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REPORT

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Lunar periodicities of diadematid echinoids breeding in Fiji

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Abstract Gonad indices for two species of *Diadema* and two species of *Echinothrix*, including two color morphs of *Echinothrix calamaris*, are described for a 12-month period on Sosoikula Reef and Nukubuco Reef, Viti Levu, Fiji. Seasonal fluctuations in salinity (36.11 ± 0.88 ppt) and water temperature (26.35 ± 0.91°C) occurred. Gonad index data showed monthly reproductive rhythms, closely attuned to the lunar cycle. *Diadema savignyi* and *Echinothrix diadema* spawned on the full moon, and *Diadema setosum* and *E. calamaris* (white and brown color morphs) spawned on the new moon. Breeding periodicities coincided with the spring tides, thus maximizing chances of fertilization and dispersal. Such breeding cycles indicate how closely related species can co-exist with minimum risk of hybridization. Unusual sex ratios were found for all species, with an exceptionally low incidence of males. Reasons for this deviation from the typically reported 1:1 sex ratio may relate to the exceptionally high levels of tributyltin (TBT) recorded in Suva Harbor.

Keywords Sea urchins · *Diadema* · *Echinothrix* · Coral reefs · Gonad indices

Introduction

Reproductive rhythm has been well documented in a number of echinoids (Orton 1923; Harvey 1947; Fox 1957; Moore et al. 1963a, b; Lewis 1966; Kobayashi 1967; Pearse 1968, 1969a, 1972; Tuason and Gommez 1979; Lessios 1981; Pawson and Miller 1982; Nichols 1984). Such rhythms have been reported to be conspicuous in species of the genus *Diadema* which have been shown to be especially sensitive to light (Yoshida 1966), even at moonlight intensities (Dambach 1969). Published reports of variations in reproductive rhythms for species of the genus *Diadema* are shown in Table 1.

Lack of appropriate sampling techniques probably accounts for failure to detect lunar periodicity in species of this genus (Halberg et al. 1987). Few reproductive cycles of other members of the family Diadematidae have been studied; however, Pearse (1969a, 1972) reported that *Centrostephanus coronatus* Verrill, 1867, off of the coast of Southern California, had a monthly reproductive rhythm. He stated that although a comparison of the 1969 and 1972 samples indicated a monthly reproductive rhythm attuned closely to the moonlight cycle, other environmental factors, tidal cycles, and food availability were likely to influence and modify the rhythm (Pearse 1975).

Many reports have shown reproductive cycles synchronized with the lunar cycle for short periods of the year. The length of the reproductive cycle is likely to be governed by the water temperature throughout the year. Yonge (1940) proposed that *Diadema setosum* (Leske, 1778) on the Great Barrier Reef was reproductively inactive at sea temperatures below 25°C. Pearse (1969a, b), working in the Gulf of Suez, supported this, finding that gametogenesis began from April to May when sea temperatures rose above 25°C. Studies in the Philippines (Tuason and Gommez 1979) revealed continuous reproduction of *D. setosum* throughout the year where temperatures were always ~25°C. The Caribbean species *Diadema antillarum* Philippi, 1845, is reported to spawn throughout the year on Curaçao and in the Virgin Islands (Randall et al. 1964), during spring in Barbados (Lewis 1966), from early summer to early winter in Bermuda (Harvey 1947; Iliffe and Pearse 1982) and in the autumn in the Florida Keys (Bauer 1976).

The fact that diadematids, in particular, show a strong association between the lunar cycle and their breeding periodicities is reflected in their morphology. One of the key morphological features used by many

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Table 1 Variations in reproductive rhythms for species of the genus *Diadema*

