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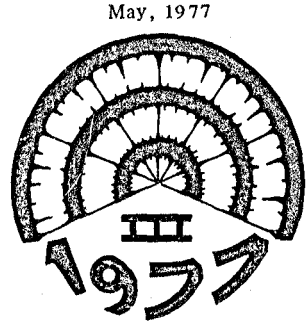

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BIOMASS AND NUTRIENT FLUX MEASUREMENTS ON HOLOTHURIA ATRA POPULATIONS ON WINDWARD REEF FLATS AT ENEWETAK, MARSHALL ISLANDS

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ABSTRACT

A population survey of the holothurian, Holothuria atra, on the interisland coral reefs of Enewetak revealed average animal densities of about 3 animals m^{-2} in the zone of small coral heads. Median fresh weight was 60 g. The size distribution of these animals was negatively correlated with water velocity along the reef.

Ammonia release rates for three species of holothurians, H. atra, H. difficilus and Actinopyga mauritiana were weight specific. The release of ammonia by H. atra on Transect II was equivalent to 9% of the total ammonia exported from the reef proper. The release of phosphorus followed the general rules set for size - metabolism relationships. Nitrogen/phosphorus release ratios are 25:1 for 1 gram fresh weight animals and 42:1 for 60 gram animals. Urea is also released.

Analysis of sediments, gut contents and fecal pellets indicates that H. atra is a selective feeder with an assimilation efficiency of about 40%. Dissolution of calcium carbonate by holothurians is estimated to be about 2.5 g $m^2 day^{-1}$, equivalent to 25% of the net calcification rate of the reef proper.

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KEY WORDS: Holothurian, Holothuria atra, Ammonia, Phosphorus, Nitrogen.

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Introduction

Coral reefs are particularly interesting ecosystems in which to study nutrient cycling because they typically exist in oceanic environments of quite depauperate inorganic nutrients. At the same time they show quite high rates of organic production and a large standing biomass of organisms. Odum and Odum (1) report that about 40% of the herbivore biomass in the zone of small coral heads at Enewetak is holothurian. While mean values of 0.5 (2) to 3 (3) individuals per square meter seem characteristic of reef flat areas, we have routinely observed Holothuria atra on shallow interisland reef flats in densities where it was impossible to walk without stepping on one (cf. also Fig. 2, reference 4).

While we recognize that it is inappropriate to equate "conspicuousness" with functional importance in ecosystems, the striking abundance of holothurians on Enewetak reef flats has led us to ask how great a role these organisms play in the flux of nitrogen and phosphorus in such locations. In an attempt to answer that question, we initiated this study while on the R/V Alpha Helix Symbios Expedition (5) in 1971. In keeping with our ultimate desire to produce a computer simulation model of nutrient flux on an Enewetak coral reef, the data presented here will be expressed in a format suitable to such an aim.

Methods

Animal incubations and sampling:

All experiments were carried out with freshly collected animals. Incubations were usually started within one hour and never longer than three hours of collection. Incubations during Symbios were performed in a one liter plexiglass chamber in a fashion used by D'Elia (6). Zero time and terminal one half hour samples were taken for N & P analysis on seventeen individually incubated animals. Similar incubations were carried out during January 1973 on Holothuria difficilis Semper collected from the quarry at the north end of Enewetak Island. Six animals were paired in each of 10 incubations in 500 ml specimen jars. Water was sampled at the start and after one hour. Temperature was controlled by running seawater around the chambers exposed to north sky light, at the Mid-Pacific Marine Laboratory (MPML).

Between 21 September and 6 October 1976, specimens of H. atra were collected from the interisland flat north of Enewetak Island. Actinopyga mauritiana was collected from the algal ridge at the same location. Animals were incubated in groups of from 1 to 9 depending upon size in a 6 liter recirculating incubator in the laboratory. Temperature was controlled at 27±1°C by placing the recirculating pump and delivery tubing in a temperature controlled heating/cooling water bath. Incubations were aerated. Samples were taken at 5 minute intervals for ammonia and at 15 minute intervals for total nitrogen (TN) and total phosphorus (TP) for periods of between 2 and 4 hours.

Chemical analyses:

Analyses for N and P species during Symbios are as reported previously (6, 7). Urea was analyzed by the method of Newell et al (8). The 1976 analyses utilized a scaled down 5 ml sample size for ammonia and modification of the Solórzano method (9) utilizing dry bleach and color development in the dark for TN and TP. Persulfate oxidation (10) was carried out on 10 ml samples which were subsequently analyzed for PO_4^{-3} and NO_3^-. Semi-automated methodology was used for NO_3^-, a peristaltic pump being used to control flow through the Cd column and to add reagents (11), 5-6 ml samples were collected for reading manually in a 1 cm cuvette on a Beckman DU spectrophotometer.

