Reproductive Cycle of Edible Echinoderms from the Southwestern Indian Ocean

I. *Tripneustes gratilla* L. (Echinoidea, Echinodermatata)

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Abstract—The reproductive cycle of the edible echinoid *Tripneustes gratilla* (Linneaus 1758) was investigated in a population located in the southwest coast of Madagascar (Toliara). The reproductive cycle was followed for two consecutive years (October 1999 to October 2001) and characterised by means of gonad and maturity indices. *T. gratilla* has an annual reproductive cycle mediated by seawater temperature, day length and feeding activity. The histology of the gonads revealed six different maturity stages grouped in three main reproductive phases: growing and maturing, spawning, and spent and recovering. Reproduction is seasonal. Growing and maturing stages occurred from late summer to early winter correlating with decreasing temperatures and day length. Spawning occurred during mid and later winter, when temperatures and day lengths were lowest. Spent and recovering urchins were observed from late summer to early winter. Besides, the reproductive cycle was also influenced by food quantity in the gut, the repletion index was higher after spawning and gametogenesis started when sufficient amount of nutrient reserves accumulated in gut tissues and nutritive phagocytes of the gonads. This study reveals that the maturity index, based on histological analysis of gonads, is more reliable than gonad index in characterising the reproductive cycle of echinoids. In view of fishery management, we recommend harvest of *T. gratilla* from November to February, when individuals are in the pre-gametogenic stages.

INTRODUCTION

The role of echinoid grazing in determining subtidal community structure is well established (Lawrence, 1975; Lawrence & Sammarco, 1982; Harrold & Pearse, 1987). A marked increase in echinoid population has been shown to transform kelp beds to barren grounds while a decrease resulted in a return to an algal dominated system (see Harrold & Pearse, 1987 for review). Many echinoids are edible and thus of economic interest. An increasing demand for echinoid roe over the past 15 years has led to overfishing and depletion of natural stocks (Sloan, 1985; Keesing & Hall, 1998). In view of these ecological and economic aspects, considerable interest has developed in the reproductive biology of echinoids. Reproductive success has a direct influence on larval supply, which is one of the factors affecting recruitment and establishment of echinoid population (Pedrotti,

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1993; Lopez *et al.*, 1998; Balch & Scheibling, 2001). Gonad quality is an important criterion for market value. Echinoid gonads are usually eaten at the pre-gametogenic stage and therefore, knowledge of the reproductive cycle is essential for fishery management (Byrne, 1990; Lawrence, 2001). Variability in reproductive patterns is common among shallow-water echinoid species and even within populations of a single species (Himmelman, 1978; Pearse & Cameron, 1991; Lozano *et al.*, 1995). This variability is explained by the fact the reproductive cycle is determined by environmental cues (temperature, photoperiod, food availability, and hydrodynamism), which are themselves subjected to periodic cycles whose intensities and patterns vary according to geographical localities.

Tripneustes gratilla (Linneaus 1758) is a-shallow-water echinoid, ubiquitous in the tropical Indo-West Pacific region. Like many other littoral echinoids, it is of economic and ecological interest since it is edible and considered as a primary herbivore in the various habitats it occupies (Lawrence & Agatsuma, 2001). Studies dealing with its reproductive cycle indicated variable spawning periods according to its geographical distribution (Chen & Chang, 1981; Lawrence & Agatsuma, 2001). A single previous study in the Indian Ocean, from the north coast of Madagascar, reported continuous gametogenic activity with a peak spawning period in late winter (Maharavo, 1993). In some tropical echinoid species, the breeding season is spread throughout the year when the population is located near the equator, but farther away, spawning occurs over a restricted period (Pearse & Cameron, 1991). We report on an investigation of the reproductive cycle of a population of *T. gratilla* located in the southwestern coast of Madagascar. The study covers two breeding seasons and reproductive patterns were determined by means of gonad and maturity indices and attempt to relate these to environmental factors and to the known seasonal variability in the feeding activity of the population (Vaïtilingon *et al.*, 2003).

