AN ABSTRACT OF THE THESIS OF David R. Hopper for the
Masters of Science in Biology presented March 27, 1990.

Title: Reproductive Biology of <u>Actinopyga</u> <u>mauritiana</u>
(<u>Echinodermata</u>: <u>Holothuroidea</u>) on Guam.

Approved: _____
Robert H. Richmond, Chairman, Thesis Committee

<u>Actinopyga</u> <u>mauritiana</u> is a dioecious,
aspidochirote holothurian of Indopacific reefs,
typically found in high energy, intertidal areas.
Females and males both show seasonal peaks in gonadal
index during spring and summer months. Mature gametes
could be found in some animals throughout the year, but
spawning occurred in late spring and summer. Most
animals reach sexual maturity at a weight of 200 g
(drained weight) or greater. Fecundity, as measured by
the number of eggs per unit wet weight of ovary, is high
compared to other tropical sea cucumbers, the mean value
being 6.2×10^5 eggs g^{-1}. The fecundity index (mean ovary
wt/oocyte diameter^3) for <u>A</u>. <u>mauritiana</u> was 3.1×10^4

during periods of peak gonadal index with a mean of 1.3×10^4 annually. Following fertilization of eggs, gastrulation is observed within 24 hr. The planktotrophic auricularia larval stage is reached by day 3 and may persist for over 20 days. Metamorphosis to the auricularia to the second larval stage, the doliolaria, may begin as soon as 6 days after fertilization, but generally was not observed until after 2 weeks following fertilization. The second metamorphosis results in shrinkage of the larvae in overall length by 41%. Recruitment of juveniles was observed, but these animals had already attained relatively large sizes, the smallest being 10 g whole wet weight, and the most being greater than 50 g.

Data suggest that to properly manage this species, harvest should be seasonal, following the completion of spawning. Reproductive populations can be maintained by taking adults larger than 400 g (whole wet weight), and by ensuring high adult densities.

REPRODUCTIVE BIOLOGY OF <u>ACTINOPYGA</u> <u>MAURITIANA</u>

(<u>ECHINODERMATA</u>: <u>HOLOTHUROIDEA</u>) ON GUAM

BY

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A thesis submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

IN

BIOLOGY

UNIVERSITY OF GUAM

APRIL 1990

ACKNOWLEDGEMENTS

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INTRODUCTION

There is an extensive literature on the reproductive
biology and life histories of many echinoderms (Hyman,
L.H. 1955; Giese and Pearse, 1974; Jangoux and Lawrence,
1982). These studies have concentrated on echinoids and
asteroids, while the remaining classes within the phylum
have received relatively little attention. Sea
cucumbers, class Holothuroidea, are frequently a
conspicuous and abundant component of the benthic fauna
in shallow tropical waters (Bakus, 1973; Kohn, 1987).
In many habitats they serve as agents of bioerosional
processes and play a role in cycling of nutrients
through detrital breakdown (Bakus, 1973; Birkeland,
1989; Hammond, 1981; Rhoads, 1973; Massin 1980).
However, much about their basic biology and ecology
remains unknown. In addition to their role in the
community there is growing interest in sea cucumbers
(beche-de-mer) for their value as a fisheries resource
(Beardsly, 1971; Gentle, 1979; Eyes, 1986). Thus for
ecological as well as economic reasons, a better
understanding of holothurian biology is needed.

To date, studies concerning the reproductive cycles
of tropical Pacific holothurians have largely been
conducted on species in the southern hemisphere (Conand,
1981, 1982; Harriott, 1985; Pearse, 1968). Their

results indicate that most species have a single annual reproductive peak as determined by stage of gamete development and gonadal index (gonad mass/whole animal mass, as a percentage). Both Conand (1981) and Harriott (1985) found that sympatric, congeneric species were asynchronous in their reproductive cycles. Conand (1981, 1982) found a correlation between reproductive cycles and ambient sea temperatures, while Pearse (1968) noted what appeared to be continuous reproductive effort in populations of <u>Holothuria</u> <u>atra</u> located nearer the equator. Little work has been conducted to determine size at first reproduction or the fecundities of sea cucumbers. Conand (1981) reported estimates for size at which sexual maturity was reached for three species, and noted some associated morphological changes. Harriott (1985) derived a fecundity index by dividing the mean weight of ovaries by the cube of the mean egg diameter. This index for three species of <u>Holothuria</u> varied by as much as an order of magnitude, but different reproductive strategies may explain this finding.

Although asteroids and echinoids are frequently reared under laboratory conditions, similar work has not been done with sea cucumbers (Kobayashi and Ushima, 1982), and none has been reported for tropical holothurians. Removal of holothurians for fishery export has virtually eliminated this resource on some

islands (Gentle, 1979). Rearing of larvae could find practical application in restocking of such reefs.

To date, studies that address or document the recruitment of larval sea cucumbers are lacking, and none are available for tropical species. Any observations concerning this phenomenon would be of value.

Actinopyga mauritiana is a conspicuous sea cucumber in intertidal and subtidal habitats throughout the tropical Pacific. Its great abundance (areas of Tumon Bay, Guam, were found to have as many as 0.67 m^{-2}, pers. obs., 1988) makes it an important member of the reef community. Its value in the beche-de-mer industry is also significant (Eyes, 1986; pers. obs, 1988), making the biology of this species an important factor in ecological considerations and in stock management.

The reproductive biology of Actinopyga mauritiana is of primary importance both in terms of community ecology and fisheries economics. This study will address the following basic questions concerning the reproductive biology of this species:

(1) What is the reproductive periodicity of
A. mauritiana, and does it exhibit a seasonal reproductive peak?

(2) At what size do individuals reach sexual maturity?

(3) What is the fecundity of A. mauritiana as measured by the number of eggs per unit of female weight and the weight of the ovary.

(4) Can gametes be fertilized and the larvae reared successfully in the laboratory?

(5) What is the developmental chronology?

(6) Can recruitment of A. mauritiana be observed in the natural habitat, and are there any observable trends?

MATERIALS AND METHODS

Adult Reproduction

Twenty-five to 30 individuals were collected from the study site at Tumon Bay, Guam (Fig. 1) at four to six wk intervals from April 1988 to October 1989. Animals were transported to a holding tank at the University of Guam Marine Laboratory in coolers filled with seawater. Individuals which spawned in transit were noted and removed from the tank to minimize the loss of gametes since spawning generally ceased when the animals were removed from water. These animals were included in the analysis if their gonadal index was similar to or above that of the animals which did not spawn. Individuals were later removed from their tank, allowed to drain for 10-20 min, and weighed (whole wet weight) to the nearest gram. Length measurements to the nearest cm were recorded prior to handling. The gonads were removed, coelomic fluids were drained from the body, and all sand removed from the gut. The weights of both the gonads and the drained tissues (drained weight) were then recorded. Gonadal indices were calculated as the ratio of wet weight of the excised gonads to the whole wet weight of the animal. A second gonadal index was also calculated using the drained weight.

