1	Brown 2 Bottom	16.56	13.08	11.14
1.94	2	Brown 2 Bottom	19.18	12.96	11.02
1.94	3	Brown 2 Bottom	117.50	70.78	68.84

120

Sand Sampling Results					
Filter Paper /g	Sieve Level	Bottom Type	Water and Sand /g	24 hrs after Drying	Sand after Drying
1.94	4	Brown 2 Bottom	11.50	5.92	3.98

Sampling Method	Organism	Species	Location
Dipnet	Hippa	3	small area on other side of dock
			(5, -14)
Dipnet	Penaeid shrimp	1	small area on other side of dock
			(7, -14)
	Portunus sp		small area on other side of dock
			(7, -14)
Dipnet	Arenaeus cribrareus		(4, 8)
	Polychaetes		
	Armandia sp		
	Terrebelid?		
Dipnet	Arenaeus cribrareus	1	(7, 8)
	Polychaetes		
	Armandia sp. -many		
	large ?Terrebelids		
Yabby Pump	B.A. Sipunculid	1	(7, 8)
	Neocallichirus	1	(7, 8)

Discussion- The study of the bottom types existing at Guana Island illustrated seven rather distinct bottom types. In certain areas however, the brown and green algal areas were at times difficult to decipher. This was due to the fact that certain areas had small and low patches of green algal growth. Those made them resemble the brown algal covering, which is also low, but generally covered a larger area than the patches of green would. The gradually changing topography of the sea floor was also noticeable not only as you ventured out towards the sea, but also along the beach. It varied from algal covering, to sandy areas, then reef areas and finally rocky areas. It was also observed that at the extremes of the beach area studied, the topography of the seafloor is quite different and these changes can be attributed to the wave action.

As observed in both (i.e. surface and bottom) sand samples of the swash and ripple zones, rubble in swash zone, brown two (2) zone and in reef two (2), sieve level three contained the most sand particles. Whereas sieve level four contained more sand particles in the green algal area, the finger reef and in reef one (1) samples. In these areas, the sand has a relatively identical consistency throughout. However, in the brown and green two (2) areas, sieve four (4) was found to have the greatest weight in the bottom sample. This indicates that the larger particles of sand in this region are found beneath the surface sand. In general, despite these slight changes in some areas of the beach, both bottom and surface sand sets have relatively equal consistencies throughout.

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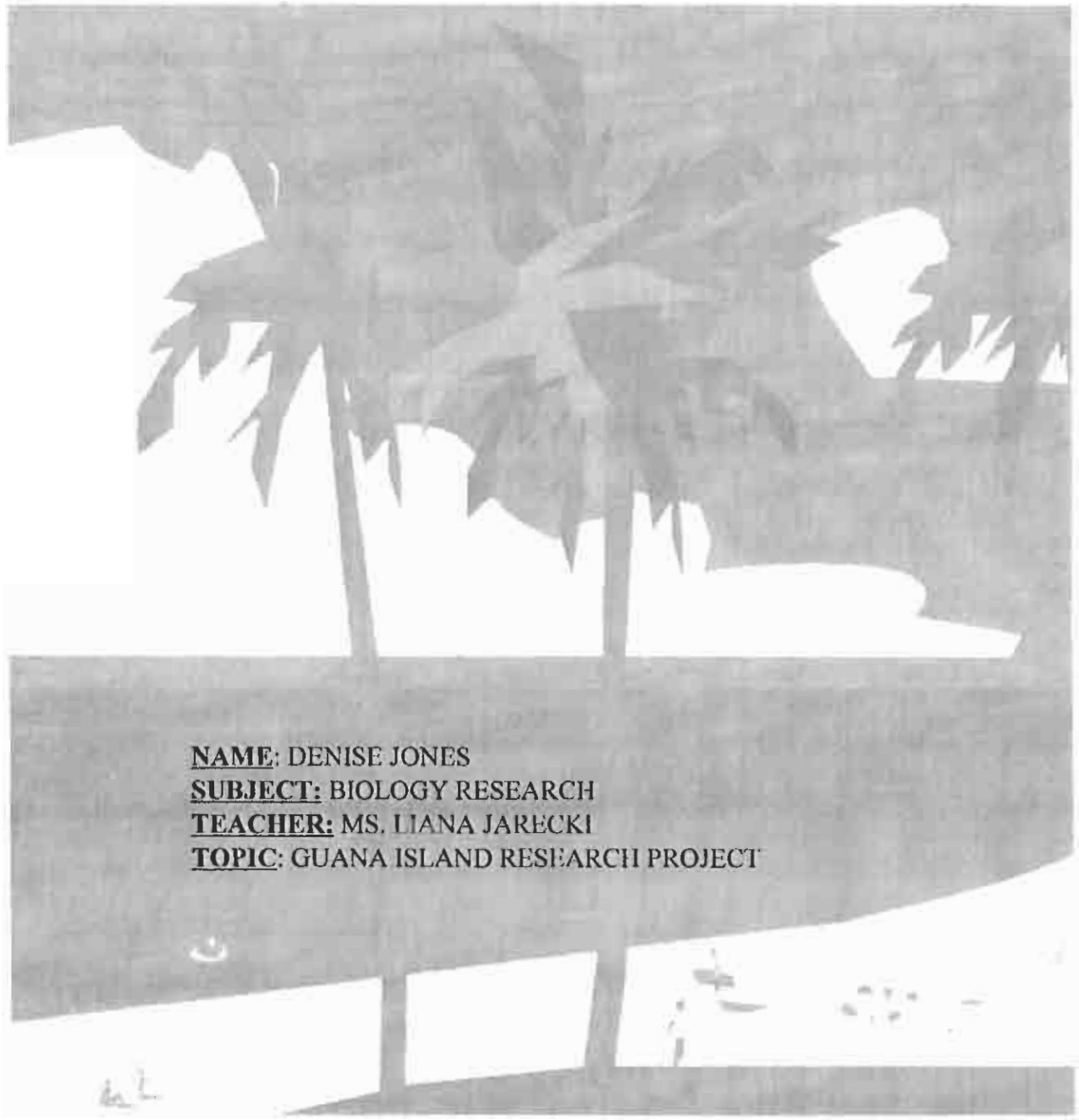