Animals were allowed to void their guts before being dried for tissue analysis. Tissue, sediment, gut contents and fecal pellets were analyzed for particulate CHNP. CHN was analyzed on either the Perkin-Elmer 240 or F & M 185 CHN analyzers. P was determined according to the method of Ketchum and Vaccaro (12).

Population survey:

Population surveys of H. atra were carried out on both transects II (tr II) and III (tr III) during the Symbios expedition. A metal one meter square was moved to cover successively every square meter from 0 to 245 m on tr II and from 0 to 140 on tr III; at every 5th meter along the transects, three lateral m^2 quadrats were also counted alternating left and right of the transect line. All the individuals of every fifth quadrat along the transect, ignoring lateral samples, were weighed individually on a dual pan balance in an accompanying skiff. These procedures were carried out once along tr II and twice along tr III.

Seventeen individual animals along tr II were observed in situ for 1 hour each and the number of fecal pellets produced recorded. Most

of the fecal pellets were successfully collected, weighed and frozen for subsequent CHN analysis. Fresh weights of the observed animals were also determined after the defecation observations.

Results and Discussion

Population survey:

Mean fresh weights and frequency of H. atra along tr II and III of the Symbios expedition (5, 13) are reported in Table 1. On tr II we observed an average of 3.03 animals m^{-2} for the zone from 130 to 230 meters. This zone approximates that of the zone of small coral heads described by Odum and Odum (1) who reported 1.9 animals m^{-2} of approximately the same size for this location. Larger animals (to about 1 kg fresh weight) occurred further downstream within the zone of larger coral heads.

Table 1. Size and frequency distributions of Holothuria atra along two transects during the Symbios expedition. Data columns are meters along the transects, mean g fresh weight ± standard error and mean number of animals per square meter ± standard error. Number of observations in parentheses.

Transect II

Meters	$\bar{X}$ weight		$\#/m^2$	
0-129	206	(2)	0.1	(118)
130-149	66	± 8.7(8)	2.7 ± .55	(24)
150-169	62	± 9.0(14)	4.06±0.58	(32)
170-189	57	±14.6(8)	2.22±0.44	(32)
190-209	31	± 7.4(12)	3.59±0.69	(32)
210-229	79	±26.9(13)	2.53±0.40	(32)
230-245	151	±38 (2)	0.56±0.13	(25)

Transect III

0-39		(0)	0.83	(29)
40-59	61.3± 5.9	(14)	2.73±0.284	(64)
60-79	111	± 9.6(13)	1.48±0.199	(61)
80-99	121	±25.4(7)	0.48±0.098	(67)
100-119			.24	(34)
120-139	287	(1)	.32	(25)

Tr III consisted principally of open algal pavement (13) with few structures extending into the water column. Here the maximum density occurred between 40 and 60 m behind the algal ridge and decreased gradually downstream. Weight of animals was linearly related to distance down the transect according to the equation:

$$y = 2.04x - 28.6 \qquad (1)$$

where y is g fresh weight and x is the distance in meters behind the algal ridge. The regression coefficient, 0.65, was significant at the <0.001 level. It appears that along this transect individuals of H. atra are segregated on the basis of current energy of the environment, as the current force diminishes with increasing depth along the transect.

The median sized animal observed was 60 g fresh weight which agrees well with the report by Bonham and Held (4) for Rongelap Atoll in September 1961. Unlike that report our data show a larger percentage of small animals. We are of the opinion that one sampling in time is inadequate to say anything about apparent age classes and the presence or absence of sexual reproduction.

Nutrient regeneration:

Metabolic rate (M) can be expressed as a power function of body size (W) according to the equation:

$$M = KW^b \qquad (2)$$

where K and b are constants. For data manipulation we have used a linear transformation:

$$\log M = b \log W + \log K \qquad (3)$$

This relationship has been widely investigated for oxygen consumption and body weight (Table 5-2 in reference 14) with the value of b generally around 0.75. A value of 1 for b indicates a constant weight specific metabolic rate for various sizes of organisms. Although it has been recognized (15) that this relationship should exist for parallel metabolic processes such as release of carbon dioxide and waste products, the principal extension has been in the area of phosphorus excretion (16). Such data formats are convenient in that they are quite compatible with inputs for ecosystem modeling.