During June and July of 1989 _Actinopyga_ _mauritiana_ covering a range of sizes were collected from Pago Bay
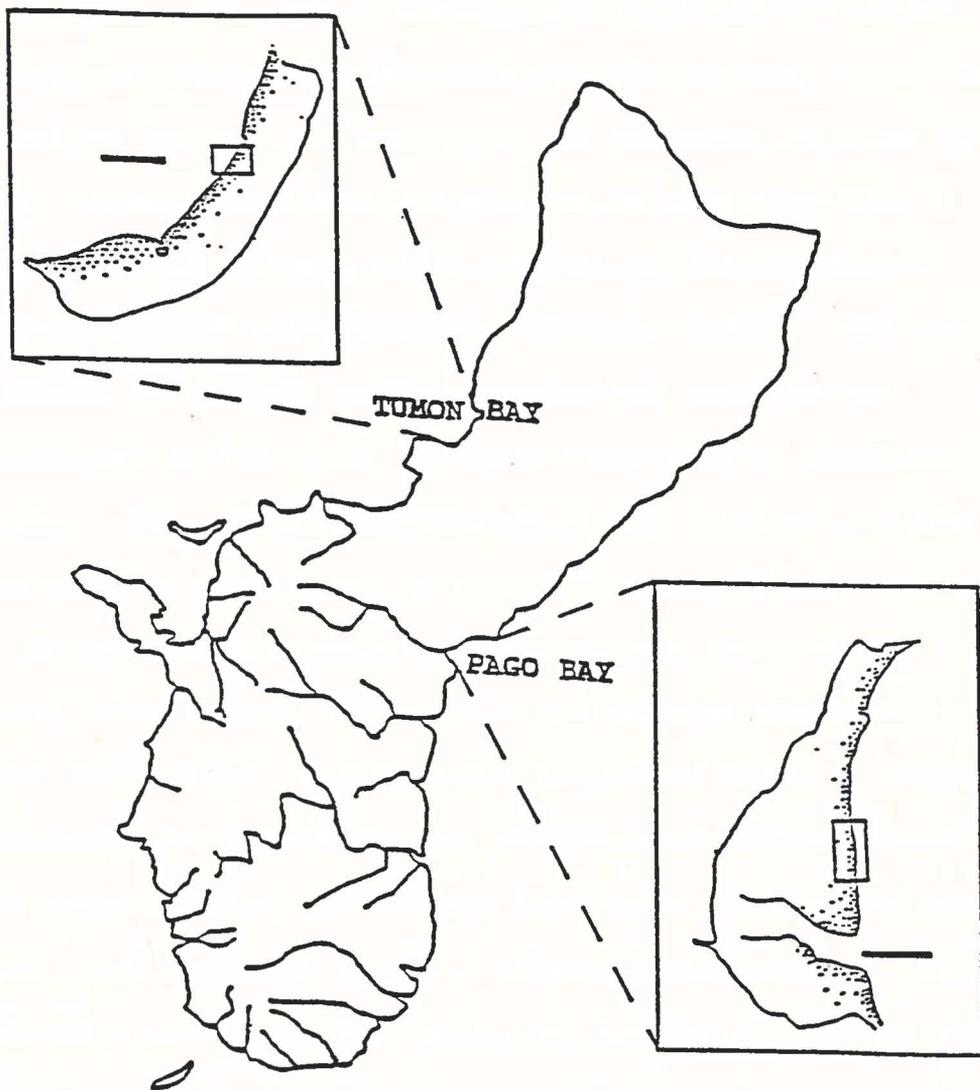
Figure 1. Collecting sites for <u>Actinopyga</u> <u>mauritiana</u> on Guam. Boxed areas within insets represent areas of collection. Scale bars are equivalent to 500 meters.

to determine the minimum size at which reproductive maturity is reached (body size when gonads with ripe gametes first appear). These collections concentrated on smaller individuals since large individuals which possess gonads were collected for the gonadal index data described above. In addition, an estimate of size at first reproduction was calculated by plotting the percentage of animals which contained discernable gametes against size classes (Conand, 1981). The point at which 50% of the animals contained gametes was designated as the size at which they become sexually mature. This method assumes that all animals are the same age, or that size is directly proportional to age.

Excised gonads were used to obtain information on gamete development, oogenesis and spermatogenesis, and adult fecundity. Testes were inspected under a compound microscope to determine the presence of active sperm. Eggs were sampled from three locations within the ovary, mixed, and measured under a compound microscope. Egg diameters were obtained using an ocular micrometer from the first 30 eggs encountered on a depression slide. Any additional portions of the gonad that appeared to represent different stages of development were sampled and additional microscopic features such as the presence of germinal vesicles, nucleolus, and egg membrane were noted.

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Mature ovaries were used to obtain an estimate of adult female fecundity, by determining the number of eggs per unit ovary weight. A small portion of ovary was removed and weighed to the nearest 0.1 g, then all eggs were rinsed free of the ovary wall with filtered seawater. The egg suspension was mixed thoroughly to suspend the eggs homogeneously, and was then poured into a dish marked with a 0.25 cm^2 grid. In six of these subgrids total egg counts were made and averaged to estimate the total number of eggs per unit weight. The mean wet weight of the ovary was divided by the cube of the mean oocyte diameter to obtain an index to allow comparisons of fecundity among holothurian species (Conand, 1981; Harriott, 1985).

Larval Biology

Attempts to raise sea cucumbers from gametes were conducted subjecting large adults to heat stress, a method known to induce spawning (pers. obs.). Spawned gametes were collected and mixed to obtain developing embryos. Embryos were kept in flasks (500, 1000, and 2000 ml) of gently aerated, filtered sea water. Penicillin was added at a concentration of 8000 units/ml at each water change to help control bacterial and ciliate infections. Water was changed every 2-3 d and the growth and development of the larvae were observed.

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The algae _Isochrysis_ spp., _Rhodomonas lens_, and the
diatom _Chaetoceros gracilis_ were used for larval food.
Larval densities were maintained at or below 1.0 ml^{-1},
and phytoplankton densities were kept between 5,000-
30,000 cells ml^{-1} (Lucas, 1973; Chen and Run, 1988).

Recruitment

Visual, qualitative searches for _Actinopyga_
mauritiana were conducted in Tumon and Pago Bays on the
reef margin and adjacent zones where the adults of _A._
mauritiana are most abundant. Searches were conducted
on exposed substrate, the under-surface of rocks, in
macroalgae, and in crevices while wading or snorkeling.
Since the smallest specimens of this species have been
found on the reef margin searches were most intensive in
this area.

RESULTS

Reproductive Periodicity

Actinopyga mauritiana exhibited a seasonal reproductive periodicity with regard to the gonadal index (Fig. 2). Sample means for gonadal indices (drained weight) ranged from 0.5% during the resting period to over 14%. Low values of 0.5% and 0.7% (sample means) were obtained for October of 1988 and August of 1989, respectively. By comparison, mean high values were 13.6% in June of 1988 and 14.0% in April of 1989 (Table 1). The indices for females and males were very similar, tracking one another throughout the study period (Fig. 3). Due to the variable quantities of liquids and sediments within the coelomic cavity and gut, drained weights were used as the most consistent measure of weight (Conand, 1981).