MATERIALS AND METHODS

Samplings and measurements

The population of *T. gratilla* was located among the seagrass beds of the Beloza fringing reef lagoon (23° 29.32' S; 43° 45.05' E). This reef forms part of the Toliara reef complex, at the southwest coast of Madagascar (see Vaïtilingon *et al.*, 2003 for further details about the sampling site). Sampling at low tide was monthly from October 1999 to October 2001, except in November 2000 and February 2001 (heavy rains were observed killing numerous echinoids and in order not to introduce errors in our results, echinoid samplings were avoided on these two months). At each sampling time, 30 individuals of test diameters between 7 and 9 cm were randomly collected, brought alive to the laboratory and measured immediately (Table 1). Two perpendicular ambital test diameters were measured using vernier calipers. Individual fresh weight was determined after leaving the specimen on absorbent paper for three minutes to remove excess water. They were then dissected to determine the fresh weight of the digestive tract with its contents and of the gonads. One of the five gonads was fixed in Bouin's fluid for further histological investigation (see below).

To characterise the reproductive cycle of *T. gratilla,* the gonad index (GI) and the maturity index (MI) were calculated for each month studied. The ratio of gonad fresh weight to total fresh weight was used to calculate the gonad index (GI):

$$GI = \frac{FW_g}{TW} \times 100 \tag{1}$$

where FW_g is the weight of the gonads in gram and TW is the weight of the whole animal in gram. The MI was determined after histological analysis of the fixed gonad.. A preliminary study confirmed the homogeneous gametogenic state in *T. gratilla* which is in agreement with previous echinoids studies (Byrne, 1990; King *et al.*, 1994; Spirlet *et*

al., 1998). Hence histology was performed only on the middle portion of one of the five gonads from each sampled individual. The fixed gonad was rinsed, dehydrated, embedded in paraffin wax and sectioned at 7 μm. Serial sections were placed on glass slides, dewaxed and stained with Masson's trichrome. All slides of gonad sections were examined under light microscope for sex determination, and photographed using a Q-imaging Micropublisher digital camera. Maturity stages were classified according to previous studies of echinoid gametogenesis (Byrne, 1990; Spirlet *et al.*, 1998) based on oocyte size (females), thickness of peripheral spermatocyte layer (males) and the amount of nongerminal nutritive tissues (males and females). For each monthly sample, measurements of at least 100 oocyte diameters and the widths of at least 30 spermatocyte layers were performed using Scion Image analysis software. The maturity index (MI) represents the monthly mean gonadal stage calculated after transforming the maturity stage of every individual to a circular scale (Zar, 1996; Spirlet *et al.*, 1998). Each monthly mean value is represented as a vector in a circle where the direction of the vector points to corresponding gonadal stage and the vector length represents the homogeneity of the sample.

To assess the possible relationship between the reproductive cycle and the variation in gut content, the repletion index (RI), was calculated each month as follows:

$$RI = \frac{FW_{dt}}{TW} \times 100 \qquad (2)$$

where FW_{dt} is the fresh weight of the digestive tract (including contents) in gram. The variation in the RI was compared to that of the reproductive cycle (GI and MI) in order to detect any correlation. Some basic environmental parameters such as, seawater temperature and salinity were recorded at time of sampling using a combined salinometer-thermometer apparatus (WTW LF 330, Bioblock Scientific). Photoperiod was also considered and its value on the sampling date was calculated from the Online-Photoperiod Calculator V 1.94 (http://www.sci.fi/~benefon/sol.html) and expressed as day length in hours.

Table 1. Synopsis of monthly measurements taken from *Tripneustes gratilla* individuals throughout the two-year study period (n = 30). Test diameter expressed in cm and fresh weight in gram.

