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Reproductive cycle of two commercial species of sea cucumber (Echinodermata: Holothuroidea) from Caribbean Panama

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Abstract The reproductive status of the holothuroid species *Isostichopus badiotus* (Selenka, 1867) and *Holothuria mexicana* (Ludwig, 1875) was studied over 16 months in Bocas del Toro (Panama), from November 1999 to February 2001. Sexual reproduction was evaluated by the gonad index method, and by histology of gonad development. In addition, population structure was assessed based on sex ratio, minimum reproductive size, and length and weight distributions of males and females. The sex ratio in both species was 1:1, with a unimodal population distribution composed mainly of mature individuals. The minimum reproductive length and weight were 13–20 cm and 150 g, respectively, for both species, although reproductive individuals 10 cm in length were also found. A consistently higher gonad index was observed in *H. mexicana*, due to a high proportion of mature females and males and high gonad indices in most monthly samples. Gametogenesis and spawning patterns seemed to occur throughout the year, with periods of enhanced activity. Two periods of maximum reproductive activity were tentatively identified: July–November for *I. badiotus* and February–July for *H. mexicana*, but neither species had a single, sharply defined annual spawning event. Further work on these exploited holothuroids should examine the relationships between reproduction and environmental factors and between reproductive status and recruitment.

Introduction

During the two last decades, numerous holothuroid species have been fished to critical levels, causing the imminent collapse of local and regional populations (Sloan 1985; Richards et al. 1994; Conand 1990, 1997; Herrero-Pérezrul et al. 1999). The demand for Bêche-de-mer in the Asian market, which has led to overfishing, has underlined the need for basic studies on the natural history of holothuroids so that the resource can be managed wisely. In particular, the reproductive biology of species of commercial importance has been a priority in different areas of the world, especially for the families Stichopodidae and Holothuriidae (Conand 1993a; Chao et al. 1994; Reichenbach et al. 1996; Herrero-Pérezrul et al. 1999; Ramofafia et al. 2000). Other studies have focused on the extrinsic factors influencing the reproductive cycle of the Holothuroidea, such as temperature (Ong-Che 1990; Sewell 1992; Hopper et al. 1998), precipitation (Kubota and Tomari 1998), salinity (Ong-Che 1990), monsoon runoff (Krishnaswamy and Krishnan 1967), lunar phase (Kubota and Tomari 1998), and active photosynthetic radiation (Catalan and Yamamoto 1994).

The information available on this fundamental process for commercially important Caribbean holothuroids, however, is mostly limited to subtropical areas (Engstrom 1980; Mosher 1982; van Veghel 1993; Pires-Nogueira et al. 2001), and more precise studies on reproduction have been recommended (Buitrago and Boada 1996; de la Fuente-Betancourt et al. 2001). The highest gonad index for *Isostichopus badiotus* was observed during late spring (October–November) and summer (January–February) months in subtropical Atlantic Brazil, and spawning was reported only in January when water temperature was maximal (Pires-Nogueira et al. 2001). Spawning in *I. badiotus* was observed after the full moon of August in tropical Bonaire, Netherlands Antilles (Graaf et al. 1999). Similarly, gametogenesis and spawning of *Holothuria mexicana*

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from southern Florida occurred during spring–summer and late summer, respectively (Engstrom 1980; Mosher 1982). Resorption of relict gametes followed, but the breeding season was considered unclear due to the presence of enlarged gonads full of mature gametes from September–November (Engstrom 1980). Van Veghel (1993) reported spawning for >70% of *H. mexicana* individuals within the first 5 days following the full moons between August and October on Curaçao reefs, perhaps the only information available for tropical areas. The minimum length at maturity for *I. badionotus* is reported to be 18 cm (Rodríguez-Milliet and Pauls 1998), while lengths of 57.9 and 75.7 cm have been reported for *H. mexicana* males and females, respectively, from Florida (Engstrom 1980).

Non-traditional fishing areas in the Caribbean have been targeted for supplying the Asian market in recent years (Buitrago and Boada 1996; Rodríguez-Milliet and Pauls 1998; de la Fuente-Betancourt et al. 2001), with species of commercial importance. In the present study, we describe aspects of the reproductive biology of two of these species, *I. badionotus* and *H. mexicana*. Both species are widely distributed throughout the Caribbean. They commonly inhabit shallow waters with sandy, coral, or interspersed seagrass areas, and prefer seagrass meadows (Humann 1992; Hendler et al. 1995). Both species can reach lengths of up to 50 cm (Hendler et al. 1995). Both species, like most of the Holothuroidea, are gonochoric.

The goals of the study were: (1) to describe the annual reproductive cycle, using the gonad index (GI) method, confirmed by histological examination of gonads; (2) to compare the annual reproductive cycle data with environmental variables (rainfall, temperature); and (3) to analyze the population structure (size frequency by sex, sex ratio, minimum reproductive size) in the Archipelago of Bocas del Toro, Panama.