NAME: DENISE JONES
SUBJECT: BIOLOGY RESEARCH
TEACHER: MS. LIANA JARECKI
TOPIC: GUANA ISLAND RESEARCH PROJECT

ABSTRACT

A beach is an accumulation of sediments along the sea or lake shores. The shape and contours of the beach depends on the action of coastal processes, the kind of sediment involved, and the rate of delivery of this sediment. Each beach has a combination of bottom types. A bottom type is a specific habitat or environment on the ocean floor. Some common bottom types are swash zone, ripple zone, green algal area, brown algal area and reef. The swash zone is the area where wave breaking and run up occurs. It is characterized by the highest water movement and consequently has a high degree of mechanical stress and possibility of rapid sediment removal. The ripple zone is the area on the ocean floor where the pattern of sand is one of ripples caused by the action of waves before they break. The green algal area is an area in which a type of green algae covers the surface of the sand. The brown algal area is an area in which brown algae covers the surface of the sand. A reef is a ridge of coral or rock.

Guana Island is located in the Caribbean and is one of the many islands in the British Virgin Islands. The beach I studied is located on Guana Isalnd and is called White Bay. It is located on the western side of the island and is approximately 550m in length.

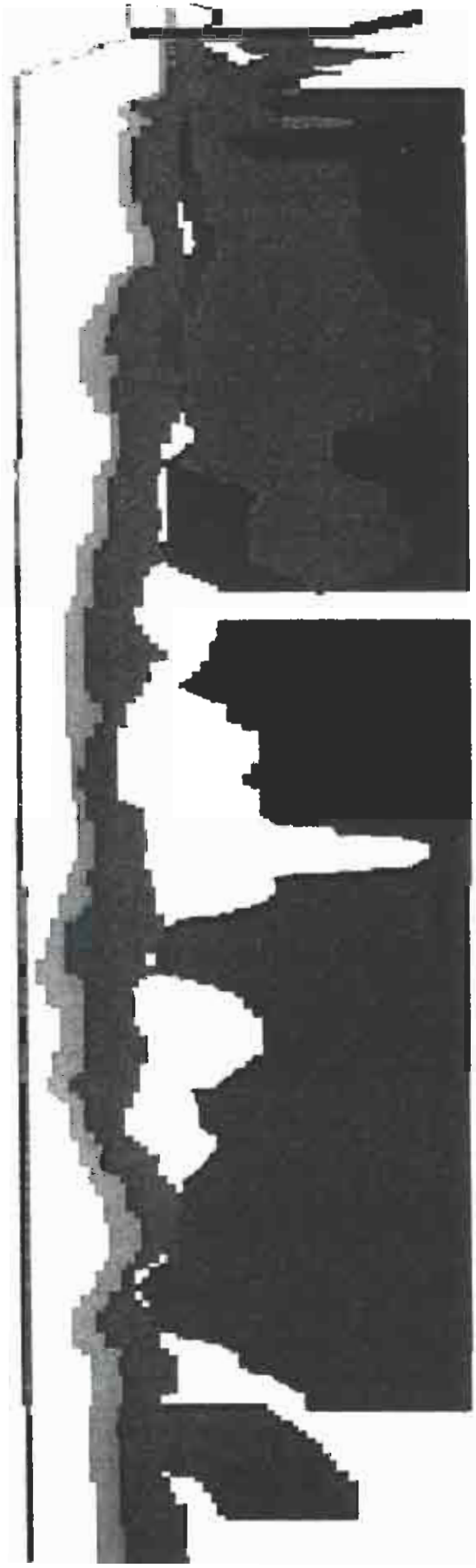
Usually when studies of the beach are done, they are done on a large scale, taking the entire beach as one habitat. This study however, aims to investigate the various bottom types of White Bay as separate habitats. The bottom types in the beach are mapped, the sand composition of the different bottom types is determined and the organisms supported in the bottom types are sampled.

This study is important for two main reasons. Firstly, the map of the beach that is made can serve as a comparison for future beach studies. This means that changes in the

locations and size of the various bottom types can be studied over time, after natural disasters such as hurricanes or human interference. Secondly, by using sand composition and organism sampling, it can be shown just how different the bottom types are in their composition and marine life supportiveness, revealing how important it is for them to be studied separately.

METHOD

1. A 100m length of tape was laid out along the length of the beach, using the vegetation as a line of reference.
2. The distance of the various bottom types i.e. swash zone, ripple zone, green and brown algal areas and reef, from the reference line were measured every 10 m along the entire length of the beach.
3. A map of the beach was drawn from the results obtained.
4. From each bottom type two samples of sand were taken, one from the surface and one from 15 cm below the surface.
5. The sand samples were sifted and weighed so that the composition of the sand for each bottom type was obtained.
6. Sampling of the organisms that live in the various bottom types was done using a yabby pump and a dip net.



KEY

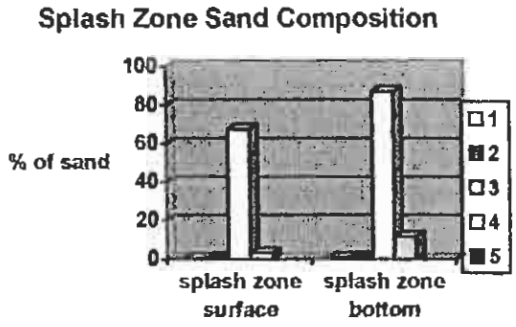
- Brown algal area
- Green algal area
- Ripple zone
- Swash zone
- Reef
- Vegetation
- Dock