Species	Reproduction	Spawning	References
D. antillarum	Annual	New moon	Kennedy and Pearse (1975)
D. antillarum	Annual	Between New & Full moon	Bauer (1976)
D. antillarum	Annual	New moon	Iliffe and Pearse (1982)
D. mexicanum	Annual	Full moon	Lessios (1984)
D. savignyi	Annual	New moon	Drummond (1995)
D. setosum	Monthly	Full moon	Fox (1923)
D. setosum	No synchrony	Irregular	Stephenson (1934)
D. setosum	No synchrony	Irregular	Mortensen (1937)
D. setosum	Monthly	Full moon	Yoshida (1952)
D. setosum	No synchrony	Irregular	Macnae and Kalk (1958)
D. setosum	Monthly	Full & New moon	Kobayashi and Nakamura (1967)
D. setosum	No synchrony	Irregular	Pearse (1970)
D. setosum	Monthly	Full moon	Tuason and Gommez (1979)
D. setosum	No synchrony	Irregular	Hori et al. (1987)
D. setosum	Monthly	Full & New moon	J. S. Pearse, personal communication
D. setosum	Monthly	Irregular	J. S. Pearse, personal communication

authors (Clark 1925, 1966; Mortensen 1940; Clark and Owen 1965) to identify *D. setosum* is the presence of five naked white spots on the inter-radials. These are visible both night and day, situated directly over the gonads. Mortensen (1940) reported that the epidermis is very thin in this region. Pearse (1970) made histological sections of the test of *D. setosum*, both from the region of the white spots where no melanocytes were found with a very thin epidermis and strongly eosinophilic cytoplasm, and from the typical aboral area where a thick epidermis contained numerous pigmented granules. *Diadema savignyi* Michelin, 1845, typically does not have these five white markings during the day; however, at night these marks become clearly visible (personal observation, Figs. 1 and 2). The test in general becomes paler at night; however, around the time of spawning the test is dark, whereupon the white markings become evident, indicating the possible association between these naked areas of the test and the moonlight as a stimulus for spawning. Chromatophore expansion in diadematids has been associated with the presence of light (Yoshida 1966). Pearse (1972) noted that on the night of the new moon *D. setosum* had a bleached, pale gray appearance, while on the night before full moon most animals were relatively dark and similar to their nearly black appearance during the day. Pearse's (1972) observations substantiate those of Dambach (1969) who found significant chromatophore expansion in *Centrostephanus longispinus* Philippi, 1845, near full moon light intensities (two lux).

Echinothrix calamaris (Pallas, 1774) has naked green bands down the median areas of the interambulacra, where no spines or pedicellariae are present. These naked bands appear directly over the gonads and may act in a similar way to the white spots on *D. setosum*.

Pearse (1975) proposed that the monthly moonlight cycle could impose a monthly feeding rhythm that would lead to a monthly reproductive cycle. However, no difference in feeding activity in either *D. setosum* (Fox 1923) or *Centrostephanus coronatus* (Verrill, 1867) (Pearse 1975) has been correlated with a difference in feeding activity at either full moon or new moon.

This investigation presents analysis of the association between lunar periodicities and four species of

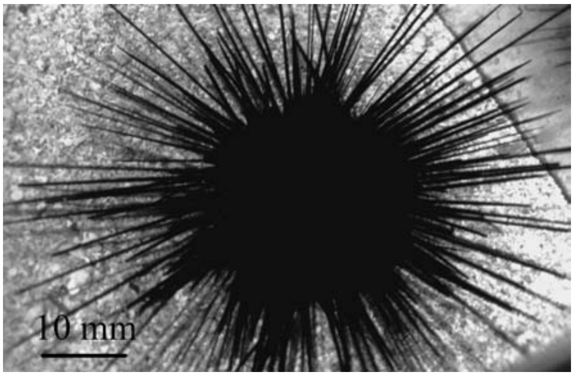


Fig. 1 *Diadema savignyi*. No white spots present on the naked regions of the interambulacra during the day

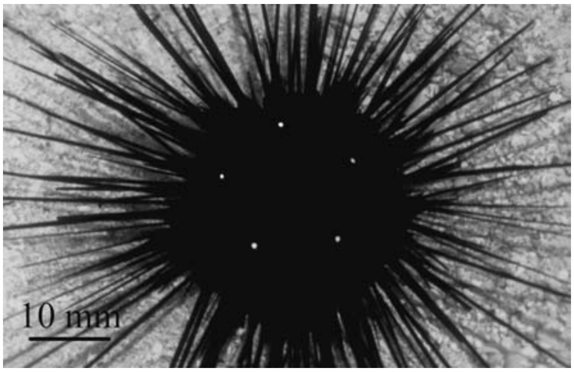


Fig. 2 *Diadema savignyi* photographed immediately after illumination of covered aquarium, after a 12-h period of darkness

diadematid echinoid (including two color morphs of *E. calamaris*) in Fiji, proposing this as a means of coexistence on a sustainable reef.