Materials and methods

Sexual reproduction assessment

Specimens of *Isostichopus badionotus* (Selenka, 1867) and of *Holothuria mexicana* (Ludwig, 1875) were collected at Punta Pondsack (09°17'19"N; 082°19'43"W) and at Punta Quarys (09°16'10"N; 082°23'18"W), in the archipelago of Bocas del Toro, Panama. Adults and juveniles of both species were commonly found in sandy areas with abundant seagrass (*Thalassia testudinum*), at depths of up to 5 m. The geology, climate, oceanography, and orography of the archipelago have been described previously (Rodríguez et al. 1993; Greb et al. 1996; Guzmán and Guevara 1998). About 30 mature individuals of each species were collected monthly just before or on the day of full moon, from November 1999 to February 2001 (475 and 479 individuals per species, respectively). We expected gonad weight to be maximal at the time of full moon according to van Veghel (1993) and Graaf et al. (1999), who observed spawning after the full moon in Curaçao and Bonaire, respectively. These holothurians were placed in buckets of ~10 l of seawater and ca. 5–10 g of MgSO₄ for 5–10 min, until completely relaxed and drained, thus facilitating biometric measurements (sensu Ong-Che 1990; Chao et al. 1993).

Body length was measured for drained individuals (to within 1 mm). For the extraction of gonads, an incision was made through the ventral body wall, and all internal parts were removed. We measured weight using gutted individuals (to within 0.01 g), defined as body wall wet weight (sensu Conand 1981; Chao et al. 1993, 1995). This weight (gutted) was chosen, since drained weight may include sediments in the digestive tract and water in the respiratory system. We used drained body weight (sensu Conand 1981; Chao et al. 1993) only to obtain the minimum reproductive size, without sacrificing more individuals (Ramofafia et al. 2000). Body length was compared to gutted weight (Fig. 1). A significant but weak relationship was observed for both species, although the r^2 was higher in *H. mexicana*. This may have been due to the relative rigidity of the body wall in *H. mexicana*, which allowed more precise measurement, compared to the smoother, more flaccid body wall in *I. badionotus*.

The extracted gonads were weighed separately (0.01 g precision) to develop a gonad index. Gonad index was calculated as follows: $GI = [(gonad\ wet\ weight)/(gutted\ weight)] \times 100$. Gonads were then fixed in Bouin's solution for 24 h, washed alternately in water and 50% ethanol several times, and preserved in 70%

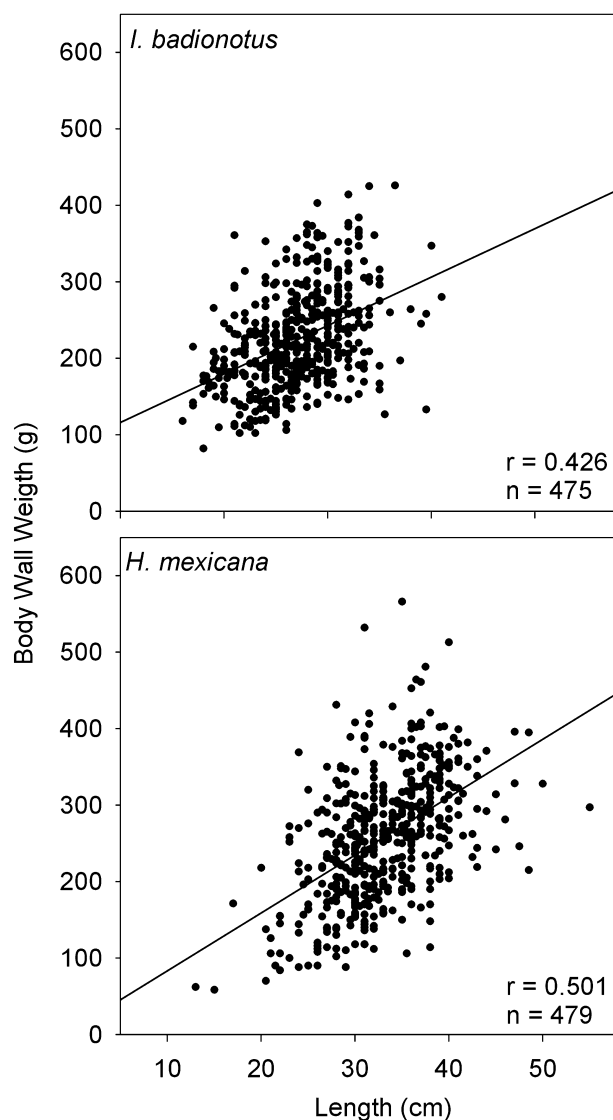


Fig. 1 *Isostichopus badionotus*, *Holothuria mexicana*. Relationship between body length and wall wet weight. Correlations were statistically significant ($P < 0.001$) as were the regressions ($F_{(1, 473)} = 104.748$ and $F_{(1, 477)} = 159.735$, respectively)

ethanol. Histological analysis of gonads was completed following standard procedures (Humason 1972); gonads were dehydrated in an ethanol series, embedded in paraffin, and sectioned at 8 μm . Sections were stained in Mayer's hematoxylin and eosin.