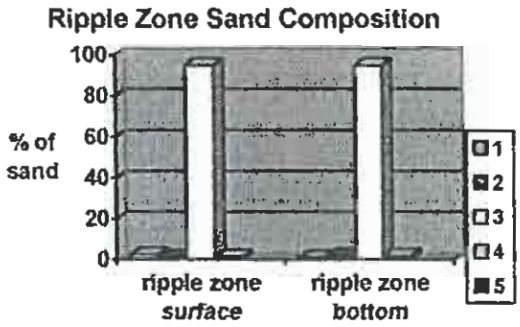
SCALE

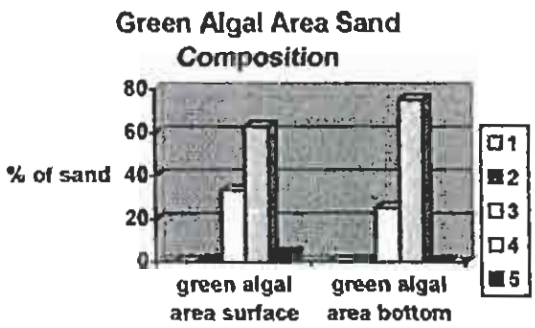
0m 46m

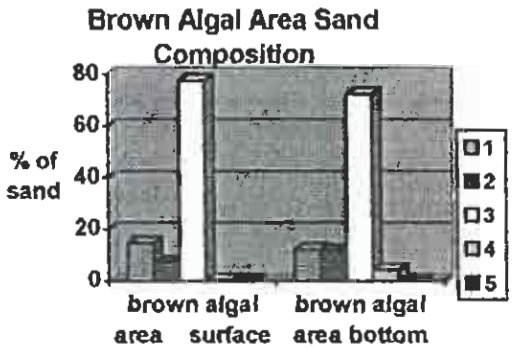
MAP OF WHITE BAY

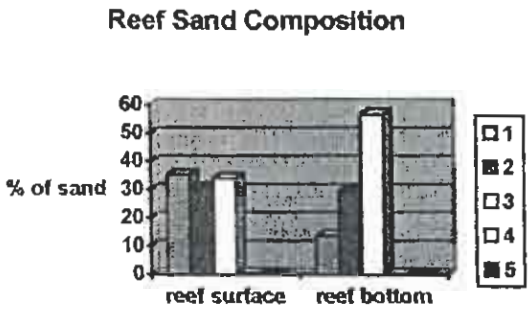
Results of Sand Composition











1- 1st seive containing largest particles
2- 2nd sieve containing 2nd largest particles
3- 3rd sieve containg small particles
4- 4th sieve containg smaller particles
5- 5th sieve containing smallest particles

RESULTS OF SAMPLING

Bottom Type	Organisms Found Using Dip Net	Organisms Found Using Yabby Pump
Swash Zone	Crustacea – Arenaeus Cribrareus. Hippa sp. Polychaetes – Armandia Terrebelid.	B.A. Sipunculid Nevcallichirus
Ripple Zone	Crustacea – Arenaeus Cribrareus. Polychaetes – Armandia Terrebelid	Penaeid Shrimp Portunus sp.

DISCUSSION

The results of the sand composition study, shows that both the splash zone and the ripple zone are quite similar in composition. They both have the highest percentage of their sand composition in sieve #3. Their similarity is also supported by the fact that the same organisms were found in both of these bottom types using the dip net. Despite their similarities however, the habitats of the splash zone and ripple zone are different, as can be seen in the yabby pump sampling which yielded different organisms for each bottom type. This therefore means that despite their similarities the splash zone and ripple zone are distinct habitats and it is worth while to study them independently of each other.

The results of the sand composition test show that despite the fact that the green and brown algal areas both support algae, they are very different habitats, and as such should be studied independently. The green algal area has the highest percentage of its sand composition in the fourth sieve, while the brown algal area has the highest percentage of its sand composition in the third sieve. Though no organism sampling was done for these two bottom types, the fact that they support two different types of algae also shows their difference as habitats.

The reef sand composition is abundantly different from that of all the other habitats. While the other habitats generally have a low composition of sand in sieve 1and 2, which means large particles, the reef area has a relatively high percentage of its composition in these sieves. Though no organism sampling was done in this area, it was observed that there is an abundance of life which differs greatly from that found in the

other habitats. This definitely merits the studying of the reef as a habitat different from the rest of the beach.

CONCLUSION

All of the bottom types of the ocean are different both in their sand composition and in the organisms they support. A great deal of valuable information in ocean mechanics and ocean life is therefore lost if the entire beach is studied as a single habitat as seems to be the trend. The map of the bottom types of White Bay show the distinct areas of splash zone, ripple zone, green algal area, brown algal area and reef. The sand composition and organism sampling show the variation in composition of the different bottom types, and show the validity of studying each bottom type separately rather than studying the beach as a single entity.

Topic: Marine Sponges

KIRSTEN LETTSOME

Question: Do marine sponges have the ability to absorb excess nitrates from seawater.

Hypothesis: Marine sponges are capable of absorbing excess nitrates from seawater.

Introduction: Elevated nitrates are directly linked to planktonic algal blooms. These planktonic algal blooms are not limited in their growth and as a result grow until they cover the entire surface of the water body. This forms a barrier which blocks light from photosynthetic bottom communities such as coral reefs and seagrass beds. When this occurs, there is a decrease in the amount of oxygen readily available to the organisms. As a result, the algae and other organisms beneath this algal shield die and are decomposed by saprobiontic bacteria, which causes a biochemical oxygen demand. All aerobic organisms in the water community regions with the exception of the upper levels regions die due to deoxygenation. (Toole and Toole 1995) In the BVI, coral reefs are exposed to nitrate-rich wastewater when boats anchor in the region. The most popular anchorages tend to have the highest nitrate levels in their water because charter-boats discharge their sewage directly into the seawater. The sewage from homes around the islands also ends up running into the seawater because there are no significant sewage treatment facilities on any of the British Virgin Islands. The negative effects of this wastewater, particularly of the nitrates they contain, are well documented, but little is known about the ability of benthic organisms to assimilate nitrates and thus possibly buffer the effects of sewage discharge over coral reefs. This study is designed to test whether a marine sponge, *Aplysina cauliformis*, is able to absorb nitrates from seawater. *Aplysina cauliformis* is a common sponge associated with coral reefs.

