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Sex-ratio and occurrence of hermaphroditism in populations of *Diadema antillarum* (Echinoidea: Diadematidae) at two contrasting habitats in Tenerife (Canary Islands)

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HERNÁNDEZ, J. C., S. CLEMENTE & A. BRITO. Sex-ratio y ocurrencia de hermafroditismo en poblaciones del equinoideo *Diadema antillarum* en dos hábitats diferentes en Tenerife (islas Canarias). *VIERAEA* 35: 155-161.

RESUMEN: Se llevó a cabo el estudio histológico de las gónadas del equinoideo *Diadema antillarum* en dos tipos de hábitats diferentes en Tenerife (Islas Canarias): «blanquizal», hábitat caracterizado por altas coberturas de algas coralináceas y ausencia de macroalgas, propio de zonas dominadas por erizos; y «borde o frente de ramoneo», hábitat caracterizado por la presencia de altas biomasas de macroalgas y pocos erizos. El sex ratio de la población de *D. antillarum* no varió significativamente de la proporción 1:1 en cada hábitat. Se registró la presencia de un individuo hermafrodita (en el blanquizal) de un total de 572 erizos examinados. Se concluye que el hábitat no influye en el gonocorismo lábil que muestra esta especie, ni tampoco en los mecanismos de determinación del sexo.
Palabras claves: islas Canarias, equionodermos, *Diadema antillarum*, sex ratio, hermafroditismo.

ABSTRACT: Gonad histological examination of the Canary Islands populations of the echinoid *Diadema antillarum* was conducted in two types of habitats («barrens», i.e. habitats characterised by high visible crustose coralline algal cover typical of urchin-barren grounds, and by absence of macroalgae; and «fringe», i.e. habitats characterised by high macroalgal biomass and few *D. antillarum*). Sex ratio did not differ significantly from 1:1 at either habitat. One hermaphrodite echinoid was found at the barren habitat out of the total of 572 echinoids examined. *Diadema antillarum* did not show labile gonochorist and sex determining mechanisms influenced by habitat.
Key words: Canary Islands, echinoderms, *Diadema antillarum*, sex ratio, hermaphroditism.

INTRODUCTION

According to Pearse & Cameron (1991) most sex ratios that have been reported for the few echinoid species adequately examined are close to 1:1. Nevertheless, there are records suggesting that a 1:1 ratio is not universal among echinoids (see review in Lawrence, 1987). Indeed, deviations from 1:1, due to excess of one sex or the other, are known even in different populations of the same species (Pearse 1968, 1969; Pearse & Phillips, 1968; Dix, 1970; Gonor, 1973). Possible deviation from a 1:1 sex ratio may reflect environmental conditions that influence sex determination (Pearse & Cameron, 1991). Boolootian (1960) suggested that pronounced temperature fluctuations may influence the sex differentiation. In terms of sex ratio, populations exist in a state of equilibrium that is affected by competition and resource (Ghiselin, 1987).

Among echinoderms there are species in which hermaphrodism is the normal sexual condition (Delavault, 1966). Nevertheless, most echinoids exhibit stable or only weakly labile gonochorism and hermaphrodites rarely occur. The frequency of occurrence of hermaphrodites is usually very low; apparently fewer than one or two per thousand (Lawrence, 1987; Pearse & Cameron, 1991). Many types of echinoid hermaphrodites have been reported. Of the 5 gonads, 1 to 4 gonads may be of one sex with the other gonad or gonads all of the opposite sex. Alternatively, one or more of the gonads may be male-female mixtures (ovotestes). When most of the gonads are ovaries (or ovarian tissue in the ovotestes), the animal is said to be a «female hermaphrodite»; when testes or testicular tissues predominate the animal is known as a «male hermaphrodite» (see review Pearse & Cameron, 1991).

In the majority of invertebrates sexuality is genetically determined. In spite of the absence of experimental proof (Delavault, 1966), the observed presence of sexual chromosomes in certain sea urchins (Harvey, 1956, Lipani *et al*., 1996) lends unquestionable support to this concept. Labile gonochrorism may be interpreted to mean that the genetic basis of sex determination is a mechanism involving multiple autosomal sex genes (Bacci, 1965). In some cases, environmental factors may be important (Gonor, 1973). Life history traits are phenotypes dictated by complex interactions between genotype and the environment (**genotype x environment (G x E) interaction-effect)**. The conditions that cause hermaphrodites are not known. Adverse environmental conditions during early juvenile stages may be involved, as suggested by Pearse & Cameron (1991). Thus, deviations from 1:1 and rare cases of hermaphrodites may provide information on biotic or abiotic factors affecting sex determination.