Nitrogen release:

Our most extensive data set is for release of ammonia for the three species, H. atra, H. difficilis and A. mauritiana, covering a fresh weight range of about 3 orders of magnitude. These data are presented in Fig. 1 and Table 2, and indicate that the weight specific rate of ammonia release remains constant within our data set. These results are rather surprising, especially in view of the observed values of b between 0.6 and 0.8 or lower for oxygen consumption in Stichopus japonicus (17) and for P for H. atra (this paper) or for a variety of Echinodermata at Enewetak (18).

Thus, on the basis of some possibly tenuous assumptions, it appears that the O/N ratio will be significantly different for small species or small individuals compared to large species or large individuals. The implication is that the large animal either regenerates or produces more N per unit of oxygen consumed than does a small animal. If true, this is precisely the reverse of what might be expected on the basis of similar studies of zooplankton P excretion (15, 19). Explanations lie either in the physiology of the animal or in the possibility that nitrogen

fixation is occurring within the guts of these animals. We did, during Jan. 1973, subject a number of whole, living specimens of H. difficilis to the acetylene assay for nitrogen fixation with negative results. In retrospect we should have used excised gut contents in the assay and animals larger than H. difficilis.

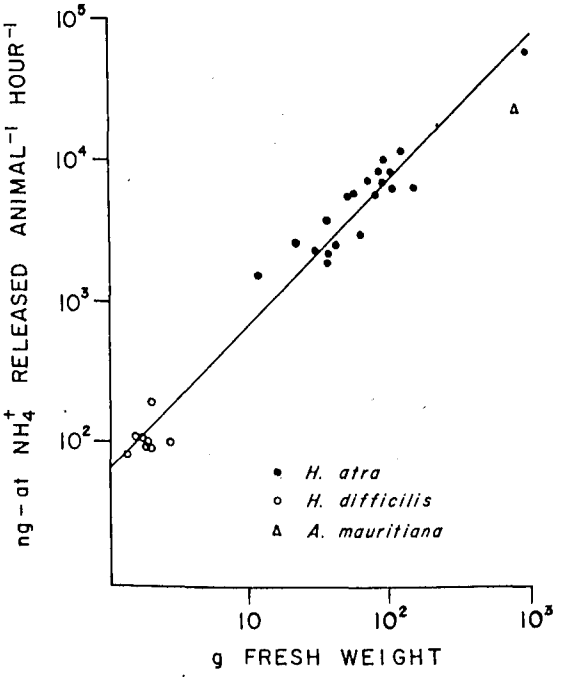

Figure 1. Relationship between fresh weight and ammonia released by three species of holothurians at Enewetak Atoll.

Our data for TN release (Table 2), although sparse, do indicate that the size metabolic rate relationship for TN is about the same as for ammonia. This is in part due to the fact that more than half the N released is in the form of ammonia, i.e. these holothurians are ammonotelic. Our results with the Newell et al (8) urea method indicated that H. difficilis releases urea-N at a mean rate 29% ($s_{\bar{x}}$ 3.8, n-10) of the ammonia rate during one hour incubations. If we apply our equations for TN and ammonia release with the urea data, the proportionality would be 57% ammonia, 17% urea-N and 26% unidentified N compounds for a 2 g fresh weight H. difficilis. This agrees well with the results reported for the echinoderm, Asterias sp., where 39% of the TN was NH_4^+ and 12% was urea-N (20). If we assume that larger animals also release urea-N in about the same proportion to ammonia as H. difficilis, then the unidentified fraction should become smaller with increasing animal size.

Evidence concerning the significance of the N released by holothurians is difficult to evaluate. Ammonia released by H. atra on transect II is equivalent to about 9% of the reported (7) total ammonia exported. If Redfield ratios are applied to predict N release based on the respiration measurements (21) on the reef, then H. atra would contribute only about 0.6% of the N released by remineralization on tr II. This indicates that one should be cautious in using Redfield ratios to predict flux of one nutrient on the basis of another.