It is deep purple to brown and grows in narrow, erect pillars of on average ten inches long in water between 3 and 20 meters deep. The sponge texture is very coarse, with very few oscula and tiny inward channels. The sponge itself is very brittle. (Funk & Wagnals 1984)

Method: The *Amphimedon compressa* sponges were collected and examined under a microscope. The organisms found on and inside the sponges were then assessed i.e. the organisms living on the surface and inside of the sponge were identified and their numbers recorded. Care was taken to count the worm heads only to avoid recounting worms. The *Aplysina cauliformis* were collected and examined under a microscope. The symbionts were then assessed and the species identified and their numbers recorded. As there were not many symbionts found on the *Aplysina cauliformis*, this sponge was chosen for the experiment. The number or capacity of the symbionts in relation to the actual sponge is important because the presence of the symbionts may be strongly influencing the sponge's behaviour.

The sponges were then weighed via seawater displacement; thus the sponges should be of equal length. A fixed volume of seawater was measured out into a graduated cylinder and the volume increase of the seawater as the sponge was immersed was recorded. These sponges were to be destroyed in order to locate all the symbionts, but the symbionts were saved and preserved.

The quality of the water was tested at both the surface and at the depth of the sponge location. The pH, salinity, dissolved O_2, temperature; depth and light penetration were all recorded at the surface and especially at the sponge habitat.

When reading the amount of dissolved O_2, the cord attached to the probe was rapidly bobbed to ensure an accurate reading. New sponges were collected to perform the experiment. The sponges were sectioned in the water and labeled and placed in their individual air lock bag. The sponge was cut from the base, but the exact section of the colony from where the section of sponge was taken need not be known. The sections of the sponge were of equal length. Four pieces from the same individual were used to set up paired experiments. One piece from each individual was placed in each of the experimental and control jars. This placement of sponges need not be done arbitrarily because variability is inevitable since the location of the sponge pieces placed in each experiment and control jar was not known. The sponge was then assembled in such a way as to allow it to heal. The pieces were assorted so that they were far enough apart to heal individually. Fishing line was run through each section of the sponge and the line wrapped to keep the pieces in close proximity.

A piece of fishing line of about 80 centimetres was cut and at one end a noose was tied. The other end of the fishing line was threaded through a needle and the needle used place the fishing line through the sponge so that it may be suspended. After the sponge was threaded with the fishing line the needle was run through the noose and the not pulled tight enough to hold the sponge. This process was repeated for the twenty sponge pieces required for the experiment. The ends of the fishing line were then tied around a PVC pipe. The PVC pipe was run across the top of the tank and the sponge suspended in the filtered seawater. An aquarium type setting was used; i.e. a glass tank, which was about three-quarters, filled with filtered seawater for the experiment. Care was taken to ensure that the aquarium had flowing filtered seawater.

After the sponges were suspended in the tank a galvanize shade was placed above the tank to regulate the light intensity affecting the sponges. The sponges were left to heal for between 48 and 96 hours.

During the healing process, the apparatus for the forty simultaneous experiments were set up. Forty deli tubs were setup in a shaded area and four hundred mls. of filtered seawater placed in each tub. Weights of varied amounts were used to keep the deli tubs stable. Aerators were used to bubble oxygen through to assist in keeping the sponges alive. Four pumps were used as well as switches to accommodate the forty deli tubs. Forty air stones were needed for the deli tubs; i.e. one air stone for each tub. The air stones were connected to the four pumps via a series of plastic tubing cut to various lengths to connect all tubs. The pressure on each T-connector was adjusted using the valves so that each tub had the same amount of air being bubbled through it. Each tub was labeled to indicate what was inside. Ten tubs contained filtered seawater only, ten contained filtered seawater and a sponge each, another ten contained filtered seawater and elevated nitrate concentration, and the final ten contained filtered seawater, elevated nitrate concentration and a sponge each. After the sponges were completely healed, or almost completely healed they were removed and placed in the appropriate deli tubs. The light intensity was maintained at the same intensity as that of the sponge's habitat.

The controls for the study are filtered seawater and seawater with nitrates elevated to ten times that of unpolluted seawater. The tests are filtered seawater and experimental seawater and also experimental sponge in seawater with nitrates elevated to ten times that of unpolluted seawater. Ten replicates for each control and experiment are done so that there are forty experiments occurring simultaneously.

The formula n=cv was used to discover how much of the stock solution was to use. After one day in the experimental conditions the sponges were removed and weighed via seawater displacement. A fixed volume of seawater was measured out into a graduated cylinder and the volume increase of the seawater as the sponge was immersed recorded. The dry weight of the sponge was also measured.

Assays for ammonia, nitrates and silica were performed and variability in the size of the sponges is taken into consideration. When using the machine, the lid was always closed before either the zero or read buttons were pressed. The Cadmium Reduction Method was used to test for the nitrates in low range (0 to 0.40 mg/L NO_3^-N). Using the machine, the number 351 was entered. After the display showed dial nm to 507, the side dial was turned until the display showed 507 nm. After that wavelength was achieved, the display showed Zero sample. Using a 50 ml. graduated cylinder 30 mls. of one of the filtered seawater was measured out. The entire contents of one packet of Nitra Ver 6 Nitrate Reagent Powder Pillow were added to the 30 mls. of seawater and covered. On the machine, shift timer was pressed and a three-minute reaction started, while the contents were shaken. After the timer beeped, shift timer was pressed again to start a two-minute reaction, which allowed the cadmium to settle. After the timer beeped, 25 mls. of the sample from the cylinder was poured into a sample cell. The entire contents of one Nitri Ver 3 Nitrite Reagent Powder Pillow were added to the sample cell and the sample shaken. Shift timer was then pressed to start a ten-minute reaction during which the sample was shaken. After the timer beeped, 25 mls. of that sample was placed into another sample cell (the blank) and the cell placed in the holder. Care was taken to keep the cell clean so that as much light as possible would penetrate the cell.