Sampling month	Mean (±sd) test diameter	Mean (±sd) total fresh weight (TW)
Oct.-99	7.92±0.31	213.01±23.14
Nov.-99	8.18±0.53	212.67±40.02
Dec.-99	8.13±0.44	220.86±38.97
Jan.-00	8.10±0.62	214.36±48.69
Feb.-00	8.58±0.57	250.76±49.35
Mar.-00	8.10±0.42	203.60±36.47
Apr.-00	8.47±0.59	250.78±49.35
May-00	8.40±0.62	246.34±50.88
Jun.-00	8.26±0.60	229.84±44.00
Jul.-00	8.62±0.51	255.19±43.98
Aug.-00	8.00±0.45	206.06±34.33
Sep.-00	8.56±0.56	248.71±41.58
Oct.-00	8.43±0.41	235.42±42.05
Dec.-00	8.24±0.45	223.86±40.14
Jan.-01	8.61±0.55	248.54±41.70
Mar.-01	7.89±0.50	218.84±34.66
Apr.-01	7.98±0.48	212.77±37.65
May-01	8.73±0.42	248.88±36.66
Jun.-01	8.05±0.55	210.59±40.82
Jul.-01	8.13±0.41	232.41±35.43
Aug.-01	8.32±0.32	234.09±26.35
Sep.-01	8.16±0.30	225.90±33.79
Oct.-01	8.52±0.52	233.93±39.43

Statistical analysis

Comparisons between monthly samples and between male and female batches of both GI and RI were done using one-way ANOVA after arcsine transformation of data (Zar, 1996). The Watson U^2 nonparametric test was used to compare MI values between two consecutive years and between male and female batches (Fisher, 1993; Zar, 1996). The correlation between temperature, daylength, salinity and MI were tested using the linear-circular rank correlation statistic Un (Fisher, 1993). In all statistical analyses, the level of significance used was $P < 0.05$.

RESULTS

Out of a total of 690 *T. gratilla* individuals collected randomly during the study period, 317 individuals were males and 373 were females, that is a sex ratio (male: female) of 1 : 1.18. This sex ratio was significantly different from 1 : 1 (Chi-square test: $\chi^2 = 4.545$, df = 1, $P = 0.033$).

Repletion and gonad indices

Although a certain degree of variability was observed within each monthly sample of repletion and gonad indices, a significant annual variation was depicted for both indices when followed throughout a two-year survey (Fig. 1). The mean repletion index (RI) varied significantly between samples throughout the study period (one-way ANOVA; df = 22, F = 8.62, P < 0.05). During the first studied year, the mean ($\pm$ sd) RI value from October 1999 to May 2000 was 14.46 $\pm$ 2.36% while significantly higher values were reported from June to September - mean RI value recorded during that period was 17.90 $\pm$ 3.07%. A similar, but less marked pattern, was observed in 2000-2001, i.e an increase from 15.11 $\pm$ 2.16 % to 16.95 $\pm$ 2.75%. Mean gonad index (GI) varied significantly during the study period (one-way ANOVA; df = 22, F = 7.72, P < 0.05). GI was lowest from October 1999 to February 2000 with a mean ($\pm$ sd) value of 11.50 $\pm$ 3.10%, then increased by March 2000 to reach peak value in June 2000 (16.07 $\pm$ 3.28%) after which, a drop to about 10% was observed. Similar pattern was observed during the second studied year, except that peak values were observed up to July 2001 (about 17%). Besides, a significant correlation was observed between the two indices (Pearson product moment correlation, R = - 0.48, P < 0.05). RI was negatively correlated to GI, that is, higher values of RI were observed when GI was at its lowest.
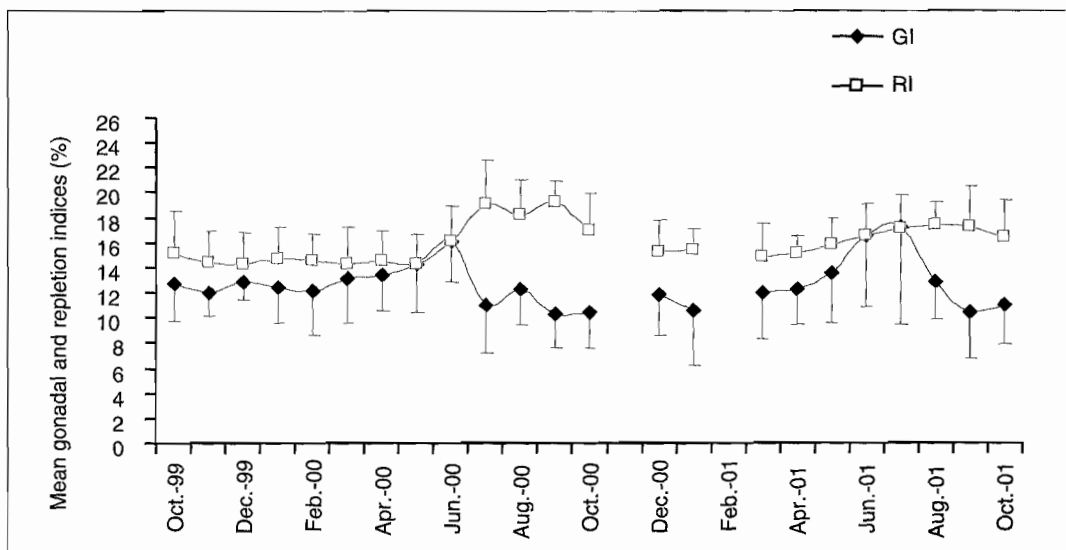