Table 2. Release of NH_4^+, total dissolved combined nitrogen (TN), PO_4^{-3} and total dissolved phosphorus (TP) where M = nanogram-atom N or P released per gram fresh weight per hour according to the formula $M = KW^b$. r is the regression coefficient for the linear transformation, equation 3. n equals the number of observations, repetitive measures on single incubations being considered single observations.

	b	k	r	n	range of fresh weight
NH_4^+	1.01	68.1	0.98	30	1.3-970g
TN	0.938	125.	0.99	3	40-970g
PO_4^{-3}	0.657	3.83	0.71	12	12-125g
TP	0.810	5.02	0.81	18	12-970g

Phosphorus release:

Our P release data is only for H. atra and is reported in Table 2. The PO_4^{-3} data are exclusively from Symbios while the TP data also include the addition of 4 serially sampled incubations from 1976. The values of b, 0.66 and 0.81 for PO_4^{-3} and TP respectively, agree reasonably well with the value of 0.67 reported by Johannes (15) for marine animals above 1 mg dry weight. Pomeroy and Kuenzler (18) also report that their echinoderm data fit the Johannes regression quite well.

Our regression equations for TN and TP, Table 2, result in N/P atomic ratios of 25:1 for 1 g fresh weight animals and of 42:1 for 60 g animals, the median sized animal on transect II. Thus at smaller weights the N/P ratio approaches the Redfield ratio and increasingly departs from it at higher weights.

N & P animal composition and turnover times:

CHNP analyses of dried whole animals from the 1976 sampling are reported in Table 3. CHN

data from Symbios are essentially identical for the smaller size class of animal. It appears from these data that N and P percentage composition may be inversely related to animal size. Mean percentages of P reported for H. atra and H. difficilis (18) are in agreement with this observation, but we feel that more extensive documentation will be necessary before N & P turnover times can be evaluated relative to body size. Preliminary calculations based on TN and TP release rates (Table 2) and percentage composition (Table 3) indicate turnover times of 284 and 116 days respectively for N & P for animals averaging 43.9 g fresh weight. This P turnover time is quite close to the published values of 120 days for H. difficilis and 150 days for H. atra (17). The similar calculation for the 127 g animals gives values of 147 days for N and 121 days for P. Thus N seems to turn over more slowly than does P in H. difficilis and the median sized and smaller H. atra.

Assimilation efficiencies:

Analysis of the organic carbon content of coral reef sediments is complicated by their calcium carbonate nature. H. atra has generally been considered within a group of non-discriminate sediment feeders which "simply shovel the surrounding substrate into their mouths by means of the tentacles" (22) although Yamanounti (2) clearly showed selective feeding by H. atra and Stichopus chloronotus. Despite the work of Emery (23), the conclusions of Trefz (23), that no dissolution of calcium carbonate occurs during passage of carbonate structures through the guts of H. atra, have also been perpetuated. Within this conceptual framework we collected surface sediments adjacent to feeding H. atra and their fecal pellets from Symbios tr II in 1971 for CHN analysis by two temperature combustion (25). This combustion is designed to differentiate between organic and inorganic carbon. The sediment was 0.40% organic C and 12% inorganic C by dry weight. The fecal pellets were 1.30% organic C and 11.6% inorganic C. There is no indication of a lower concentration of either N or organic C in the fecal pellets than in the sediment (Table 3). Thus 3% of the total C in the sediment, the presumed food, was organic. This is in reasonably good agreement with the report (26) that the ash-free weight of Enewetak quarry sediments ranged from 4.72 to 6.02%. Surprisingly, 10% of the fecal pellet C was organic. Dissolution of calcium carbonate, selective feeding, or both could account for this observation.

During September, 1976, we separated the gut contents of H. atra into fore, mid and hindgut fractions for CHNP analysis. These data are also included in Table 3. A one-way analysis of variance showed highly significant differences for C, H and P but not for N. Perhaps the higher relative variability for N obscured any differences or alternatively, N does not decrease during gut passage. The apparent assimilation efficiencies, (foregut-hindgut) (foregut) (100), are 9.5, 37. and 36% for C, H and P respectively. These values for H and P are very similar to the 40% calculated for H. difficilis (27) from the data of Bakus (26). Thus, we will in our subsequent data manipulations assume a 40% assimilation efficiency for C as well.

An evaluation of possible carbonate dissolution using the gut content data, assuming that assimilation efficiency is 40% and that 3% of the sediment carbon is organic, indicates 8.5% dissolution of the ingested carbonate between the fore and hindgut. A correction based on this value would raise the P assimilation efficiency to 41.5%. Similarly the organic C content of the food can be projected to be about 24% of the total C using the fore and hindgut C content and assuming no dissolution, i.e. (13.73-12.43) (0.4) (13.73) = 0.237. The lower limit would be 9.5% organic C of total C with a 100% assimilation efficiency. The alternative calculation with the fecal pellet data, (1.3% organic C, 11.59% inorganic C by dry weight), projects the C in the food to be 14.8% of total C if 8.5% dissolution is assumed or 15.8% if no dissolution is assumed. Our data therefore seem consistent with the hypothesis that H. atra is a selective feeder and that some dissolution of calcium carbonate takes place in the gut.