The lid was then closed and on the machine, the Zero button pressed. When the symbol 0.00mg/L NO_3^- LR appeared, the blank was removed. After the blank was removed, 25 mls. of all samples, one after the other, containing filtered seawater and sponge only were placed in a cell and the read button pressed for each sample. The process was repeated for all remaining samples. Care was taken to wash all cells with distilled water if being reused. Upon achieving results, 0.01 mg/L NO_3^- must be subtracted from them.

The Heteropoly Blue Method was used to test for the silica in low range (0 to 1.600 mg/L). Using the machine, the number 651 was entered. After the display showed dial nm to 815, the side dial was turned until the display showed 815 nm. After that wavelength was achieved, the display showed Zero sample. A 10 ml cell riser was inserted into the cell compartment. Two sample cells were filled to the ten-ml. line with filtered seawater and to each 14 drops of the Molybdate 3 Reagent and each solution swirled to mix. Shift timer was then pressed to begin a four-minute reaction. After the timer beeped, the contents of one Citric Acid Reagent packet were added to each sample cell. Shift timer was then pressed to begin a one-minute reaction during which the sample is shaken. After the timer beeped the contents of one Amino Acid F Reagent Powder Pillow were added to one of the samples. Shift timer was then pressed to begin a one-minute reaction. After the timer beeped, the display showed mg/L SiO_2 LR and the blank (the solution without the Amino Acid F) was placed into the cell holder. The zero button was then pressed and the display read 0.00mg/L SiO_2. The other 10ml. sample (the solution with the Amino Acid F) was placed into the cell holder and the read button pressed. The process was repeated for the remaining samples, but only one sample cell was used because the machine was already zeroed.

Thus each sample to be tested contained 14 drops of the Molybdate 3 Reagent, one packet of Citric Acid, and one packet of Amino Acid Reagent F.

The ammonia was collected using fractional distillation. 25mls. of each sample were placed in a 100 ml. round bottom flask. The normal set up for fractional distillation was used. 25 mls. of distilled water along with two drops of HCl were placed in the 100 ml. collecting jar at the other end. When the collecting jar was about half way full the distillation was complete and the solution was collected for testing. To each solution, a few drops of both NaOH and HCl were added to bring the pH back to around 7.

The Nessler Method was used to test for the ammonia. Using the machine, the number 380 was entered. After the display showed dial nm to 425, the side dial was turned until the display showed 425 nm. After that wavelength was achieved, the display showed Zero sample. 25 mls. of a sample were placed in a graduated cylinder while another sample cell was filled with 25 mls. of distilled water. 1 ml. of Nessler Reagent was pipetted into each cylinder, each covered and shaken. Care was taken not to get the Nessler Reagent on the skin. Shift timer was pressed to begin a one-minute reaction, during which the solutions were shaken. Each solution was poured into a separate sample cell. After the timer beeped the solution with distilled water was placed in the machine and the zero button was pressed. The prepared sample was then placed into the cell holder and the read button pressed. The process was repeated for each prepared sample. The machine did not require zeroing again because it was already zeroed.

Results:

To discover the final concentration of the solutions with the elevated Nitrate content:

X=volume of stock solution

Y=volume of seawater

The volumes of x and y are in the same units.

A=new concentration

100mg of NO_3 in stock solution

0.2mg of NO_3 in seawater

0.02mg of NO_3 is the desired concentration

N=CV

$100x + 0.02y = a(x+y)$

$100x + 0.02y = a$

$(x+y)$

$(100)(0.75) + (0.02)(450)$

$(450 + 0.75)$

$A=0.1864s$

Samples one and two are filtered seawater and samples three and four are the filtered seawater with the elevated nitrate concentration. These are the initials.

Test	Sample	1	2	3	4
Silica		0.338	0.339	0.203	0.201
Nitrate		0.02	0.02	0.08	0.07
NH_3 (pH)		7.4	7.4	7.4	7.1
NH_3 volume		61	64	62	63
Ammonia		0.08	0.07	0.23	0.25

These are the filtered seawater samples.

Test	Sample	1	2	3	4	5
Silica		0.167	0.169	0.170	0.180	0.175
Nitrate		0.02	0.02	0.02	0.02	0.03
NH_3 (pH)		7.1	7.1	7.0		
NH_3 volume		65	63	64		
Ammonia		0.08	0.06	0.07		

These are the filtered seawater with elevated nitrate concentrations samples.

Test	Sample	1	2	3	4	5
Silica		0.035	0.034	0.035	0.036	0.030
Nitrate		0.05	0.06	0.07	0.06	0.05
NH_3 (pH)		7.2	7.1	7.1		
NH_3 volume		62	60	60		
Ammonia		0.15	0.14	0.11		

These are the filtered seawater and sponge samples.

Test	Sample	1	2	3	4	5
Silica		0.093	0.094	0.090	0.089	0.093
Nitrate		0.2	0.22	0.21	0.26	0.23
NH_3 (pH)		7.1	7.1	7.2		
NH_3 volume		60	61	60		
Ammonia		0.23	0.24	0.22		

These are the filtered seawater with elevated nitrate concentrations and sponge samples.

Test	Sample	1	2	3	4	5
Silica		0.33	0.36	0.33	0.32	0.32
Nitrate		0.094	0.096	0.093	0.096	0.094
NH_3 (pH)		7.0	7.0	7.1		
NH_3 volume		60	62	63		
Ammonia		0.27	0.30	0.29		

Theses are the volume displacement results from the sponges.

Sponge	Volume Displaced
Filtered Seawater and Sponge 1	6
Filtered Seawater and Sponge 2	6
Filtered Seawater and Sponge 3	5
Filtered Seawater and Sponge 4	9
Filtered Seawater and Sponge 5	6
Filtered Seawater and Sponge and elevated Nitrate 1	6
Filtered Seawater and Sponge and elevated Nitrate 2	7
Filtered Seawater and Sponge and elevated Nitrate 3	5
Filtered Seawater and Sponge and elevated Nitrate 4	15
Filtered Seawater and Sponge and elevated Nitrate 5	7

These are the dry weight results of the sponges.