Method

Species identification

No difficulty was found in distinguishing *D. savignyi* from *D. setosum*. Identification of the two species of the genus *Echinothrix* initially proved difficult in the field. The white color morph of *E. calamaris* was easily identifiable, but this was not true for the brown color morph and *Echinothrix diadema* (Linnaeus, 1758). These two species have commonly been mistaken, to the extent that field guides often misrepresent the species (e.g. Gosliner et al. 1996). Correct identification initially required close examination of the diagnostic test, spine, and tridentate pedicellariae morphology as reported by Coppard (2002) and Coppard and Campbell (2004).

Specimen collection

Specimens of *D. savignyi*, *D. setosum*, *E. calamaris* (white and brown color morph) and *E. diadema* were collected from Sosoikula Reef and Nukubuco Reef, Suva, Fiji. Six to eight specimens of each species and color morph were collected by snorkeling using a small hand rake and transported alive in large plastic containers to the University of the South Pacific (USP). Water temperature and salinity readings were recorded on the reef; tidal data were obtained from the Fijian Meteorological Department. Echinoid samples were collected on the day of the moon's first quarter, full moon, last quarter, and new moon of each month, and at intermediate times for a 12-month period.

Specimens were also collected at Tambua Sands, 109 km west of Suva and at Namatakula, 92 km west of Suva. These specimens were only collected three times during the study period; however, a much larger sample size was collected consisting of 20 to 30 specimens for each species. The gonad index data from these samples were compared with the gonad index data from Suva for the same lunar phase of the same month, to determine whether different populations were in synchrony.

Sex determination and specimen dissection

The majority of reports of sexual dimorphism in echinoids have used external features such as the position of the gonopores (Swann 1954; Tyler and Tyler 1966), the size of the genital papillae (Tahara et al. 1958, 1960), and the color and texture of the gonads (Harvey 1956). However, each of these methods has been shown to be unreliable, particularly when cross-referenced with gamete smears or histological examination (Pearse and Cameron 1991). The only reliable method for determining the sex and reproductive condition is to examine gametogenic cells or gametes.

Specimens were transferred into holding tanks upon arrival at USP, before being dissected in the laboratory within 4 h of collection, using the following protocol. The test was cut open and the five lobes of the gonad were pried away from the test wall and placed on filter paper. One lobe was then carefully dissected and a gametes smear taken on a slide. This was then observed under a microscope for the presence of oocytes (Fig. 3), sperm (Fig. 4), and nutrient phagocytes. A second slide was made and stained with haematoxylin and counterstained with eosin, thus making sperm and oocytes easily identifiable.

The four remaining gonad lobes and test material were dried on filter paper in an oven at 50°C for 48 h. The dried gonads and test material were then immediately weighed and recorded. The gonad index was calculated using the formula:

$$\frac{\text{Dried gonads}*}{\text{Dried test material}} \times 100$$
$$= \text{Gonad Index (Grant and Tyler 1983)}$$

* The mean weight of the four gonad lobes was calculated, and multiplied by five, thus compensating for the one lobe that was dissected for the gametes smear.

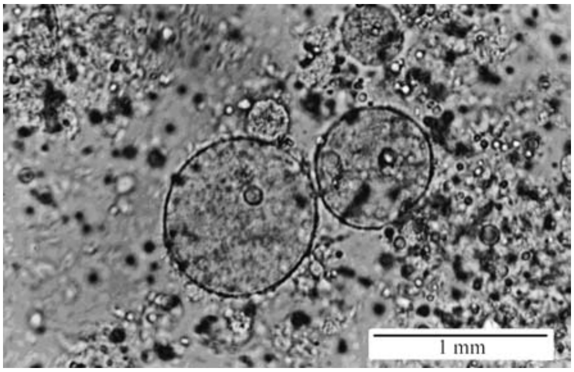


Fig. 3 Oocytes and nutrient phagocytes of *D. savignyi*, 2 days prior to spawning