Developmental stage of gametes was not, by itself a good indicator of reproductive activity. Testes contained sperm which showed swimming activity throughout the year (Table 1). Oocyte diameters were measured from all females within each sample for one year, October 1988-September 1989. Although there was an increase in mean oocyte diameter which corresponded closely with the increase in gonadal index (Table 2), the variation in oocyte diameter within samples was sufficient to make average oocyte diameters between
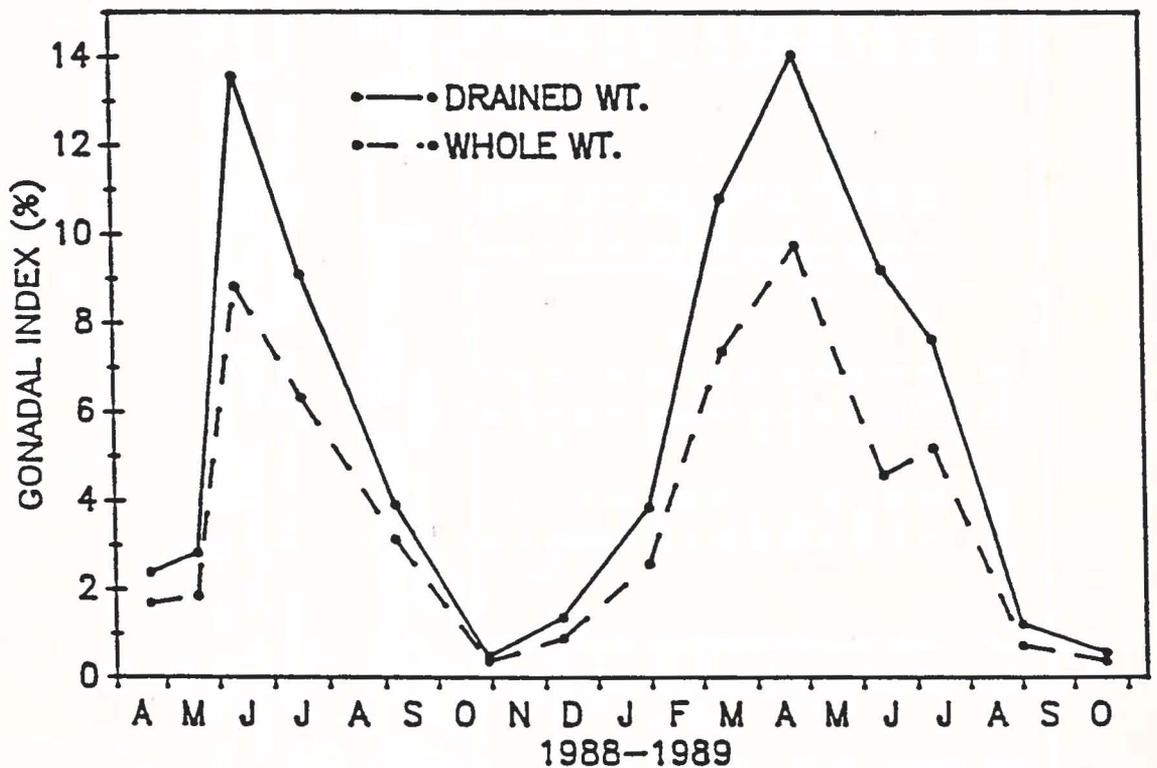
Figure 2. Mean gonadal index over time. Comparative
values for drained and whole wet weight (see Table
2 for samples sizes).

Table 1. Reproductive status of <u>Actinopyga</u> <u>mauritiana</u> during study period. Mean gonadal index, oocyte diameter, and approximate activity of sperm. ND: no data; NS: none present in sample; UNKNOWN: undetermined sex; S: standard deviation.

| DATE SAMPLED (DAY MO. YR.)	MEAN GONADAL INDEX (DRAINED)					MEAN OOCYTE DIAMETER (μm)	PERCENT SPERM ACTIVITY
	FEMALE	MALE	UNKNOWN	MEAN	S		
21 APR. 88	3.0	3.6	0	2.4	(3.4)	ND	ND
18 MAY 88	2.2	4.9	0.7	2.8	(2.9)	ND	ND
9 JUN. 88	8.8	10.7	0	13.6	(7.6)	ND	ND
15 JUL. 88	11.3	8.2	1.7	9.1	(6.8)	ND	ND
3 SEP. 88	5.5	4.5	0.1	3.9	(3.7)	118	ND
25 OCT. 88	0.7	0.8	0.1	0.5	(0.5)	107	100
6 DEC. 88	1.8	1.5	0.3	1.4	(1.4)	113	100
24 JAN. 89	3.6	4.1	0.4	3.8	(3.2)	109	100
8 APR. 89	11.9	9.6	NS	10.8	(5.3)	115	100
20 APR. 89	14.3	13.6	NS	14.0	(5.2)	112	100
7 JUN. 89	7.6	9.8	0.3	9.2	(12.7)	128	100
5 JUL. 89	8.3	7.1	NS	7.6	(5.6)	126	100
23 AUG. 89	1.6	1.0	0.2	1.2	(1.0)	121	76
10 OCT. 89	0.9	1.5	0.3	0.4	(0.2)	117	34
MEAN OF MEANS	5.8	5.8	0.4	5.8		117	
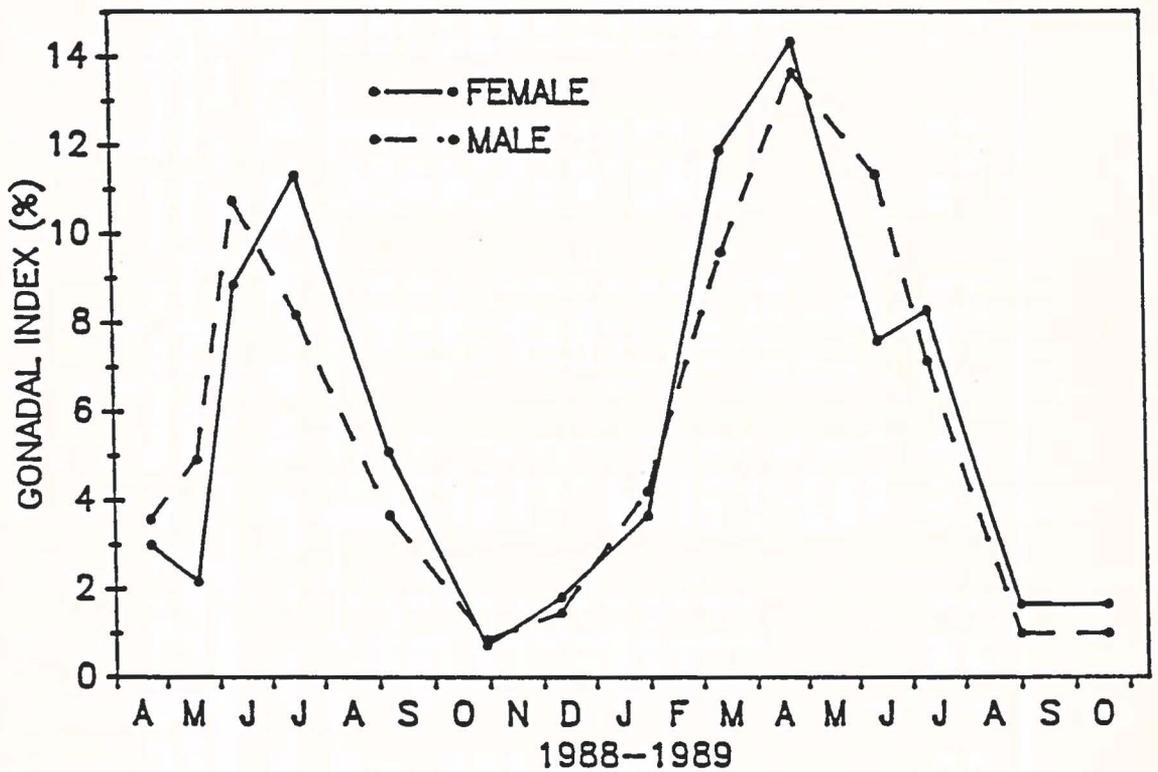
Figure 3. Mean gonadal index over time. Comparative values of female and male sample means (see Table 2 for sample sizes).