Males and females were identified by gonad morphology and color (sensu Engstrom 1980; Herrero-Pérezrul et al. 1999; Ramofafia et al. 2000); ovaries were pink, orange or red, with relatively uniform diameter, while testes were creamy white and nodose throughout their length. Sex and gonad developmental stage were confirmed histologically for each individual. Reproductive individuals for both species were classified into five stages of sexual development following the criteria of Ramofafia et al. (2000): recovery (I), growth (II), maturity (III), partly spawned (IV), and spawned (V). However, individuals in recovery (I) were never found in our study, due either to the specimens' small size (immature), poor histological quality of the slides, or to the apparent re-absorption of tubules after spawning (see Sewell and Chia 1994).

The reproductive cycle of the sea cucumbers was compared with average monthly rainfall, using data obtained from a meteorological station at the airport on Isla Colón, and with mean monthly water temperatures. Temperature was measured continuously with a thermograph at 3 m depth, located south of Isla Colón (09°00'57"N; 082°15'51"W).

Minimum reproductive size and size distribution

Monthly collections were designed to collect only larger, mature holothuroids, so those individuals were not used to evaluate minimum reproductive size. Size at first reproduction was estimated to be when 50% of the sampled individuals had recognizable gonads (sensu Conand 1981). Both species were collected from outside the monthly collection areas (south of Isla Colón) between January and March 2001, with emphasis on smaller sizes (<25 cm length), which were assumed to be juveniles. A small incision (<2 cm) was made in the dorsal region of each specimen to record gonad presence (Ramofafia et al. 2000). Length and drained body weight were measured and the individuals were released. These individuals and the ones collected monthly were combined only to evaluate population sex ratio, and the size distribution of reproductive and non-reproductive holothuroids.

Statistical analyses

Parametric statistical tests were performed. Data were transformed appropriately when the variance requirements (homoscedasticity and normality) were not fulfilled (Sokal and Rohlf 1995). Pearson product moment correlation and regression analysis were used to test for relationships among the different biometric parameters (length, weight, gonad weight). Species differences in body size were tested with a Student's *t*-test. To compare the monthly variation in the gonad index and between sexes, a two-way ANOVA test was applied, and the differences between months were evaluated using Tukey's a posteriori test for multiple comparisons.

Results

We observed an overall mean (\pm SE) length of 27.05 \pm 0.19 cm (range: 16–41 cm) and 32.93 \pm 0.25 cm (13–55 cm), in *Isostichopus badionotus* and *Holothuria mexicana* respectively. Mean gutted weights were 224 \pm 2.9 g (82–426 g) and 257 \pm 3.7 g (58–566 g), respectively.

Minimum reproductive size

From the 55 small individuals of *I. badionotus* haphazardly collected to determine the minimum reproductive

size, we obtained an overall mean length of 15.5 \pm 0.43 cm, of which 51% were non-reproductive with a mean length of 13.6 \pm 0.48 cm. For the 71 individuals of *H. mexicana*, a similar overall mean length of 15.9 \pm 0.46 cm was observed, with 35% being non-reproductive with a mean length of 13.3 \pm 0.59 cm. No differences were found when comparing the overall lengths between species ($t = -0.778$, $P = 0.438$). Both species had some individuals <10 cm in length with mature gonads. Using the criterion of 50% of first reproductive occurrence, a length range of 13–15 cm was established as the minimum reproductive length for both species (Fig. 2).

Using drained weight of individuals as the criterion for estimating size at reproductive maturity (gutted weight was not measured), we obtained non-reproductive weights of 68.4 \pm 4.41 g for *I. badionotus* and 60.9 \pm 5.05 g for *H. mexicana*. The mean weight of all individuals was 98.1 \pm 7.1 and 97.9 \pm 5.4 g, respectively (55 and 72 individuals, respectively). The observed minimum weight of reproductive individuals was 50–100 g in both species; however, 50% of the sea cucumbers weighed ca. 150 g in both species (Fig. 2). In spite of the wide variance observed in the populations, significant differences were not found between the weights of the two species ($t = -0.089$, $P = 0.929$).