Fecal pellet production and sediment reworking:

The 17 animals observed on tr II released fecal pellets of mean dry weight of 0.4 g at a rate of 8.5 h^{-1} (range 0-15, $s_{\bar{x}}$ = 1.03). This daily rate of 82 g agrees well with the published value (2) of 86 g day^{-1} of calcareous sand eaten per individual H. atra. Thus, undoubtedly individual holothurians ingest kilogram quantities of sediment $m^{-2}yr^{-1}$. The effects this has on the microbiota of the sediments are likely to be extensive and to our knowledge are uninvestigated.

Dissolution of calcium carbonate by holothurians would appear to be measurable directly from short term incubations and total alkalinity determinations. Although we have not done this, earlier in this paper we established limits of 0 to 8.5% of the ingested material for dissolution by H. atra. Mayor (28) reports an experiment directly measuring dissolution which indicates values of 0.64 and 1.13 g day^{-1} $animal^{-1}$. In the absence of additional data, conservative estimate of 1% dissolution of the ingested material seems very reasonable for preliminary calculations. Thus on tr II, the holothurian zone from 130 to 230 m in back of the algal ridge might be expected to produce about 2.5 g m^{-2} day^{-1} dissolution by H. atra. This appears to equal what might be expected from a 50% cover of boring sponge and greatly exceed that contributed by fish (see p. 117 in reference 13). As the calcification rate by

Table 3. Elemental analysis as weight percentage of dry weight of tissue, sediment, gut contents and fecal pellets of H. atra ± standard error with number of observations in parentheses. Each gut sample consists of material pooled from 5 individuals.

	C	H	N	P
Tissue**	27.0 ± 1.64 (5)	4.23 ± 0.238 (5)	6.95 ± .41 (5)	0.156 ± 0.0127 (5)
Tissue***	17.8 (2)	2.02 (2)	2.77 (2)	0.109 (2)
Sediment	0.40*± 0.149 (4)	1.24 (4)	0.39 ± 0.0192 (4)	--
Foregut	13.73 ± 0.178 (6)	0.72 ± 0.047 (6)	1.2 ± 0.30 (6)	0.060 ± 0.0063 (6)
Midgut	12.61 ± 0.051 (6)	0.483 ± 0.0287 (6)	0.72 ± 0.200 (6)	0.0415 ± 0.00182 (6)
Hindgut	12.43 ± 0.108 (6)	0.457 ± 0.0272 (6)	0.75 ± 0.202 (6)	0.0383 ± 0.00126 (6)
Fecal pellets	1.30*± 0.251 (7)	3.42 ± 1.14 (3)	0.154 ± 0.0098 (7)	--

*based on 2 temperature method (25) for organic carbon
**$\bar{x}$ fresh weight of animals, 43.9 g. ± 3.5 (5); dry weight, 5.95 g. ± 0.209 (5).
***$\bar{x}$ fresh weight of animals, 127 g; dry weight 21 g.

the alkalinity reduction technique for the whole transect is about 10 g m^{-2} day^{-1} (13, 29), the rates associated with dissolution of calcium carbonate by holothurians are large enough to warrant inclusion in carbon flow models of the coral-reef ecosystem (30).

Summary

Median sized individuals of H. atra on interisland transects of Enewetak were about 60 g. fresh weight. Along these transects the size of the animal appeared to be inversely related to the velocity of the water. Population density averaged about 3 individuals per square meter from 130 to 230 meters behind the algal ridge on the transect adjacent to that of Odum and Odum (1).

Analysis of sediment, gut contents and fecal pellets indicates that H. atra is a selective feeder, ingesting and egesting materials considerably richer in organic carbon than the adjacent sediment. Assimilation efficiency is about 40%.

A major ecological role of H. atra appears to be reworking of sediments. The median sized animal passes about 80 g. dry weight of sediment per day. A preliminary estimate of 1% dissolution of ingested calcium carbonate indicates a dissolution rate equal to 25% of the net calcification rate on the reef flat.

Mineralization of phosphorus by 3 species of holothurians appears to follow classical body size metabolism relationships. In contrast, weight specific nitrogen release was found to be the same over three orders of magnitude of body size.

The holothurians investigated are primarily ammonotelic; however, 17% of the total nitrogen released is urea nitrogen. They account for an estimated 9% of the net ammonia regeneration on Symbios transect II.

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