Table 2. Mean oocyte diameters from females of each sample period. Each value is the mean of 30 eggs taken randomly. Mean values and standard deviations of sample means and standard deviations are presented at the bottom of each column.

SEP. 88	OCT. 88	DEC. 88	JAN. 89	MAR. 89	APR. 89	JUNE 89	JULY 89	AUG. 89	OCT. 89
199.2	118.5	118.5	121.4	116.3	118.3	126.6	131.4	121.7	110.3
115.7	110.9	119.8	109.8	119.5	116.9	120.5	126.2	118.7	118.4
125.6	101.0	107.3	114.3	120.0	115.9	129.2	131.4	122.7	124.9
117.4	69.6	102.8	117.7	119.1	120.0	126.5	124.6	119.4	107.7
115.9	120.7	122.5	119.6	114.1	117.7	134.0	131.0	120.1	123.1
116.5	103.6	177.5	120.4	118.6	120.9	131.8	131.1	121.0	121.4
121.2	114.0	95.6	115.6	118.7	124.5	134.0	131.0	123.7	
114.9	115.4	118.4	23.9	120.4	120.5	129.2	124.9	121.8	
117.4		100.4	108.9	110.1	121.6	119.5	129.6	122.0	
123.2		130.9	114.6	120.7	121.0	130.9	130.0	121.4	
116.1		72.4	111.7	113.1	124.4	127.5	133.4		
118.1			114.3	123.4	118.9	121.4	129.7		
120.4			119.6	111.7	122.7		130.5		
				110.8	120.5		77.1		
				109.0	118.7				
				108.1	117.3				
				98.3					
118.2	106.7	109.6	109.0	114.8	120.3	127.6	125.7	121.0	117.6
3.1	15.4	16.3	23.9	6.1	2.6	4.8	13.7	1.7	6.4

samples not significant (single classification ANOVA, F=4.23, 8 df)(Sokal and Rohlf, 1981), and a great degree of variation was evident. Additionally, a regression analysis of mean oocyte diameter against mean gonadal index does not show a significant correlation between these parameters (r-value=0.54; 8 df). The ovaries from sample periods with low gonadal index frequently bore few eggs, and many ovaries contained eggs in the process of degeneration.

Size at First Reproduction

Animals collected from Pago Bay and from Tumon Bay in June and July of 1989 were placed in weight classes of 50 g intervals (whole wet weight), and the presence or absence of distinguishable gonads was noted (Fig. 4). The data indicate that the majority of animals (92.5%) above 200 g are mature, while about 47.4% of the animals between 100-200 g bore mature gonads. Actinopyga mauritiana is predicted to become sexually mature at approximately 153 g (Fig. 5) by the method employed by Conand (1981).