Sex ratio

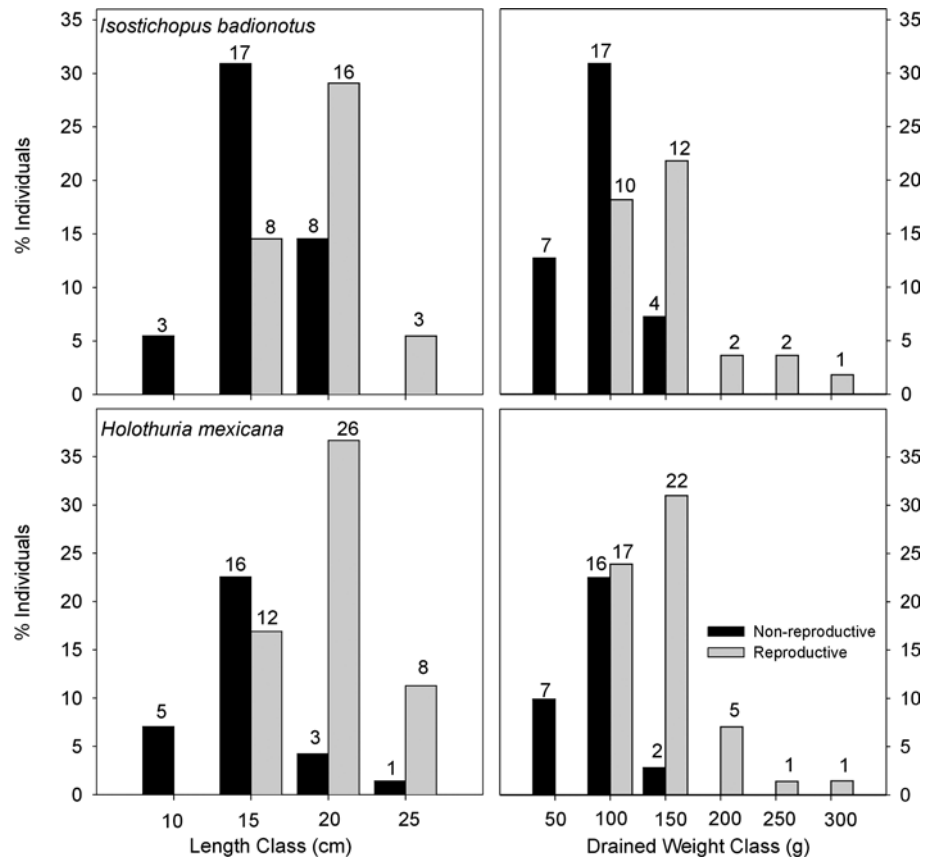
Both species were gonochoric and sexes were distinguished by color and histology. Of the total number of sea cucumbers collected in this study, 89.6% of *I. badionotus* ($n = 530$) and 92% of *H. mexicana* ($n = 551$) were reproductive (with gonads in various stages of development). The sex ratios for the two species were similar, 1:1 as expected ($\chi^2 = 0.490$, $P = 0.484$ in *I. badionotus*; $\chi^2 = 0.434$, $P = 0.510$ in *H. mexicana*).

Weight and size distributions

The size distributions of mature individuals in monthly samples of both species were evaluated using body wall weight (gutted) and body length. A Kolmogorov–Smirnov normality test indicated normal distributions for body wall weights in both species (Fig. 3A–D), while length classes were normally distributed only for males (Fig. 3F, H). The modal weight for *I. badionotus* ($n = 448$) was ca. 200–250 g, unimodal, and somewhat skewed in females and males (Fig. 3A, B). For *H. mexicana*, the modal weight was ca. 300 g ($n = 460$), also unimodal, but nearer normal (Fig. 3C, D). When comparing the two species based on body length, unimodal distributions were observed (25–30 cm and 35 cm, respectively), similar to those for weights (Fig. 3E–H). Again, the tendency for *H. mexicana* to be heavier and longer may be a result of the rigidity of the body.

Both gutted weight and length were very similar between the sexes in both species. Mean lengths of

Fig. 2 *Isostichopus badionotus*, *Holothuria mexicana*. Length and drained wet weight distributions in reproductive and non-reproductive individuals (%) used to calculate minimum reproductive size (only individuals with <25 cm). Values on top of bars indicate number of individuals. Sample sizes were 55 and 71, respectively



27.3 ± 0.28 and 27 ± 0.28 cm were observed in females and males of *I. badionotus*, respectively, whereas mean body wall weights were 223.5 ± 3.9 g in females and 230 ± 4.5 g in males. In *H. mexicana*, mean length was ca. 33 ± 0.35 cm for both females and males, while mean weights were ca. 258.0 ± 5.7 and 260 ± 5.2 g in females and males, respectively.