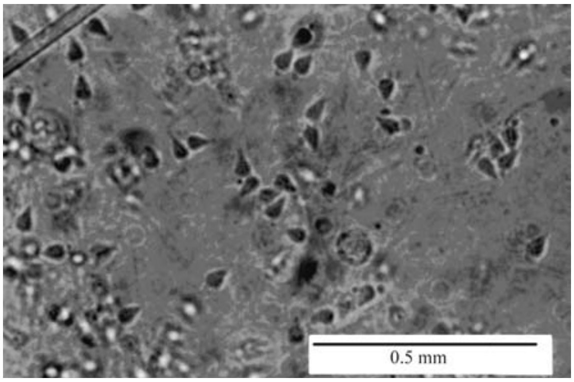


Fig. 4 Sperm (*E. calamaris* brown color morph)

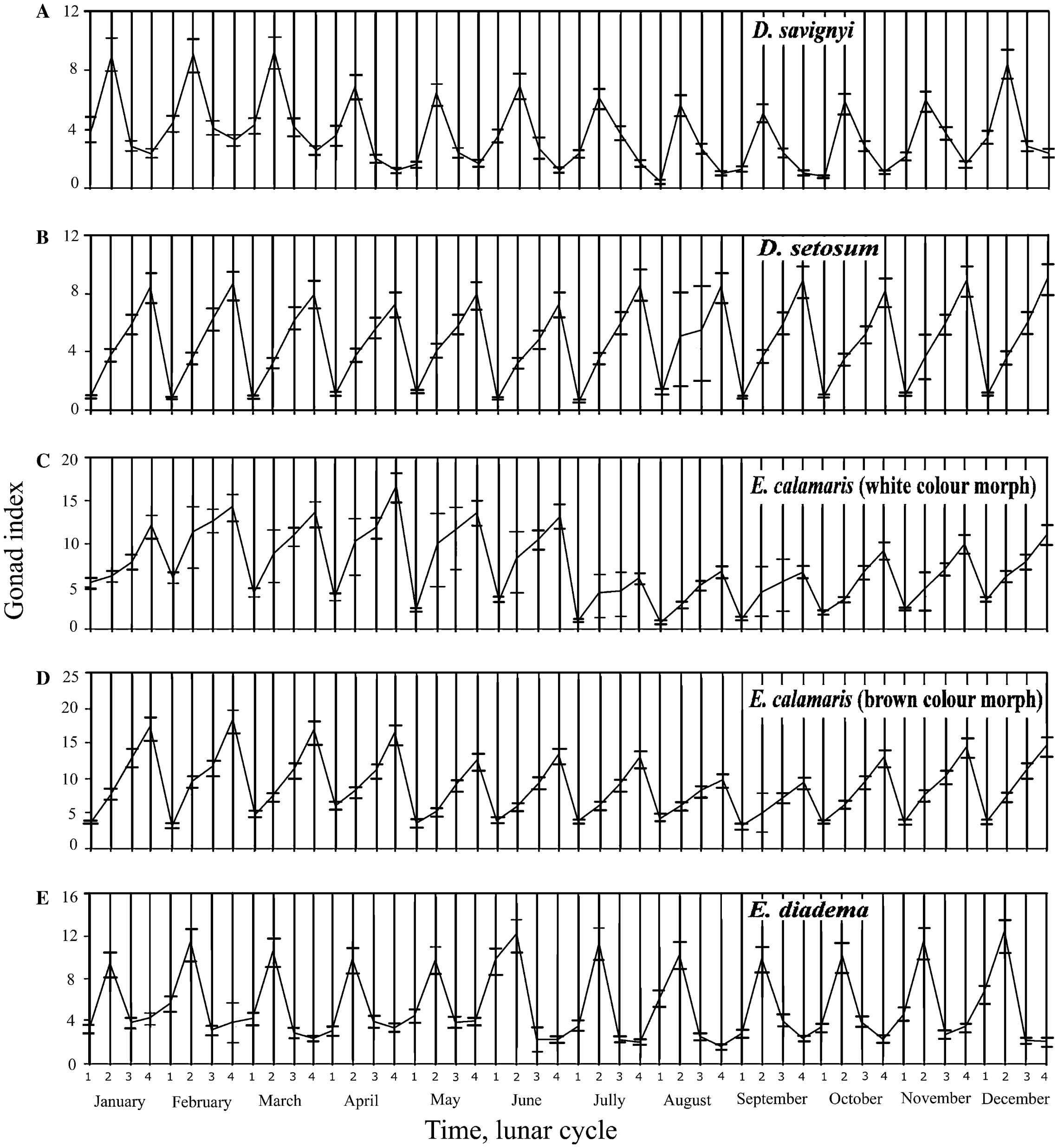


Fig. 5 Mean gonad indices for diadematid echinoids on Sosoikula Reef and Nukubuco Reef, Suva, Fiji (1998); *1* First quarter, *2* Full moon, *3* Last quarter, *4* New moon

The dried weight of the filter paper had already been calculated by working out the mean weight of ten dried pieces of equal size and density. This was necessary due to the very high humidity in Suva, which increased the weight of the filter paper.

Results

All breeding periodicities described in this investigation are for females, with the identification of oocytes from gonad smears. Male gonad indices were not obtained because of the exceptionally low incidence of males. The female to male ratio for the different species were: *D. savignyi* 10:1, *D. setosum* 12:1, *E. calamaris* (white color morph) 20:1, *E. calamaris* (brown color morph) 20:1, *E. diadema* 15:1. Both species of the genus *Diadema* were found on the fore-reef to mid-region of the back-reef, while *E. diadema* were found throughout the reef depending on the age and thus size of the specimen. Both color morphs of *E. calamaris* were predominantly found on the back-reef.

Standard deviations of the mean gonad index were low for each sample of each species collected (Fig. 5).

Diadema savignyi Michelin, 1845