At high densities occurring in Canary Islands (Aguilera *et al*., 1994; Brito *et al*., 2004; Tuya *et al*., 2004a, b), effects of the grazing activity of sea urchins become intense and food becomes limiting, forming «barren ground areas» dominated by the echinoid and crustose coralline algae. When sea urchins are in low abundance, algal beds flourish in the rocky subtidal zone. As sea urchins increase in number, they begin to aggregate along the edge of algal beds forming a destructive grazing «front» (Whartog & Mann, 1981) or «fringe» (Byrne *et al*., 1998). Therefore, the different habitat in which *Diadema* occurrs in the Canary Islands is an interesting framework to test sex ratio variation between different food availability regions and population density situations.

METHODS

Over the course of one year, eight individuals of *D. antillarum* were collected each month from each of two contrasting habitats (described below) in two localities (Abades: 28º08'26''N/16º26'04''W and Boca Cangrejo: 28º24'22''N/16º18'52''W). As *D. antillarum* does not exhibit any sexual dimorphism, sex was determined by microscopical examination of histological slides of the gonads. Urchins were dissected and one gonad was preserved in Bouin's fixative for histology. A portion of the preserved gonads were rinsed, dehydrated, embedded in paraplass (MERKS) and sectioned into 7 mm sections. Sections were stained with haematoxylin and eosin (H/E). The sex ratio of urchins collected from both habitats was examined by chi-square test ($\div^2$) to determine if it deviated from 1:1 (Lessios, 1979; King *et al*., 1994). To adjust significance levels for multiple tests, we used the standard Bonferroni technique (Miller, 1980) and therefore the significance level was lowered.

Barren ground areas are dominated by encrusting coralline algae with scattered patches of filamentous algae and microalgae (mainly *Blennothrix lynbyacea* (Kutzing ex Gomont) Anagnostidis and Komárek, *Lyngbya lutea* (C. Agardh) Areschoug, *Ceramium* sp. and *Pseudochlorodesmis furcellata* (Zanardini) Boergesen. Other species such as *Padina pavonica* (Linnaeus) Thivy, *Dictyota dichotoma* (Hudson) J.V. Lamouroux, *D. pfaffii* (Schnetter) and *Lobophora variegata* (J.V. Lamouroux) Womersley *ex* E.C. Oliveira are present in crevices.

The fringe areas consisted of a dense bed of *Dictyota* sp., including *D. dichotoma*, *D. liturata* J. Agardh, *D. pfaffi*, and also *Asparagopsis taxiformis* (Delile) Trevisan de Saint-León. There was up to 75 % cover of erect algae in the fringe habitat. This band was generally located shallower in the subtidal with an algal community dominated by *Lobophora variegata* and *Stypocaulon scoparium* (Linnaeus) Kützing, and occasionally mixed with *Padina pavonica*. In the Canary Islands, urchin populations at barrens had higher density (9.44 m^{-2} ± 0.43, n = 100) than those at fringe (3.62 m^{-2} ± 1.52, n = 100) (unpublished data). Sea urchins from barrens were smaller (mean test diameter ± standard desviation: 36.88 mm ± 11.05, n = 360) than those from fringe (mean test diameter: 63.83 mm ± 11.98, n = 360) (unpublished data).

RESULTS

One «male hermaphrodite» (Pearse & Cameron, 1991) of *Diadema antillarum* (n=375) was found at Abades in the barren habitat; the gonad was mosaic and predominantly male with one entire mature female acinus (Fig. 1). The status of the remaining four gonads of this hermaphrodite is unknown. No hermaphrodites were found in the rest of study. The frequency of hermaphroditism was 0.0035% in barrens (n=188) and zero in fringe habitats (n=187).

Not significant skewed sex ratio was found in the studied months and habitats and neither for pooled habitat data (Table I). In some cases, an excess of females was discernible for *D. antillarum*, but values were not significant. Sex ratios of *D. antillarum* did not deviate significantly from 1:1 in barren (x^2 = 16.450, p<0.124, n = 284) or in fringe populations (x^2 = 17.628, p<0.090, n = 288).

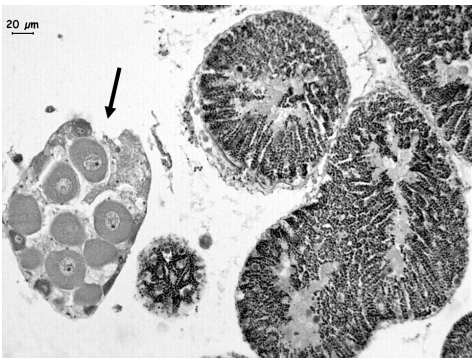

Fig. 1. «Male hermaphrodite» from Abades barren ground. (arrow = mature female acinus).