Changes in gonad developmental stages and gonad index

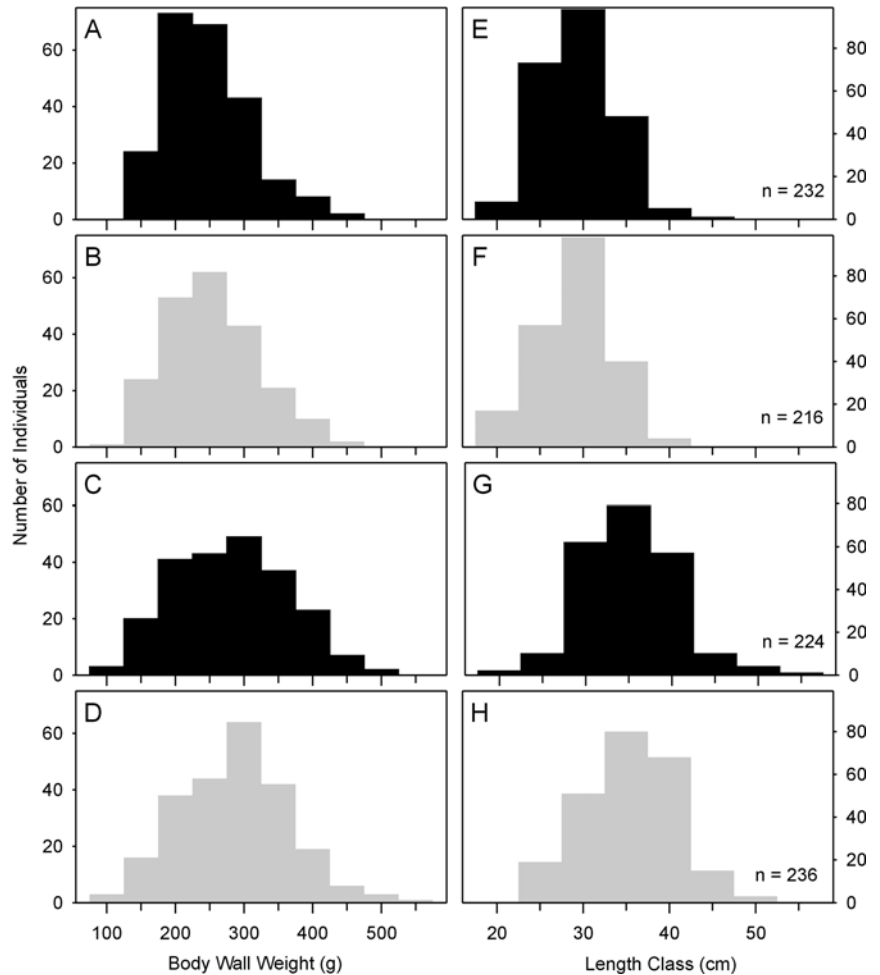
Gametogenesis appeared continuous, with periods of enhanced activity, but there was no clear annual pattern in either species. The mature stage (III) was observed in both sexes and both species, but not throughout the study (Fig. 4). In *I. badionotus*, males in stage II dominated throughout the year, and the proportion in stage III peaked only in November in both years and in June (Fig. 4A), while females had more individuals in stages III (mature) and IV (partly spawned) (Fig. 4B). Over 50% of females were partly spawned in May, July (100%), and August, and <40% in January 2000 (Fig. 4B). In *H. mexicana* both sexes were mature (stage III) throughout most of the study, particularly females (Fig. 4C, D). Stage II was never observed in females of *H. mexicana* and sporadically in males, though 100% of males were in stage II in October and November. The proportion of partly

spawned males (stage IV) peaked in January, August, and September (100%), while <40% of females were in stage IV only in November (Fig. 4C, D). Only 40% of males were observed in stage V (spawning) during December 1999.

Average gonad index based on body wall weight was always lower in *I. badionotus* (2.63 ± 0.24 and 4.73 ± 0.44 in females and males, respectively) than in *H. mexicana* (3.2 ± 0.39 and 7.66 ± 0.92 in females and males, respectively). Differences between sexes and among months were significant in both species, though the interaction was not significant for *I. badionotus* (Table 1). A Tukey test revealed a similarity between the peaks in GI observed in March, May–November (both years), and January 2001 for *I. badionotus*, while for *H. mexicana*, July's peak of maximum activity was different from all other months, but marginally similar ($P=0.054$) to June and February 2001.

Overall mean GI (regardless of sex) in *I. badionotus* showed two apparent peaks of reproductive activity, one very short in March and another more prolonged and difficult to define between July and November (Fig. 5A). Partly spawned females were observed after the first peak and, unexpectedly, at the beginning of the second peak (Fig. 4B). GI in females (Fig. 5B) and males (Fig. 5C) followed the same seasonal pattern as for total individuals, although a more pronounced reduction in reproductive activity was observed in females from November onwards. December's decline did not

Fig. 3A–H *Isostichopus badionotus*, *Holothuria mexicana*. Frequency distributions (number of sexed individuals) based on wall wet weight (left) and length (right) for female (closed bars) and male (gray bars) in *I. badionotus* (A, B, E, F) and *H. mexicana* (C, D, G, H)



coincide with the presence of partly spawned (IV) or spawned (V) stages (Fig. 4A, B).

Reproductive activity in *H. mexicana* seemed more defined. A gradual increase in GI was observed from February onwards, with a peak during July (Fig. 5). Partly spawned males (stage IV) were observed immediately after this peak (Fig. 4C). The GI in males was greater than in females (Fig. 5C). There was a marked inter-annual difference for both species, noticeably between January–February 2000 and 2001. This last observation obscured any clearcut seasonal interpretation, since we could not define whether a peak in reproductive activity really occurred at the beginning of every year, though spawning individuals (stage V) were only observed after that peak (Fig. 4C).