The gonad indices showed a clear monthly cycle, with spawning peaks at the time of the full moon (Fig. 5a).

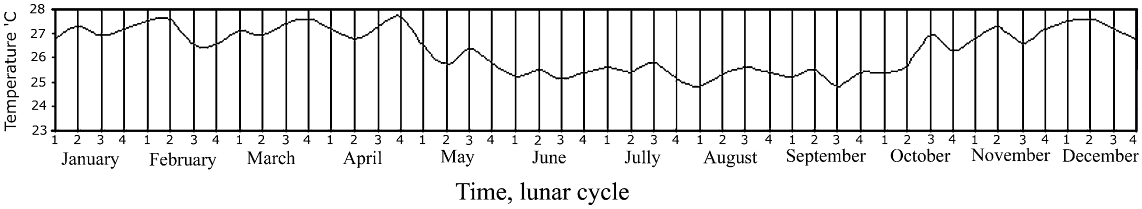


Fig. 6 Water temperature on Sosoikula Reef and Nukubuco Reef, Suva, Fiji (1998); *1* First quarter, *2* Full moon, *3* Last quarter, *4* New moon

These peaks were greatest in the summer months (December–March), which corresponded to elevated water temperatures (Fig. 6), but had no relationship to slight changes in salinity (Fig. 7). The gonads increased in size rapidly from the moon's first quarter to the full moon whereupon spawning occurred (observed both in aquaria and on the reef). After expulsion of the gametes, the gonads became small, dark, and shriveled in appearance. At the time of the moon's first quarter, the gonads were still small but no longer shriveled and dark, resembling a small version of the ripe gonads.

Diadema setosum (Leske, 1778)

Gonad indices showed a clear monthly cycle, with spawning occurring on the new moon, except in August when a few individuals spawned on the full moon (Fig. 5b). These echinoids were clearly out of synchrony with the rest of the population. Their test, spines, and pedicellariae were closely examined to confirm that they were not hybrids. Spawning peaks were greatest between November and December, which corresponded to elevated water temperatures (Fig. 6), but with no apparent association with changes in salinity (Fig. 7).