Fecundity

Estimates of fecundity were obtained from two replicate samples from each of four females during peak gonadal index. There was a fairly narrow range in the
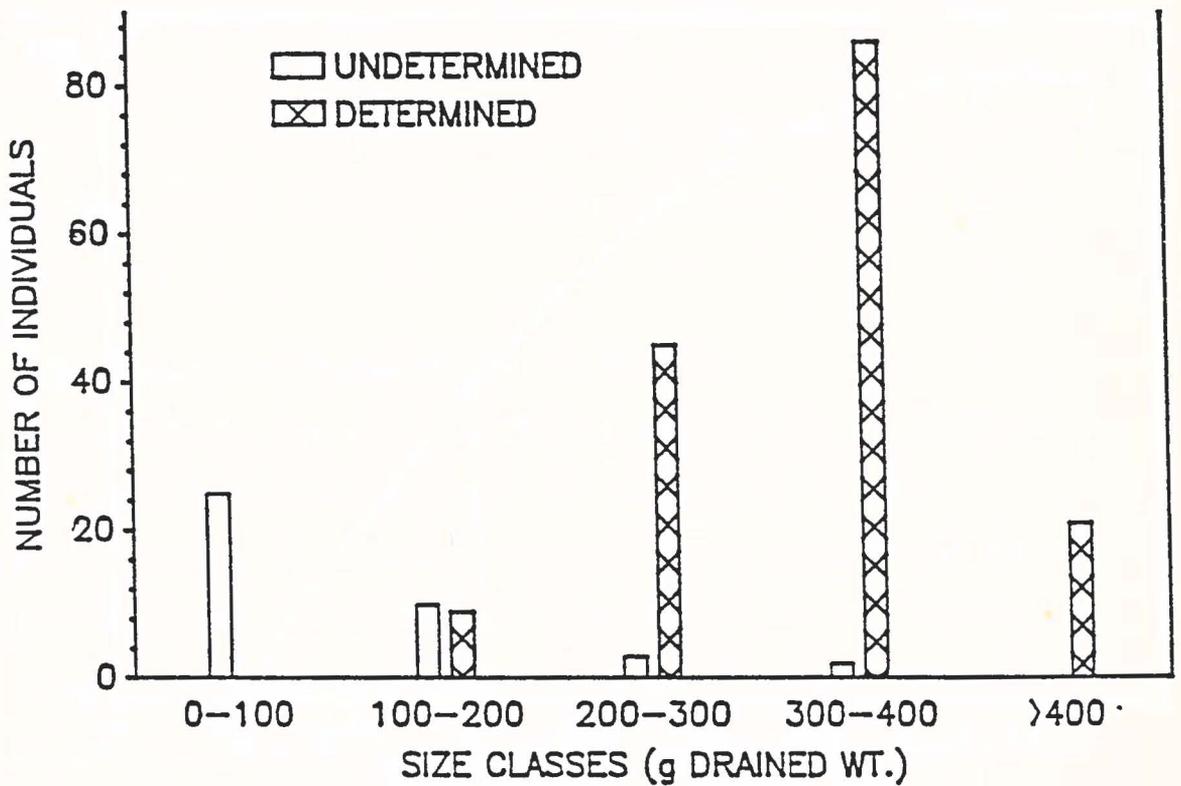
Figure 4. Number of animals within size classes which were determined as mature (with discernable gametes) and immature (without discernable gametes).
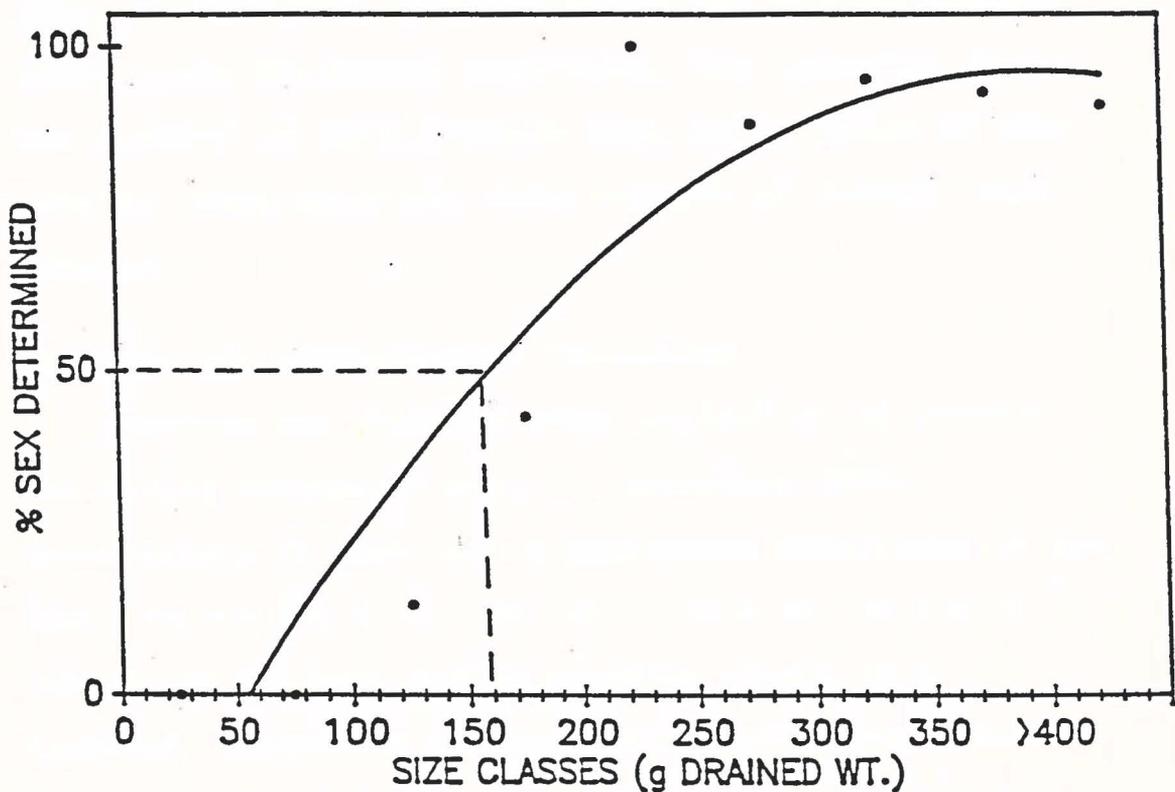
Figure 5. Estimated size at first reproduction.
Determined by point at which 50% of individuals
posses discernable gametes. Regression value of
$Y=-33.46+32.67X-2.06X^2$.

number of eggs per unit weight ovary. Among individuals, fecundity ranged from 5.0×10^5 to 7.8×10^5 eggs g^{-1} ovary weight. Mean fecundity was $6.2 \times 10^5 \pm 1.2 \times 10^5$ eggs g^{-1}.

A fecundity index can be calculated for Actinopyga mauritiana by dividing the mean wet ovary weight by the cube of the mean oocyte diameter (Harriott, 1985). This measurement was obtained for all females throughout the year (annual mean), and during periods of high gonadal index. For the annual mean value, the fecundity index was 1.35×10^4 (3 Sept. 1988-23 Aug. 1989), while at peak gonadal index (20 April 1989) a value of 3.09×10^4 was obtained.

Spawning and Developmental Chronology

Spawning was observed after collection of animals and during successive days in laboratory tanks. Observations of spawning of Actinopyga mauritiana in the field verify the following description as the normal behavioral patterns during spawning events (R.H. Richmond, University of Guam Marine Laboratory, pers. comm., 1989). As with other echinoderms, the egg and sperm of A. mauritiana are negatively buoyant upon release from the parent. Spawning is preceded by swellings along the animals' anterior region. This swelling is most pronounced in the female although it

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often occurs in the male as well. Release of gametes is frequently accompanied with elevation of the anterior half of the body above the substratum. Males typically release a steady flow of sperm while females often release large quantities of egg in a less prolonged (2-3 sec), forceful burst.

The eggs of Actinopyga mauritiana had a mean diameter of 124±4 um (± S.D.) during their reproductive peak (April to July). The first cleavage occurred approximately 1 hr after fertilization and is synchronous within cohorts, with subsequent divisions occurring approximately every 30 min (C. Hunter, Hawaii Inst. Mar. Biol., pers. comm., 1989). Blastulation is followed by a massing of mesodermal cells at one pole. Gastrulation is complete within 24 hr after which continued development occurs, noted in an elongation of the embryo along the axis of the archenteron. By day 2 there was an increase in size of the embryos with a mean length of 384±17 um, and width of 302±9 um (n=6). Both the blastula and gastrula stage were ciliated and positively buoyant. All developmental stages are transparent, but acquire their first pigment granules in the gastrula stage.