Weight of filter	Sponge Type	Number	Weight of sponge and filter	Dry Weight
0.88	F. S. & sponge	1	7.66	5.59
0.92	F. S. & sponge	2	8.96	7.42
0.92	F. S. & sponge	3	5.46	3.89
0.90	F. S. & sponge	4	11.0	9.76
0.92	F. S. & sponge	5	6.98	5.03
0.90	F.S. & sponge + Nitrate	1	6.42	4.25
0.84	F.S. & sponge + Nitrate	2	8.56	7.12
0.94	F.S. & sponge + Nitrate	3	8.86	7.51
0.86	F.S. & sponge + Nitrate	4	14.72	11.87
0.88	F.S. & sponge + Nitrate	5	7.90	5.18

Discussion: The average volume displaced by the sponges placed in the filtered seawater only was 6.5 mls. The average volume displaced by the sponges placed in the filtered seawater with elevated nitrates was about 8mls.

The weight of both the sponges placed in the filtered seawater only and the sponges placed in the filtered seawater and the elevated nitrates diminished upon drying. Thus the dry weight for both sets of sponges decreased.

The silica levels in the initials were higher than in the filtered seawater tested with the other samples. The silica levels between the initials and the filtered seawater samples tested diminished on average by almost 0.168. The silica levels diminished even further when the sponges were placed in the filtered seawater. On average, the silica levels between that of the filtered seawater and the filtered seawater with the sponge diminished by almost 0.77. The silica levels between the initials with elevated nitrate and the samples tested without the sponge diminished by on average almost 0.169. When the sponge was added however, the silica levels diminished by less than they did without the sponge. The levels actually only diminished by 0.107.

The levels of ammonia between the initials and the sample filtered seawater remained constant. When the sponge was added to the filtered seawater however, the levels of ammonia increased on average by 0.15. The levels of ammonia for the initials decreased when the nitrate concentration was increased. On average the levels decreased by 0.11. When the sponge was added to the solution containing filtered seawater, the ammonia levels were kept relatively constant in comparison to the initials.

The nitrate levels of the sample filtered seawater in comparison to that of the initials remained constant. The nitrate levels of the filtered seawater increased when the sponge

was added to it in comparison to the initials. The nitrate levels increased by a factor of 10. The nitrate levels of the filtered seawater with elevated nitrates remained constant in comparison to the initial with elevated nitrates. The nitrate levels of the filtered seawater with elevated nitrates increased when the sponge was added to it in comparison the initial, which contained filtered seawater and elevated nitrates only. The nitrate concentration increased by 0.25. It is apparent from this data that the sponges are putting out nitrates into the seawater.

Appendix 1:

Program for MSM Symposium at HLSCC, July 13th, 2001

Welcome

Remarks:

Dr. Charles Wheatley,
President of the H. Lavity Stoutt Community College

Introduction of research students:

Aasha Flax
Denise Jones
Kirsten Lettsome
Shanie Dasrath

Presentations:

Todd Zimmerman
Los Angeles County Museum of Natural History
"UNSEEN WONDERS OF SHALLOW WATERS"

Don Cadien
County Sanitation Districts of Los Angeles County
"SCUDS, PILLBUGS, CUMACEANS, AND OTHER MICROCRUSTACEANS"

Rick Ware
Coastal Resources Management, San Clemente, California
"SEAGRASS DISTRIBUTION AROUND GUANA ISLAND"

Break with refreshments catered by Guana Island

Presentations:

Rachel J. Petrik-Finley
University of Rhode Island
"PARASITES IN REEF FISH: HARMLESS COMPANIONS OR LETHAL FOE?"

Lianna Jarecki
H.L.S.C.C. and the University of Kent at Canterbury
"SALT PONDS IN PERIL; WHY SHOULD WE CARE"

Caitlin O'Connell-Rodwell
Center for Conservation Biology, Stanford University
"FLIRTING FLAMINGOS; CAN WE MAKE IT HAPPEN?"

Appendix 2:

Local News Articles

The BVI Beacon, July 12, 2001. *Scientists to research on Guana.*

H. Lavity Stoutt Community College Info Update, October, 2001. *Biology students visit biological research programme on Guana Island.* Volume 5, Issue 3.

The BVI Stand Point, October 29, 2002. *Guana Island student research programme has led some to careers in science.*

The BVI Beacon, November 7, 2002. *Century plant devastation.*

The BVI Beacon, November 14, 2002. *Students, teachers tour Guana Island.*

Scientists to research on Guana

• 4 BVI students are assisting

Four HLS Community College students are conducting research with scientists from several US universities during Marine Science Month on Guana Island this July.

Aasha Flax, Kirsten Lettsome, Denise Jones and Shanie Dasrath will contribute to several long-term studies by asking such questions as: Can we monitor long-term changes in our coral reefs? What creatures inhabit the sandy bottom between reefs? What is the role of sponges on coral reefs? Can we control the reproduction of wetland mosquitoes?, according to Lianna Jarecki, an HLSCC senior lecturer in biology.

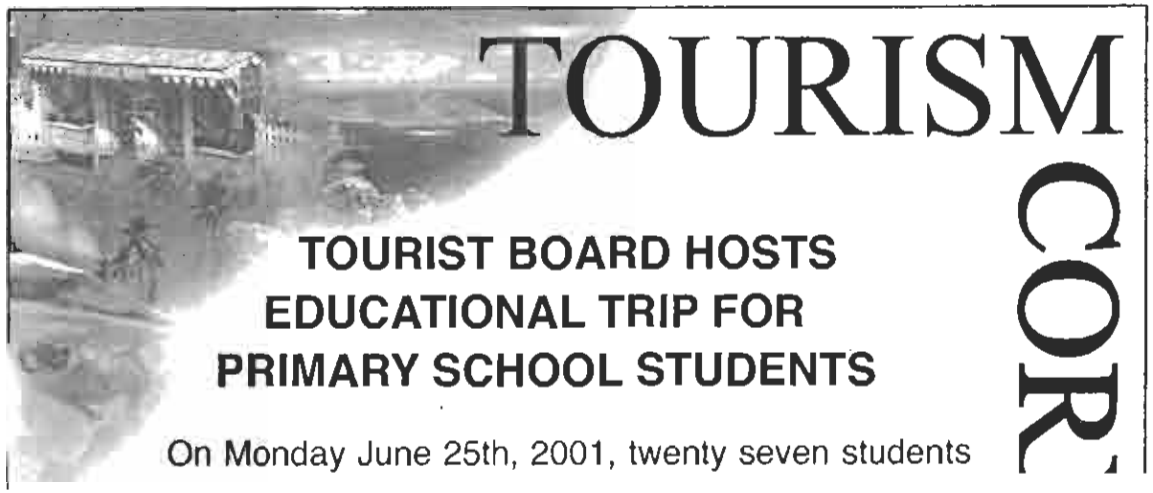
Marine Science Month on Guana is a time when marine biologists visit the island to study the ecology of coral reefs, seagrass habitats, rocky shores and mangrove wetlands. Ms. Jarecki coordinates this programme every year. Now in its 10th year, the programme has hosted many important studies and enjoys increasing recognition by the international scientific community, according to her. A comprehensive report of research results and publications is compiled yearly and distributed to relevant offices in the BVI, the biology lecturer said.