Echinothrix calamaris (Pallas, 1774), (white color morph)

A monthly spawning rhythm was clearly defined, with spawning occurring at and around the time of the new moon (Fig. 5c). Gonad indices were greater between February and June, where water temperatures were raised (26–28°C). The gonad index was significantly reduced when temperatures fell between 25°C and 24.5°C and is possibly related to an observed decrease in feeding. The gonads during the time of the new moon were largely swollen, with gametes 'oozing' out of the lobes from the inside of the test if the sea urchins were disturbed. Spawning was observed at this time, both on the reef and in aquaria (housed outside, exposed to moonlight). During the time of the full moon, the gonads were small and 'spongy' in appearance, later becoming thin and shriveled. However, during the month of August two specimens were found which were completely out of phase with the rest of the population, having ripe full gonads at the time of the full moon. These specimens were not obvious hybrids, having all morphological features typical of the species. However, by having such a marked contrasting reproductive rhythm, this could explain the possible hybridization with *E. diadema*, which spawned on and around the full moon.

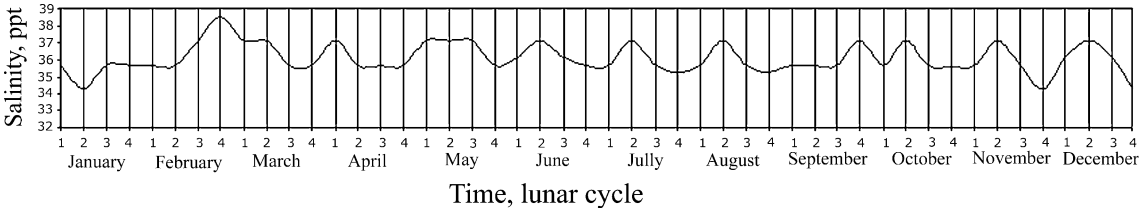
Echinothrix calamaris (Pallas, 1774), (brown color morph)

A very similar rhythm was observed to that of the white color morph, spawning on and around the new moon (Fig. 5d). Gonad indices were greater than for the white color morph from January to April, but still showed the same trend, with a close association between water temperature and gonad index. The lobes of the gonads were generally longer and larger than those of the white color morph, and associated with an increase in test size. The appearance of the gonads was also very similar i.e., being large, swollen and 'oozing' around the time of spawning, and small and spongy around the time of the full moon before becoming very thin, membranous and shriveled.

Echinothrix diadema (Linnaeus, 1758)

Spawning occurred on the full moon throughout the Fig. 5e) and did not appear to be influenced by changes

Fig. 7 Water Salinity on Sosoikula Reef and Nukubuco Reef, Suva, Fiji (1998); *1* First quarter, *2* Full moon, *3* Last quarter, *4* New moon



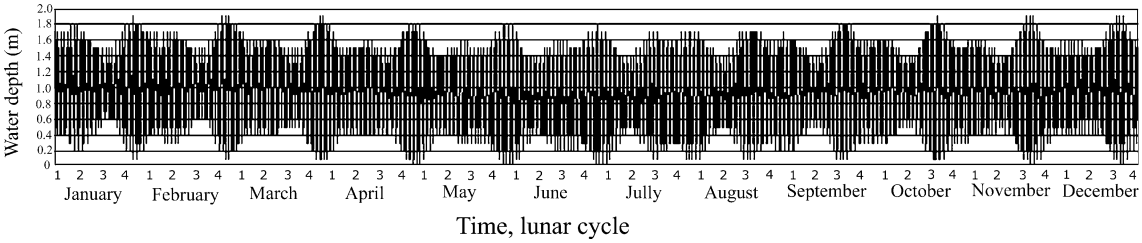


Fig. 8 Tidal prediction of high and low water (January, 1998–December, 1998) for Suva, Fiji; Latitude 18°08′S, Longitude 178°26′E; *1* First quarter, *2* Full moon, *3* Last quarter, *4* New moon

in water temperature or salinity. Breeding synchrony was in phase for all specimens which occurred as individuals on the reef and not in aggregations. The gonads were typically smaller, but broader in relation to the length of the central lobe, than those seen in *E. calamaris*, but had a similar appearance at the different stages of the reproductive cycle.