In both species, there may be a relationship between GIs and sea-surface temperature, but not with rainfall (Fig. 5D). The two species did not have overlapping periods of reproductive activity, but the periods seemed to coincide with warmer temperatures (average $>28.5^{\circ}\text{C}$) (Fig. 5A). If precipitation in 1999 were anomalous, it would seem that most reproductive activity in *I. badionotus* occurred during periods of lower rainfall (yearly average of <276.9 cm), contrary to what was observed in *H. mexicana* (Fig. 5D).

Discussion

Reproductive cycle

Isostichopus badionotus and *Holothuria mexicana* are very common Holothuroidea throughout the Caribbean, but little is known about their ecology in tropical areas, which makes any attempt to compare our data with other populations in the region difficult. However, this study shows that the reproductive behavior of both species was similar in some respects to most tropical Holothuroidea; the sexes are separate, gametes are released, and fertilization is external, and there may be an annual reproductive cycle with a continuous and broad peak of reproductive activity when seasonal water temperatures are higher (Conand 1982, 1993a; Smiley et al. 1991; Chao et al. 1995; Hendler et al. 1995; Hopper et al. 1998).

Comparing the limited information available on the reproductive biology of the two species studied here (GI only), we found that the timing of maximum activity for *H. mexicana* in Bocas del Toro (May–July) occurred up to 2 months before that in Florida (August–September) (Engstrom 1980). As in other studies (Engstrom 1980;

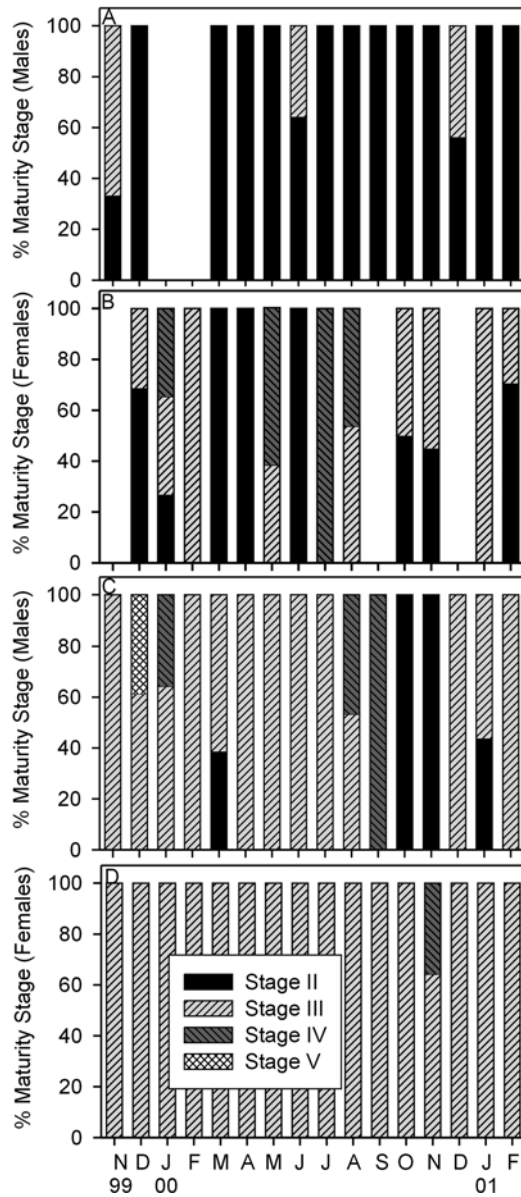


Fig. 4A–D *Isostichopus badionotus*, *Holothuria mexicanana*. Percentage of individuals with gonads for each of four stages (II–V) by sex, from November 1999 to February 2001. **A, B** *I. badionotus*; **C, D** *H. mexicanana*. Samples of < 5 individuals not included (sensu Ramofafia et al. 2000)

Hopper et al. 1998; Reichenbach 1999), we observed numerous sea cucumbers with mature gonad stages throughout the year, suggesting that individuals may reproduce continuously or asynchronously several times during the year. The broad period of enhanced activity (2–3 consecutive months) as seen for both species, particularly *I. badionotus*, has been reported for congeneric species (Ong-Che 1990; Conand 1993b; Chao et al. 1994, 1995; Hopper et al. 1998; Herrero-Pérezrul et al. 1999; Ramofafia et al. 2000). This suggests that within broader annual peaks, multiple spawning events might occur, rather than one single event per year. This inference is based on two previous reports on *H. mexicanana* at different latitudes. In Curaçao, with a latitude and temperature range similar to Panama and Venezuela, *H. mexicanana* spawns in September and October (van Veghel 1993), whereas in Florida (under laboratory conditions) spawning was induced from July to September (Mosher 1982).