The participating scientists work closely with the community college, as well as mentor local high schools through the 'Marine Science Mentorship Programme.' There, between three and eight students work in collaboration with scientists in all aspects of their research from designing experiments to collecting data and analyzing results.

This year, scientists from the Los Angeles County Museum of Natural History, in cooperation with Ms. Jarecki, secured funding from the U.S. National Science Foundation to support three HLSCC students to conduct research full-time on Guana Island for this month. A fourth student has been funded by the Environmental Health Department to study the effects of high salinity on mosquito larvae. Each student will design his or her own research project in conjunction with the scientists involved in Guana's long-term marine monitoring programmes.

An ongoing component of Marine Science Month has been the participation of the LA County Museum and University of Rhode Island, Ms. Jarecki said. This July, the LACM has sent out Todd Zimmerman, Dr. Joel Martin and several other researchers to continue their survey of small marine invertebrates around Guana. The study reportedly aims to describe the physical characteristics, habitat and general ecology of small invertebrate species, most of which are less than one inch long. A unique feature of this programme will be the development of a teaching tool, including full colour photographs with descriptions, within a web site that will be available to marine biology students and teachers in the BVI, according to the senior biology lecturer.

"[It] is the first time HLSCC students will conduct independent research projects during Marine Science Month, providing a rewarding experience for both the students and the scientists on Guana this year," Ms. Jarecki noted. Tomorrow, the scientists and students participating in Marine Science Month are to present a public symposium on marine research at the community college in Paraquita Bay from 5:00-7:30 p.m. All are invited to meet the scientists and students, she said.



The New Cafeteria at the HLSCC will be opened on Thursday, November 1, 2001. Please come out as we are catered to by the NECI@HLSCC students.

Bulletin

EVENT	DATE
October 26	Heads of Depts. Meeting
	"Classics in the Atrium" Jennifer Koh
October 27	College Classic - VG
October 28	Wright State University MBA Graduation
November 2	Faculty Meeting
November 3	HLSCC 2-Mile Classic
November 7	Public Lecture Series, Austin Percival
November 9	"Jazz Showcase" Duane Eubanks Quintet
November 10	College Classic - VG
November 30	Heads of Depts. Meeting

"I shall be telling this with a sigh
Somewhere ages and ages hence:
Two roads diverged in a wood, and I—
I took the one less traveled by,
And that has made all the difference."

-Robert Frost
The Road Not Taken

Info Update is a publication of HLSCC

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Student, Tashima Barzey, at a research lab on Guana Island learns to distinguish between a wasp and a moth that mimics the physical characteristics of a wasp.

BIOLOGY STUDENTS VISIT BIOLOGICAL RESEARCH PROGRAMME ON GUANA ISLAND

Two second year A-level biology classes, and a general ecology class, along with several members of faculty and staff, conducted a scientific expedition to Guana Island on October 19th. Students were introduced to several research projects by biologists participating in Guana Island's annual scientific research programme. These biologists later visited HLSCC to give presentations of their research findings at the annual Guana Island Natural Science Symposium, which took place on October 24th. On Guana, the students learned about native snakes, lizards, and a variety of insects, including some unique moths that look like stinging wasps. The expedition culminated in a long hike that involved counting hundreds of century plants under attack by a recently introduced beetle. The data collected by the expedition was used by the Guana biologists to estimate the level at which the beetles (a large species of weevil) are infecting the native century plants. Evidence of similar century plant infection has also been noted on Tortola.

After the long hike, the group donned snorkeling gear and swam over a lovely coral reef in Muskmelon Bay.

Distance Education Programme Launch

The mission of the College focuses on providing educational opportunities to the people of the Territory, and dedication to this objective was evident on Tuesday, October 2, 2001 with the launch of the Distance Education Programme at the H. Lavity Stoutt Community College. This new programme allows students at HLSCC to connect with other students worldwide using two-way video and audio in "e-classes." A wide range of courses will be available to students, from health and education to business and technology, from a number of institutions in the United States. At the launch, President Wheatley spoke of the potential of the programme, noting that 'the scope of e-classes is tremendous', and that it is hoped that availability of this medium will allow everyone to pursue his or her dream.

This programme is facilitated through a partnership with New York-based Educational Video Conferencing Incorporated, with technical support from Cable & Wireless.

Above: The launching of the Distance Education Programme at the HLSCC
Below: Faculty members Lianna Jarecki and Claude McNamarah inspect a century plant damaged by weevil larvae.

Guana Island student research programme has led some to careers in science

By SUSANNA HENIGHAN

Taking part in scientific research on Guana Island has led some of Liana Jarecki's students to choose career paths in the biological sciences.

This is one of the positive effects of the island's ongoing scientific research programme, Jarecki, a biology lecturer at H. Lavity Stoutt Community College, said during a presentation at the Guana Island Symposium on Friday, Oct. 18.