Water temperature and salinity

Water temperatures (Fig. 6) were elevated during the summer 'wet season' (November–April) and declined for the winter 'dry season' (May–October). Salinity readings (Fig. 7) indicated a peak of 38.5 ppt during the summer month of February, the hottest month of the year (Fiji Meteorological Service). The greatest troughs in salinity were found in January, November, and December with readings of 34.3 ppt. These troughs relate to the heaviest rainfall occurring during this time. Tidal data (Fig. 8) were compared with water temperatures, salinity, and breeding periodicities for the Suva samples. As expected, tidal data showed spring tides occurring on the full moon and new moon, with neap tides occurring on the moon's first and last quarter. Troughs in salinity typically occurred at the time of the full or new moon, thus spring tides. Peaks in salinity varied throughout the year, occurring on the moon's first quarter during April and May, new moon during February and September, and full moon in January, March, June, July, August, October, November, and December. Ten of the twelve salinity peaks therefore indicate an association to spring tides, with shifts possibly related to changes in seasonality.

Gonad indices obtained for specimens of *D. savignyi*, *E. calamaris* (white color morph) *E. calamaris* (brown color morph), and *E. diadema* collected from both Tambua sands and Namatakula had very similar mean values to those obtained from Suva. These values fitted the same monthly breeding rhythms as expressed by specimens of the same species in Suva. This shows gametogenic and spawning synchrony among different populations in Fiji, indicating a spawning rhythm directly related to the lunar cycle.

Discussion

Different breeding cycles allow genetically similar species to co-exist, minimizing the risk of hybridization. The fact that hybridization occurs when individuals of similar species spawn simultaneously supports such a conclusion. Different breeding cycles would allow very similar species (*D. savignyi*/*D. setosum* and *E. calamaris*/*E. diadema*) to occupy the same or similar habitats when environmental factors such as food and crevice space are not limiting.

This study found clear distinct monthly breeding periodicities attuned with the lunar cycle for all four species. *Diadema savignyi* and *E. diadema* had breeding rhythms linked to the new moon (spring tides), while *D. setosum* and both color morphs of *E. calamaris* had a breeding rhythm attuned to the full moon (spring tides). Such a precise rhythm within a population, particularly spawning on the spring tides, would appear advantageous to maximize chances of fertilization. A decline in water temperature produced a reduction in gametogenesis in *D. savignyi* and both color morphs of *E. calamaris*; with a more limited effect in *D. setosum* and little or no effect in *E. diadema*. Continuous reproduction was observed for the 12 months of this study and can possibly be attributed to water temperatures continually above 25°C. The role sea temperatures play in regulating reproductive cycles in Indo-Pacific echinoderms is not always clear. A few authors have reported that they believe that water temperatures alone do not play a key role (Pearse and Cameron 1991; Drummond 1991, 1994, 1995). Water temperatures in Fiji influenced feeding rates of certain species, particularly *D. savignyi* (Coppard 2002), with feeding rates in themselves intrinsically influencing reproductive fitness, thus reproductive cycles.

A few specimens in this investigation were found to be out of phase with their conspecifics. It has been reported that in the laboratory, sperm of *D. savignyi* and *D. setosum* can fertilize the eggs of the other species at equal rates to those of intraspecific crosses (Uehara et al. 1990). Lessios and Pearse (1996) reported that the resultant hybrids have been reared to sexual maturity producing motile sperm. In this investigation, a small number of sea urchins were found that had intermediate morphologies. The majority of these for the genus *Diadema* conformed to the morphotypes 'Type 1', 'Type 2', and 'Type 3' as described by Lessios and Pearse (1996). One specimen found did not conform to these hybrid morphotypes. The mixed morphology of this specimen suggested that it might be an F2 or later generation hybrid. Hybrids morphotypes, determined by intermediate test, spine, and tridentate pedicellarial structures were also found to form between both color morphs of *E. calamaris* with *E. diadema*.

The two color morphs of *E. calamaris* present in Fiji have been shown to have distinct differences in their spine structures (Coppard and Campbell 2004). This morphological evidence suggests that they are either separate species or subspecies. This investigation found that both color morphs spawned at very similar times on and around the new moon. Possible hybrids with intermediate morphologies of these echinoids were found in low numbers, proportional to those found between other species in this investigation. This therefore presents a perplexing question: why are more hybrids not formed between these two closely related sea urchins? No evidence was found to indicate spatial isolation. Gametic incompatibilities during sperm activation, acrosmal reaction, sperm-egg attachment, or sperm-egg fusion have been demonstrated as mechanisms preventing hybridization (Metz et al. 1994). Increased sperm specificity has also been associated with closely related sympatric species (Riginos and McDonald 2003). Such a low incidence of hybridization between these sea urchins provides further evidence that they are likely to be subspecies or separate species. However, in light of the laboratory investigations by Uehara et al. (1990) it seems strange that a prezygotic isolation mechanism would have evolved in one potentially recently divergent subspecies/species, whilst not having evolved amongst previously divergent species in the same monophyletic family. If high fertilization rates are occurring, then it would appear likely that this is coupled with high mortality rates, thus explaining the low numbers of hybrids and low levels of species introgression (Lessios and Pearse 1996). The mechanism by which *E. calamaris* white color morph and *E. calamaris* brown color morph avoid hybridization remains to be determined.