Generally, temperature is an important factor for interpreting seasonal variations in the gonad index; nevertheless, Hopper et al. (1998) have suggested that “increased duration of elevated sea temperature does not appear to influence the length of the breeding period”. For *H. mexicanana* in Florida, the annual temperature range is greater and more markedly seasonal compared with Panama. In our study, maximal reproduction was observed in *I. badionotus* and *H. mexicanana* during the warmest part of the year, when the temperature was generally 2°C above the annual average and 3–4°C higher than the lowest monthly average recorded during the study period. Similarly, spawning of *I. badionotus* in Brazil occurs in January, when the temperature reaches its maximum of 30°C (Pires-Nogueira et al. 2001), a relationship widely observed in other holothuroids (Conand 1981, 1993a; Hopper et al. 1998; Herrero-Pérezrul et al. 1999; but see Ramofafia et al. 2000). We cannot discount the possibility that, in the Bocas del Toro archipelago, spawning may be induced by reductions in salinity associated with local run-off and rainfall, which regularly affect productivity in coastal ecosystems (see Krishnaswamy and Krishnan 1967; Ong-Che 1990; Kubota and Tomari 1998). Ong-Che (1990), suggests that, in the tropics, salinity could be more important than temperature, since the latter is

Table 1 *Isostichopus badionotus*, *Holothuria mexicanana*. Analysis of variance for gonad index data between sexes and among months from November 1999 to February 2001

Source	df	SS	MS	F	P
<i>I. badionotus</i>					
Sex	1	2.050	2.050	6.494	0.011
Month	15	42.091	2.806	8.888	< 0.001
Sex×Month	15	6.164	0.411	1.302	0.197
Residual	416	131.332	0.316		
Total	447	192.245	0.430		
<i>H. mexicanana</i>					
Sex	1	28.971	28.971	38.893	< 0.001
Month	15	151.339	10.089	13.545	< 0.001
Sex×Month	15	20.735	1.382	1.856	0.026
Residual	428	318.816	0.745		
Total	459	533.223	1.162		

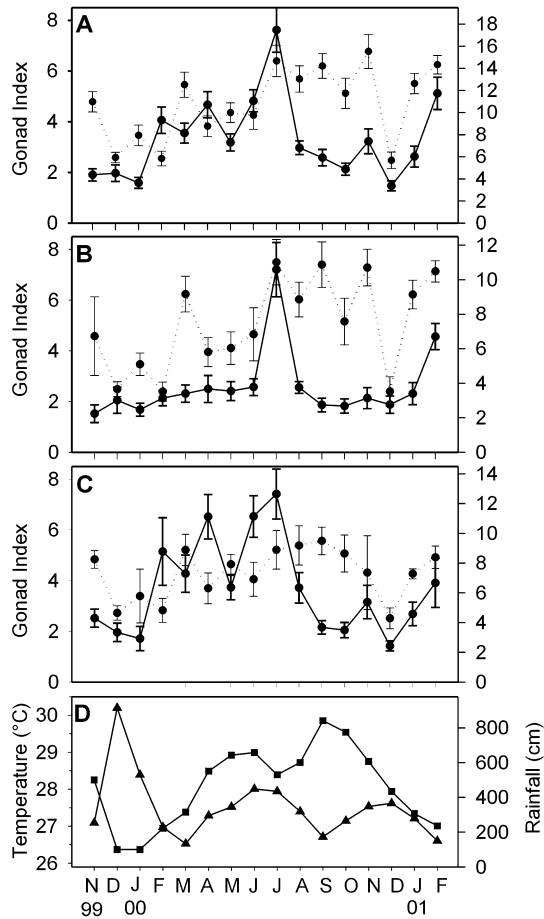


Fig. 5A–D *Isostichopus badionotus*, *Holothuria mexicana*. **A–C** Monthly mean and standard errors in gonad index based on body wall wet weight (dotted line and left axis for *I. badionotus*; solid line and right axis for *H. mexicana*), for all sexed individuals (**A**), females (**B**), and males (**C**). **D** Monthly variation in water temperature at 3 m depth (solid square) and rainfall (solid triangle), from November 1999 to February 2001, at Isla Colón, Bocas del Toro, Panama

more constant throughout the year. The average salinity in the study area is 30.6‰ (28.9–35.1‰), and clearly responds to changes in rainfall and run-off affecting the upper 1-m sea surface layer (Guzmán and Guevara 1998). Hence, we considered that these three parameters (temperature, salinity, rainfall) act together, but they are difficult to interpret for this area of Panama, where it rains practically year-round, with moments of fast run-off associated with the orography of the coastal zone. Furthermore, and less conclusively, there is considerable inter-annual variation in rainfall and its distribution within the archipelago (Gordon 1982; Rodríguez et al. 1993; Guzmán and Guevara 1998), which could contribute to year-to-year differences in spawning patterns. Interestingly, Chao et al. (1995) pointed out that different methods used to evaluate spawning periods of holothurians may have confused the interpretation of factors affecting reproduction.