DISCUSSION

The only record of hermaphroditic individuals of *Diadema antillarum* is in Caribbean populations found by Bak *et al.* (1984). Analyzing gonad smears, they found the gonads of 2 individuals to be packed with blastulae. It was attributed to parthenogenesis results. However, Pearse & Cameron (1991) suggested that blastulae could have resulted from self fertilization within aberrant hermaphroditic gonads. In this way, we have found a mature hermaphroditic specimen that probably can self-fertilize as reported for other echinoid species. Boolootian & Moore (1956) described a hermaphroditic *Strongylocentrotus purpuratus* that produced normal plutei from self-fertilized gametes. But it is important to keep in mind that the presence of blastulae may be due to contamination in the smear procedure (Boolootian & Moore 1956).

In the Caribbean, the two strange specimens found by Bak *et al.* (1984) occurred after a mass mortality event during 1982-1984, under extremely low population densities and the authors suggested it was a consequent of this mass mortality. On the other hand, our hermaphroditic case appeared under extremely high density populations in a barren ground. The lack of more cases of hermaphroditism in our study makes it difficult to atribute the ocurrence of hermaphroditisim to environmentally stressful situations that may affect *Diadema* populations.

Concurring with our results, King *et al.* 1994 found three hermaphroditic Diadematids (n=327) (*Centrostephanus rodgersii*) in barren ground habitats off the central coast of New South Wales. On the other hand, Meidel & Scheibling (1998)

Table I. Pooled number of females and males in monthly samples of *Diadema antillarum* in each habitat and chi-square tests. (NS = not significant).

	Barren grounds				Fringe			
Month	♀	♂	χ^2	Sig.	♀	♂	χ^2	Sig.
Jun-04	9	7	0.571	NS	11	5	7.200	NS
Jul-04	6	10	1.600	NS	8	8	0	NS
Aug-04	7	9	0.444	NS	8	8	0	NS
Sep-04	6	9	1	NS	8	8	0	NS
Oct-04	9	7	0.571	NS	8	8	0	NS
Nov-04	10	6	2.666	NS	9	6	1.500	NS
Dec-04	9	6	1.500	NS	10	5	5	NS
Jan-05	10	6	2.660	NS	9	7	0.570	NS
Feb-05	9	6	1.500	NS	9	7	0.570	NS
Mar-05	5	11	3.273	NS	5	10	2.500	NS
Apr-05	9	7	0.571	NS	8	7	0.140	NS
May-05	7	8	0.125	NS	8	7	0.140	NS
Total habitat	96	92	16.490	NS	101	86	17.628	NS

found three hermaphrodites (n=1968) one from each of the following studied habitat: kelp bed; grazing front; and barren ground. Therefore, hermaphroditism does not seem to be related to any of these types of habitats alone.

Boolotian (1960) found a number of hermaphrodites of *S. droebachiensis* in areas where seasonal temperature fluctuations were extreme. He never found hermaphrodites of this species in warm or cold waters exhibiting a normal temperature range. Moore *et al*. (1963a, b) reported that the occurrence of hermaphrodites in populations of both *Lytechinus variegatus* and *Tripneustes ventricosus* was due to the unusually cold winter of 1957-58, as they were unable to find this condition in other years. These authors did not include habitat descriptions, but they suggested that stressful environmental factors did facilitate occurrence of hermaphroditic cases. In view of our results we hypothetise that there may be a small percentage of hermaphrodites, all the same, which occur irrelevant of habitat condition, population density or environmental factors. In view of the rarity of the phenomenon, the total number of individuals of a species examined is an important factor (Boolootian &Moore, 1956).

In our populations of *D. antillarum* the sex ratio did not deviate significantly from 1:1. The same result was obtained by King *et al*. (1994) in two barren grounds of New South Wales (Sydney, Australia) and by Meidel & Scheibling (1998) in two barren grounds at Little Duck Island and Mill Cove of Nova Scotia (Canada). But unequal sex ratios are not rare in echinoids (see review Lawrence, 1987). They may be due to differences in growth or mortality between sexes (McPherson, 1965). Another explanation proposed by Bernards (1977) for sex-biased samples, may be linked to the tendency for sexual aggregations which can be different between habitats. Also, it may be realistic to assume that the greater availability of food will change the ratio margin one way to another, animals may utilize the same amount of food irrespective of its cost. Greater female frequency may be coupled with larger size of well fed urchins. In this sense, it is obvious to think that better food-conditions typical of fringe habitats encourage higher female numbers, as reported before by Lessios (1979) in *D. antillarum*, Gladferter (1978) in *Cassidulus caribbearum* and McPherson (1965) in the larger size classes of *Tripneustes ventricosus*. But our results do not show any evidence of the influence of habitat characteristics, or size and density of population in the ocurrence of hermaphroditism cases and skewed sex ratios. More information and precise records with publication are needed to give a basis for assessing possible causative factors of the occurrence of hermaphroditism.

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