Sex ratios in echinoids have predominantly been reported to be 1:1 (Ikeda 1931; Harvey 1956; Fuji 1960). A few reports have shown a variation from this ratio. These reports show that deviations from the 1:1 ratio do occur in echinoids, but this divergence does not stray vastly from the 1:1 ratio (Mcpherson 1965; Pearse 1968, 1969a; Pillay 1971; Hori et al. 1987).

The mechanism of sex determination in echinoids, as in other echinoderms, is not known (Pearse and Cameron 1991). Therefore, it can be hard to substantiate the cause and the mechanism by which populations have varying ratios. Possible explanations are likely to reflect extreme environmental conditions. Moore et al. (1963a, b) report how adverse weather conditions can affect and influence sex determination. In populations of *Lytechinus variegates* (Leske, 1778) and *Tripneustes ventricosus* (Lamarck, 1816) off southern Florida, an exceptionally large proportion of hermaphrodites were found. Pearse and Cameron (1991) attribute this to an unusually cold winter when such individuals were juveniles.

Suva Harbor, 10 km west from the sample sites on Sosoikula Reef, and Nukubuco Reef has one of the world’s highest recorded levels of tributyltin (TBT) from antifouling paints. Maata (1998) and Maata and Koshy (2001) reported finding high levels of TBT in the sediment of Suva Harbor in 1991, measuring 38,000 parts per billion (ppb). However, it is reported that by 1995 this value had increased to 360,000 ppb. Low levels of tributyltin have been shown to have noxious effects on many marine organisms; ’imposex’ in neogastropods (Gibbs et al. 1991), shell thickening in Oysters (Alzieu et al. 1982), and significantly reduced growth rates and probable lethal effects on the veliger larvae of the bivalve *Scrobicularia plana* (Da Costa, 1778) (Ruiz et al. 1994). There are, however, few reports on the effects of organotins on sea urchins. Ringwood (1992) reported the sensitivity of gametes and early developmental stages of the tropical sea urchin species *Echinometra mathaei* (de Blainville, 1825) to TBT, while Ozretic et al. (1998), Martin et al. (2000), and Novelli et al. (2002) demonstrated sperm and embryotoxicity of TBT in the temperate sea urchin *Paracentrotus lividus* (Lamarck, 1816). Such reports support the hypothesis that high TBT levels may be a factor in explaining the strange sex ratios. Species in this investigation found near the lagoon on the back-reef where TBT levels are reported to be highest, had the greatest difference in the sex ratios; both color morphs of *E. calamaris* 20:1 and large adult *E. diadema* 15:1. Species that inhabited the fore-reef to mid-back reef where TBT levels are reportedly reduced had lower ratios; *D. setosum* 12:1 and *D. savignyi* 10:1.

Further samples taken from Tambua sands 109 km west of Suva Harbor (currents from east to west in southern Viti Levu, P. Newell, personal communication), the source of the TBT pollution, had sex ratios (1.6:1) nearer the expected 1:1 ratio.

Other authors contacted, with regard to this strange sex ratio (J.S. Pearse, personal communication; P. Newell, personal communication), mentioned possible parasitic infection by ascothoracid crustaceans. These are well described as endoparasites on anthozoans and echinoderms (Grygier 1996). However, the presence of such parasites or damaged gonads as a result of such a parasitic infestation was never seen. Therefore, such an explanation on this occasion seems unlikely. Although other anthropogenic or natural phenomena may, in the future, be determined to cause the predominance of females, at this point the high levels of TBT seem a viable explanation for the strange male to female sex ratios.

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