"Perhaps our greatest achievement is that students leave Guana understanding that biological research is a viable and exciting career path," Jarecki said. "Several of my own students, after leaving HLSCC and continuing their studies at various U.S. universities, have chosen to follow careers in research biology. Some of these students have told me that before their experiences on Guana, they considered only a career in medicine and had not realized the breadth of career possibilities in science."

The experience is an eye-opening one, former students say. "The close interaction with other people was very beneficial - meeting people that can help you get started in their field of work," Jevaun Decastro said after working on Guana in 1998.

"Guana Island is the perfect classroom," was Lesley Husbands remark in 1999.

These observations are sure to make Henry and Gloria Jarecki happy. The owners of Guana Islands, the couple welcome research scientists to their part-time home every year. In addition, they invite local students to come observe the scientists, and carry out research of their own.

The student research programme has been going on for the last 15 years, and has been happening in partnership with H. Lavity Stoutt Community College for the last nine years.

Liana Jarecki's presentation on the impact the research programme was one of several presentations made last Friday as part of the annual Guana Island Scientific Symposium.

Other presenters discussed biological research they have carried out on Guana Island. Topics include global biodiversity, migrant birds and melons.

Over the years, local students have been exposed to a range of scientific topics at Guana, from coral reef monitoring in 1994 to marine invertebrates in 1999. In addition, the island has opened its doors to youth groups like the Pathfinders and the Youth Environmental Service corps.

In addition to welcoming students to work with them, scientists taking part in the programme have also been offering the public lectures since the programme started.

♦ HLSCC students conduct research on Guana Island in 1999 under the supervision of marine biologists from the Los Angeles County Museum of Natural History. (Photo courtesy of Liana Jarecki)

BVI named to anti-money laundering body

Region raises concerns over IMF review

By SUSANNA HENIGHAN

The British Virgin Islands has become a member of the Caribbean Financial Action Task Force Steering Committee, the body that makes recommendations to the CFATF ministers.

The decision was made during a recent meeting of the CFATF held Oct. 15-16. Attorney General Cherno Jallow represented the territory at the meeting, along with Crown Counsels Jo-Ann Williams

Monday, Chief Minister Ralph O'Neal said that during the reeent meeting, concerns were raised about the ongoing review by the International Monetary Fund of off shore business centres. The IMF is in the midst of reviewing a number of Caribbean jurisdictions.

"Standards may be applied about which the Territory had not been fully informed previously and which were not applied to jurisdictions that have already undergone IMF assessments," O'Neal said Monday. "As a

visit the BVI during the second week of November.

In other finance related news, O'Neal said he would be attending a roundtable discussion between Eastern Caribbean heads of state and World Bank President James D. Wolfensohn in St. Kitts Nov. 26-27.

As the OECS' spokesperson on financial services matters, O'Neal said he would discuss the region's efforts to meet international finance standards.

Financial services are just one

Tourism Month starts Friday

Friday, Nov. 1, marks the beginning of Tourism Month. This

Century plant devastation

• Importation of foreign species suspected

BY JAMES OSBORNE

When scientists arrived on Guana Island last month for their annual field study, the news was not good for the century plant.

Almost all of the plants, also called agaves, found on Tortola, Beef Island, Guana, and the Camanoes are dead or dying.

To blame is a 3/4-inch weevil, which burrows into the plant and lays larvae, which eat the host. Dr. James Lazell, director of the Guana Island Wildlife Agency and president of the U.S.-based Conservation Agency, thinks the weevil was first introduced to the BVI through the importation of another species of agave native to the southwestern United States commonly used ornamentally in gardens.

Those plants possess natural defences that keep the weevil at bay until they've already flowered — agave only flower once and then die.

But native agave lack those defences and are being killed off before they have a chance to flower and reproduce. Their numbers have dropped off rapidly since the Guana scientists first noticed the problem last October.

Now the weevils have been found on Jost Van Dyke, a puzzle for scientists. The creatures can fly, but usually only for short distances. In the case of the outer islands such as Guana, Dr. Lazell said, evidence suggests the weevil flew across from Tortola — a

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Century — from p. 1

much shorter distance.

"Maybe if the wind was right they could have made it (to Jost Van Dyke)," said Dr. Lazell.

The weevil epidemic hit the USVI in the mid-1980s. While century plants there were obliterated almost to the point of extinction, Dr. Lazell said they are now making a comeback.

There is hope here, too. One in 20 of the BVI agave is immune to the weevil, and while scientists are not sure why, Dr. Lazell theorised those plants might be producing a chemical repellent. There is also the tendency in any ecosystem to move towards equilibrium, namely that another species, in this case the parasitic wasp, will eventually move in and bring the weevil population under control.

"If you don't have total extinction there's a good chance you'll have recovery," said Dr. Lazell.

But there are a number of birds and insects, most notably butterflies, which rely on the century plant as a food source. While scientists haven't observed any decline in their populations yet, Dr. Lazell said it is expected to come soon.

"There are alternate food sources for these animals in the drier months. The crutch will probably be next winter," he said.

Nov. 14th 2002

Students and teachers get ready to return from a recent trip to Guana Island. (Photo provided)

Students, teachers tour Guana Island

Twelve students and five teachers representing four schools sailed from Tortola on Oct. 25 to meet scientists on Guana Island, where they also benefited from an extensive tour of the prominent wildlife sanctuary there.

The pupils were the first-place winners of the project presentations during the National Science Fair on May 28.

The students were Tiara Grant, Yafriesy Ramirez, Sonjah Smith, Shalya Springette, Tanesha Allen, Kevon Smith, Marisa Malone, Meshach Pierre, Travis Walters, Liselin Fraser, Kishira Martin and Diedra Thomas.

Beverlie Brathwaite, education officer for science and mathematics, said that, while the ministry organised the fair, sponsors' contributions were invaluable to its overall success.

The scientists who do research on Guana Island are from countries including Australia, Austria, Bermuda, Canada, China, Croatia, Dominica, England, Finland, Israel, the United States, and the BVI.

Based at research universities, public museums and government agencies, they are mostly professors, teachers, students and retirees.

The scientists study marine life as well as plants, mushrooms and animals, ranging from insects to birds and mammals.