Development to the first larval stage, the auricularia, occurred by day 3 in most larvae (Fig. 6). Ciliary tracts are evident and collections of pigment
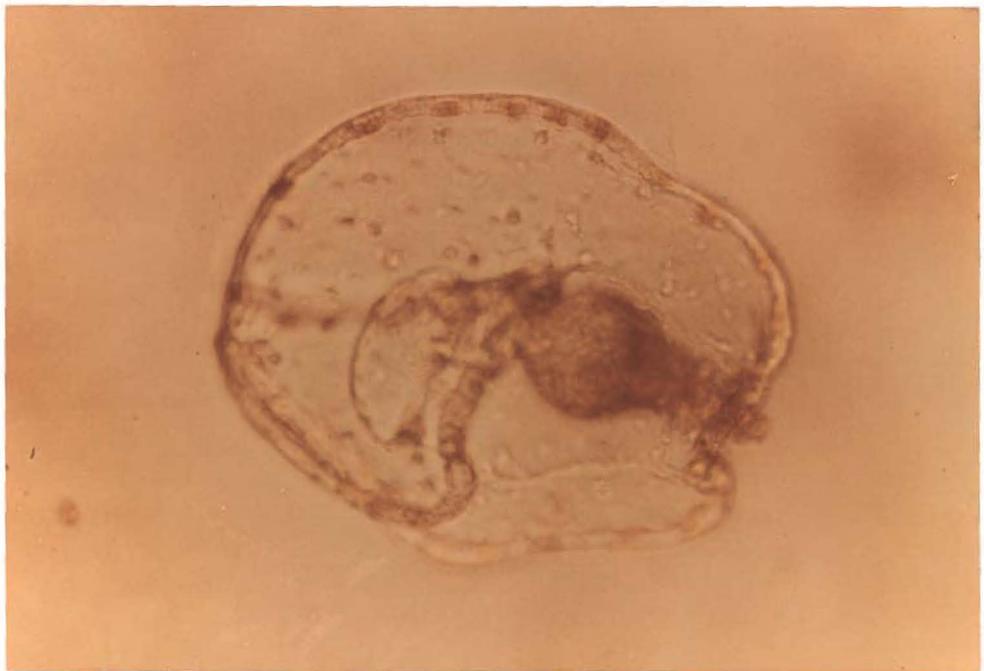
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Figure 6. Auricularia larvae.   Approximate
length=450um.

ranules had concentrated along these ciliary bands. Differentiation of the gut is evident by this stage. A large oral region below the oral hood narrows to a sphincter which separates it from the muscular, convoluted esophageal region. The posterior esophagus also possesses a sphincter prior to its opening into the stomach. An additional sphincter separates the stomach from a smaller hindgut bulb which empties via the anus. The gut of the auricularia is initially horseshoe shaped, but with continued development there is a straightening of the posterior end. The anus migrates from a position where it opens parallel to the mouth and oral hood (180°) to a more posterior position (90°). This straightening becomes complete in the doliolaria stage.

Auricularia have a mean length of 468±56 um, and a mean width of 398±72 um (n=13). The presence of hyaline spheres (Kume et al., 1968) was not observed. Between days 3-4 a single polyaxon spicule forms posterio-dorsally to the anus and increases in size over time. No other spicules were observed to form in the embryos

Complete metamorphosis to the second larval stage, the doliolaria (Fig. 7), is temporally variable as compared to the first stage. Metamorphosis to this larval stage within a cohort may begin as soon as 6 d or as late as 20 d after fertilization. The gradual

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Figure 7. Doliolaria larvae. Approximate
length=250um.

process of metamorphosis to the doliolaria is
characterized by a continued straightening of the gut as
described previously. The gut becomes more elongate,
losing its bulb-like appearance. Ciliary bands shift
and/or degenerate, forming ciliary rings around the
embryo, similar to those described by Kume et al.
(1968). Reorganization of the ciliary tracts is
accompanied by a dispersal of pigment granules which
were previously concentrated along the tracts. Pigment
granules become scattered and clumped randomly through
the larvae. Body convolutions including that of the
oral hood, characteristic of the previous larval stage
are absorbed. This absorption is accompanied by a
shrinkage in total length and width, and an increase in
thickness as the larva take on a more oval form. In
contrast, there is a slight protrusion of the anus.
Mean length and width of the doliolaria is 363 ± 95 um and
281 ± 89 um (n=8) respectively. This shrinkage results in
a reduction in length of 41%.

Due to problems in maintaining larvae, the last
larval stage (pentacula) was not observed in this study.

Recruitment

Periodic surveys were conducted from August 1988-
August 1989 along the algal ridge and adjacent habitats
of Tumon and Pago Bay. Individuals weighing <100 g

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(whole wet weight) were rare totaling only 17 individuals. Only six of those individuals were <50 g, the smallest weighing 10 g. All animals of this size class were found in Pago Bay in July-August 1989. Juveniles were most common on the reef margin in the company of adult animals although some areas with large numbers of adults were devoid of juveniles. Few juveniles were found in areas not inhabited by adults, although surveys were not as extensive in these areas.

DISCUSSION

Reproductive Periodicity

Specific periods within the reproductive cycle can
be arbitrarily assigned to stages according to the
gonadal indices. Periods when the mean index is high is
the peak period. The peak period preceeds the period
when most spawning takes place. During the spawning
period there is a drop in the mean gonadal index from or
shortly before its highest point, but does not include
that time when the index is lowest. Gonadal index may
continue to drop after spawning has ceased, here called
the post-spawning period. This is due to a degeneration
and resorption of leftover gametes that were not
spawned. Such degeneration occurs frequently in oocytes
which were observed to be in various stages of
degeneration in this study and others (Engstrom, 1980).
The post-spawning period, as well as the period
following it, when no oocytes of any stage are present,
may be refered to as a resting stage. The growth stage
is that period between the lowest and peak gonadal
index. The data show that the time of peak gonadal
indices may vary between years. The mean peak values
occurred in June 1988 and April 1989. This variation in
timing has been noted for other aspidochirote
holothurians (Conand, 1981; Harriott, 1985).

At least some individuals from each sample period throughout the year had mature gametes. However, not all individuals had recognizable gametes at all times. This can be seen in the higher proportion of individuals in which the sex could not be determined during periods of low gonadal index (Table 3). During these periods, sex could not be determined for up to 51% of the individuals sampled, whereas during periods of high gonadal index, gender in 100% of those animals collected was determined.

Temperature is often cited as an important parameter of reproductive periodicity (Kinne, 1970; Giese amd Pearse, 1974; Pearse, 1968; Conand, 1981, 1982). Correspondingly reproduction of marine invertebrates that are synchronized with temperature may occur over a longer period of time as their proximity to the equator increases, with those along the equator showing no seasonal variation (Pearse, 1968; Giese and Kanatani, 1987). Seawater temperatures fluctuate little around Guam, ranging from $26°-29°C$. However, even with such low seasonal variability there is an apparent breeding peak (Fig. 8). From the data collected in this study, it appears that Actinopyga mauritiana on Guam shows a similar pattern as noted for A. mauritiana and A. echinites in New Caledonia (Conand, 1982, 1989). Although seasonal temperature fluctuations in New

Table 3. Sample dates, quantities, and percentages of animals collected from Tumon Bay.