Maturity stages and gonad index in relation to size and weight

The gonad index is recognized as a reliable indicator for the study of sexual reproduction in Holothuroidea (Chao et al. 1995). Nevertheless, it is possible that the index is affected by small variations in body length, which are reflected as significant changes in body weight (Engstrom 1980). According to Conand (1993b), the percentage of individuals with mature gonads, and the gonad index, can be used to estimate reproductive effort or energy invested in reproduction. Furthermore, she pointed out that body weight could also be important for interpreting interspecific variations, and suggested the sexes should be analyzed separately. In this study, there was a notable contrast between the species, with greater reproductive effort observed in *H. mexicana*, similar to that in Florida populations (Engstrom 1980). Among *I. badionotus* females, 25–100% were in the mature stage (III) in 9 of the 16 monthly samples, while for males 35–70% were in this stage in only 2 of 16 samples. This contrasts sharply with *H. mexicana*, where >60% of both males and females were mature throughout the period, with the exception of males in October and November 2000. Thus, we found that maturity stages and GIs did not clearly explain the annual cycle.

Similarly, important average differences between gonad indices of both species were observed; GI for total individuals was twice as big in *H. mexicana* as in *I. badionotus* (Fig. 5A), very similar between females of both species (Fig. 5B), and greater in *H. mexicana* males than in *I. badionotus* males (Fig. 5C). Conand (1993b) suggested that such differences may be due to the fact that individuals (females in her study) consumed more food, used the absorbed material more efficiently, or used it preferentially in the production of gonads. We suggest that the continuous presence of the mature individuals for both species and both sexes (except *I. badionotus* males), and perhaps the fact that the collections were always made at full moon and apparently before spawning events, may have resulted in high gonad weights, hence GIs, during the entire study. Most studies do not provide details on the lunar phase and monthly sampling times, so no reliable comparisons can be made.

First reproductive age and population structure

In our study, as in other holothuroid studies (Conand 1993a; Chao et al. 1994), a unimodal size distribution was found, composed mostly of reproductive adults. We observed this for both sexes whether we used body wall weight or body length in our analyses. When comparing Panamanian holothuroids with the information available for those in Venezuela (Rodríguez-Milliet and Pauls 1998), similar lengths were obtained in *I. badionotus* (20–30 cm) and much smaller lengths

in *H. mexicana* (10–30 cm). The size ranges may not be entirely representative of populations in Venezuela, given the small sample size (123 individuals). Concerning sex ratio, we found the expected 1:1 female/male ratio. This result is similar to those reported for the majority of Holothuroidea, and specifically for *Holothuria* and *Isostichopus* species (Ong-Che 1990; Conand 1993a; Foster and Hodgson 1995; Herrero-Pérezrul et al. 1999).

A direct relationship between body size and reproduction is known for many marine invertebrates (Catalan and Yamamoto 1994). The minimum reproductive length found here (13–15 cm) was similar for both species and was about half the modal lengths. It is important to note that many reproductive individuals (30–50%) with lengths < 15 cm were found in both species. Furthermore, we observed reproductive juveniles with lengths of 10–15 cm, and recorded adults > 20 cm long that lacked gonads completely. The estimated minimum reproductive length for *I. badionotus* in Venezuela was 18 cm (Rodríguez-Milliet and Pauls 1998), somewhat larger than that determined in Panama.

Conclusion and management recommendations

We have described the seasonal reproductive pattern of two commercially important species for Caribbean Panama. *H. mexicana* seems to have a greater reproductive effort throughout the year compared with *I. badionotus*. Although we suspect multiple spawning events for both species during the year, it is likely that maximum spawning activity occurs between February and November. This range is wide when compared to other species, where the reproductive stage is generally confined to 2–3 months (Conand 1982; Smiley et al. 1991; Chao et al. 1994; Hopper et al. 1998; Herrero-Pérezrul et al. 1999; Ramofafia et al. 2000). This pattern might make management decisions easier for these species. However, more studies on recruitment patterns and juvenile survivorship on longer time-scales are recommended to further understand the population ecology of the two species.

The exploitation of Holothuroidea in Panama, as in many other Caribbean countries (Buitrago and Boada 1996; Rodríguez-Milliet and Pauls 1998; de la Fuente-Betancourt et al. 2001), started with the consent of the authorities and without any information on the ecology of the species. The fishing pressure exerted on this resource since 1997 forced the Panamanian government to close the fishery in the Bocas del Toro archipelago, although illegal fishing continues (Cruz 2000). Initially, the establishment of a partial or total seasonal ban in the archipelago is recommended; this might include the suggested period of maximum reproductive activity, July–November for *I. badionotus* and February–July for *H. mexicana*. Due to the present abundances and fishing preferences, we consider that priority should be given to *I. badionotus*, and another species not studied here,

which is in a critical state of scarcity, *Astichopus multifidus* (Guzmán and Guevara 2002).

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