| DATE (DAY MO. YR.)	NUMBER ANIMALS COLLECTED				SAMPLE COMPOSITION (%)		
	FEMALE	MALE	UNKNOWN	n	FEMALE	MALE	UNKNOWN
21 APR. 88	8	16	10	34	23.5	47.1	29.4
18 MAY 88	3	3	2	8	37.5	37.5	25.0
9 JUN. 88	13	5	0	18	72.2	27.7	0
15 JUL. 88	18	11	4	33	54.5	33.3	12.2
3 SEP. 88	15	9	4	28	53.6	32.1	14.3
25 OCT. 88	8	7	11	26	30.8	26.9	42.3
6 DEC. 88	11	15	6	32	34.4	46.9	18.7
24 JAN. 89	14	18	1	33	42.4	54.5	3.1
8 MAR. 89	17	15	0	32	53.1	46.9	0
20 APR. 89	17	12	0	29	58.6	41.4	0
7 JUN. 89	12	17	2	31	38.7	54.8	6.5
5 JUL. 89	14	18	0	32	43.8	56.2	0
23 AUG. 89	10	17	1	28	35.7	60.7	3.6
10 OCT. 89	8	9	18	35	22.9	25.7	51.4
TOTAL	165	169	57	391	42.2	43.2	14.6
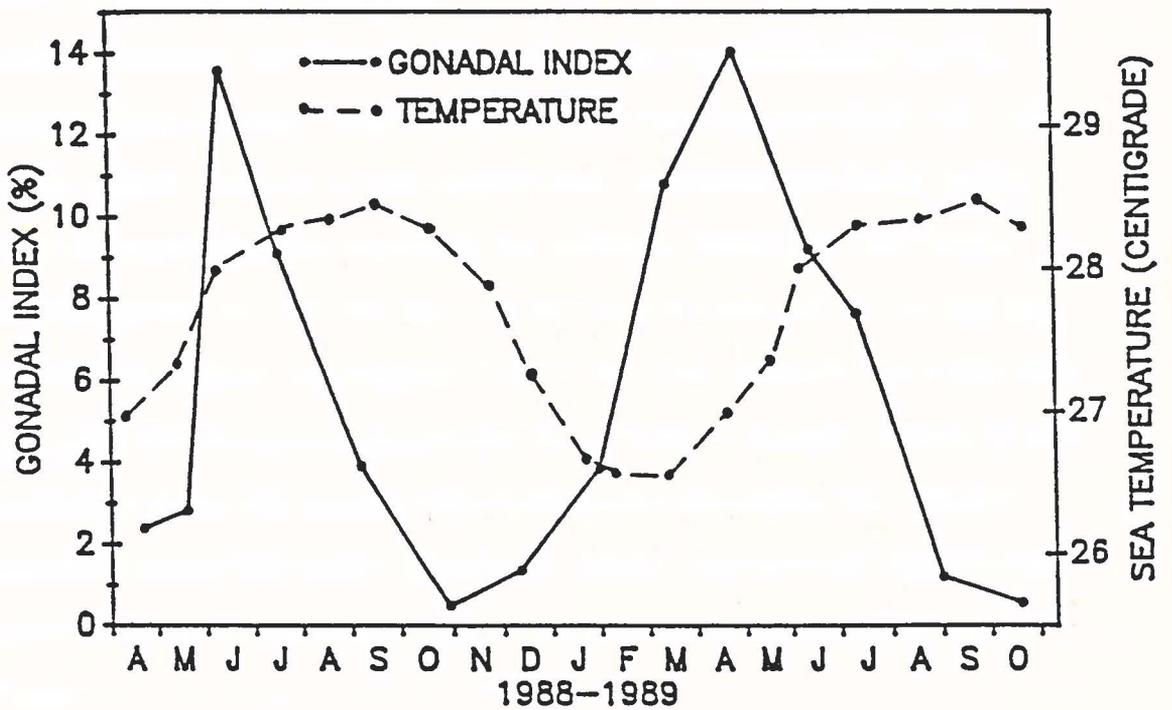
Figure 8. Mean gonadal index and ambient sea temperature (ten year mean, Jones and Randall, 1973) over time.

Caledonia are much greater than those at Guam (7°C as opposed to 3°C), all three spawn during the onset of, or early in, the period of seasonal warming. It should also be noted that the reversed seasonality between New Caledonia and Guam could be a result of reversed photoperiod between the southern and northern hemispheres as well as, or in addition to temperature cycles. In the tropics, daily temperature variations can be much greater than seasonal ones. Intertidal temperatures on Guam, where both A. mauritiana and A. echinites reside, can exceed 35°C during periods of low tide (pers. obs.). In addition, animals in this zone may frequently be exposed to direct sunlight or rain. Such exposure occurs during spring tides of late spring which continue throughout much of the summer, during the periods of intense spawning activity. Large temperature changes such as these can initiate spawning in other marine invertebrates (Sastry, 1963; Loosanoff and Davis, 1963), and could serve as a triggering mechanism on Guam.

Size at First Reproduction

Due to the difficulty of determining age of holothurians (Conand, 1983), many studies relate size to the parameter being considered. Size at first reproduction has been little studied in holothurians.

Harriott (1985) notes that individuals of Holothuria atra, H. edulis, and H. impatiens typically bore no gonads at weights less than 100 g, 100 g, and 80 g respectively. Some researchers have used size at which most individuals bear gonads as an indicator of size at first reproduction (Harriott, 1980; Franklin, 1980; both in Conand, 1982). Conand (1982) states that the method that she utilized to determine size at first reproduction presents a more precise estimate, but, since this method requires placement of individuals into size classes to obtain a percentage, it still only allows for an estimate. For use in management policy, a frequency histogram (Fig. 4) serves this purpose adequately since it indicates at what size animals will most likely be of reproductive size. Actual calculations of size at first reproduction for Actinopyga mauritiana from this study and those using Conand's (1989) method are in fairly close agreement, with her estimate being about 125 g (drained wt.), 27 g less than determined in this study.

Fecundity

Little information has been published on fecundity of tropical holothurians. The use of a fecundity coefficient or index as used by Conand (1981, 1989) and Harriott (1985) allows for comparisons among

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holothurians. Of the other species studied, Holothuria (Microthele) nobilis and Actinopyga echinites exhibits an index within the same range as A. mauritiana. The data presented here supports Conand's findings that Actinopyga mauritiana is a highly fecund species relative to most other tropical holothurians (Conand, 1989). Mean oocyte diameters for tropical aspidochirote holothurians range from 88 um for H. atra to 200 um for Thelenota ananas (Harriott, 1985; Conand, 1981). The mean oocyte diameter of Actinopyga mauritiana lies between these values (Tables 1 & 2).

Relationships between egg number and size are often suggestive of the reproductive strategy of the animal in question. It is generally accepted that there is an inverse relationship between relative egg size and number. The large number of eggs present in Actinopyga mauritiana, combined with there medium size, elevates the relative fecundity of this species when compared to other aspidochirote holothurians. This does not conform to the reproductive allocation model described by Stearns (1976), and discussed for holothurians by Harriott (1985). Large numbers of medium sized eggs might seem to provide an evolutionary advantage to the offspring, but might be necessary to off-set factors which result in high mortality of the embryos and larvae. A. mauritiana predominantly resides on the reef

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margin, which is a narrow, high energy habitat that might limit recruitment on a spatial scale. The rapid turnover of water in this zone may limit recruitment on a temporal scale as well. Other abiotic factors which might prove restrictive include low tides which often result in elevated temperatures and exposure to fresh water or brackish conditions.

Spawning and Developmental Chronology

The embryological development of Actinopyga mauritiana is similar to that of some other aspidochirote holothurians. Rate of development is much like that described for Stichopus japonicus (Kobayashi et al., 1987), where the auricularia stage was reached at day 1-2. Development throughout embryonic and larval stages, although variable for both of these species, occur over the same time range.

Previous authors have described the doliolaria as a short-lived stage (2-3 d), changing quickly to a pentacula. In this study, the doliolaria of Actinopyga mauritiana persisted and no pentacula were observed to develop. This could have been due to several factors including improper diet or lack of proper settling cues. Due to the settling specificity of many marine invertebrates (Crisp, 1965; Scheltema, 1974; Chia, 1978), development of proper techniques to rear larva

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past the doliolaria stage will require continued work.

Of note is the large size and location of the embryonic spicule. Although speculative, it seems likely that the single large spicule of _Actinopyqa_ _mauritiana_ may represent the precursor to the large anal teeth which are characteristic of this genus.

Recruitment

Conand (1981, 1983) states that juvenile sea cucumbers are seldom encountered, this being the case with _Actinopyqa_ _mauritiana_ on Guam as well. The difficulty of locating juvenile holothurians can partly be attributed to the lack of complete pigmentation which may persist past the doliolaria and well into the juvenile stage (R.H. Richmond and B. Smith, U.O.G. Marine Laboratory, pers. comm., 1988). Juveniles may also exhibit more cryptic behavior than that of the adults. It is also possible that larval mortality is quite high and that successful recruitment is a relatively rare event.

Of the two population sites surveyed, small sea cucumbers were only found on a small portion of the reef margin in Pago Bay. It is not known if these individuals represent recruitment from the 1988 reproductive season or if they are the result of prior or subsequent spawnings. Recruitment of _Stichopus_

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<u>japonicus</u>, by artifical means, has been reported by Kobayashi and Ushima (1928).   However, there are few reports of recruitment of holothurians.

MANAGEMENT IMPLICATIONS

The data presented here can be used for management of populations of <u>Actinopyga</u> <u>mauritiana</u> in <u>beche-de-mer</u> fisheries throughout the tropical Pacific. Although reproductive timing may vary among islands and even among locations on an island (Harriot, 1985), the similarity of seasonal gonadal development in this species in both Guam and New Caledonia indicates that reproductive peaks in different areas may be determined without conducting extensive research. Knowledge of reproductive cycles can be used to help optimize recruitment of the animals, and thus maintain the resource locally or regionally. For example, harvest should be seasonal, and take place when the animals are in their post gonadal peak condition, rather than before it. This allows for the annual reproductive effort by adults before being harvested, and should increase the number of larvae (=potential recruits).

Size limits need to be established and regulations implemented to ensure that reproductive adults are always present to help maintain healthy populations capable of perpetuating successful, sustainable recruitment of new individuals. Although size is not easily measured, some accepted standard should be adopted that only allows for the removal of large adults, enabling smaller reproductive adults to remain

in the population for future reproductive seasons. Not enough is known about the rate of growth of sea cucumbers to determine how large they may grow after sexual maturity is reached. Without these data, estimates of the best havestable size for management of the resource remain tenative. With the data in figures 4 and 5, an estimate can be made of the size at which animals can be harvested to leave an appropriate size class of reproducing adults on the reef. Assuming 250 g (drained weight, 400 g whole wet wt.) to be a size which will likely ensure that numerous reproductive animals are left in the population, Figure 9 shows the length at which harvest might not affect the population (data from Tumon Bay, June and July 1989). This assumes that animals covering a range of sizes are present. However, it must be remembered that the great plasticity in weight and shape of these animals combined with the lack of knowledge about their life-span or recruitment makes any such predictions quite speculative. Of important consideration is the fact that most of the adults sampled and other populations are well in excess of the approximate 13+ cm size limit. Thus, harvest limits that are based on length or weight criteria alone will not guarantee the maintenance of a stable, reproductive population. Sufficient numbers of adults, regardless of size, must be present and in adequate density to allow
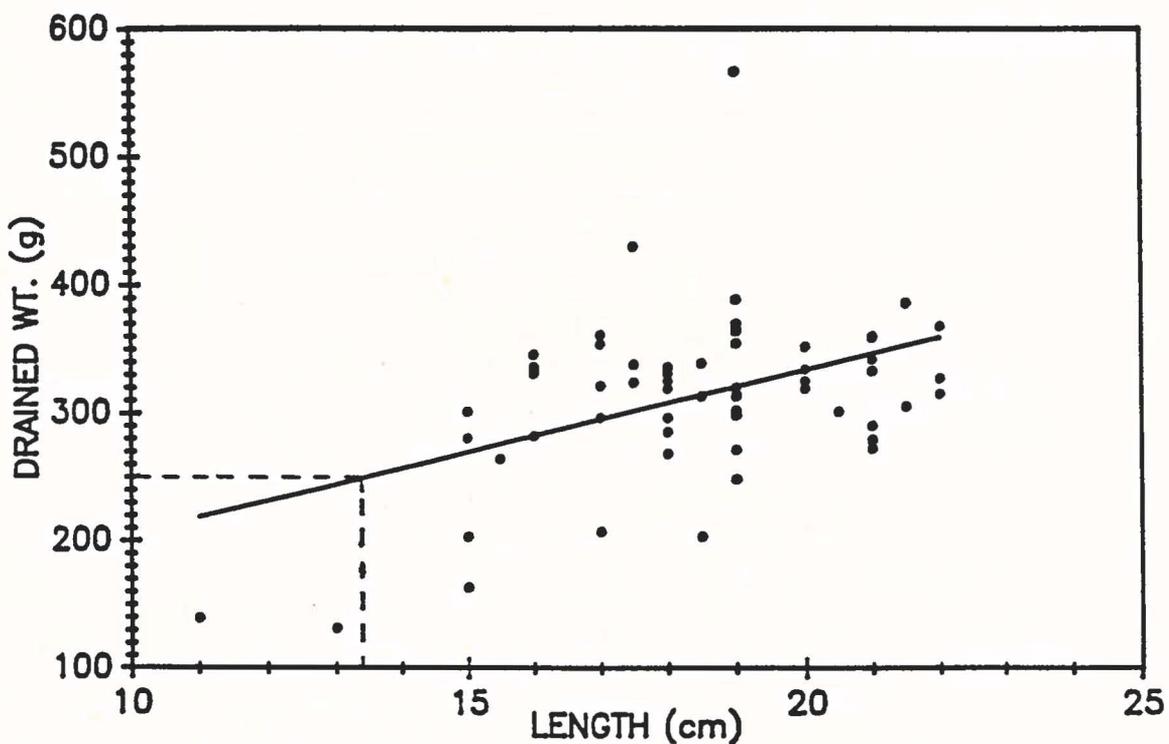
Figure 9. Length to weight regression for
<u>Actinopyga</u> <u>mauritiana</u>, Y=7.64+1.29X: r-value
=0.44535; P<0.01. Dashed line indicates length at
approximately 250 g, approximate, minimum adult
length recommended for harvest on Guam.

for successful spawning events. However, such minimum viable population sizes data have not yet been reported. Such work is currently under way in this laboratory for application to appropriately manage this resource.

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