Fig. 1. Mean gonad (GI) and repletion (RI) indices for *Tripneustes gratilla* over the two year survey. Values of RI and GI represent mean + or - SD, respectively (n = 30). Data in November 2000 and February 2001 are missing

Gonad histology

Six different maturity stages were recognised after histological analysis of both female and male gonads. These stages were observed successively during the two studied years and are described below.

Ovaries

Stage 1: Spent
Ovaries in the early spent stage are characterised by a thin layer of nongerminal nutritive phagocytes lining the acinal walls (Fig. 2A). Unspawned ova and few vitellogenic oocytes largely occupy the lumens. Both ova and vitellogenic oocytes are destined to be resorbed by the phagocytes. Resorption of germinal cells happens throughout the spent stage so that the lumens of late spent ovaries are completely deprived of any germinal cells and fill with dense meshwork of nutritive phagocytes.

Stage 2: Recovery
The appearance of the first previtellogenic oocytes, defines the recovery stage. This stage happens either at mid-late or late spent stage. Figure 2B shows a recovering ovary at the mid-late spent stage where some relict ova are still present at the centre of the lumen and are being resorbed by phagocytes while previtellogenic oocytes differentiate along the acinal wall. Most of the ovaries (76%) recovered at this stage of development, while, the rest recovered only when all the ova have been resorbed, that is, after late spent stage (Fig. 2C). The diameter of the previtellogenic oocytes range from 10 to 30 μm.

Stage 3: Growing
This stage is characterised by growth in size of the previtellogenic oocytes due to the onset of vitellogenesis. The growing ovaries contained numerous vitellogenic oocytes that are still in contact with the acinal wall and are surrounded by the nutritive phagocytes (Fig. 2D). The size of these vitellogenic oocytes ranges from 30 to 60 μm in diameter. Some previtellogenic oocytes may still be present along the acinal wall and the lumen is still filled by nutritive phagocytes.

Stage 4: Premature
In premature ovaries, vitellogenesis is a continual process and oocytes of all stages are present. Vitellogenic oocytes continue to grow and as they become bigger, they get detach from the acinal wall to migrate in the centre of the acini (Fig. 2E). Fully grown vitellogenic oocytes undergo maturation and the resulting ova accumulate in the lumen, which is now devoid of any nutritive tissues.

Stage 5: Mature
The ovarian lumen is filled with densely packed ova and most of the nutritive tissues have been used up, leaving a thin layer at the periphery of the acini (Fig. 2F). The mature ova have a small nucleus and measure from 65 to 80 μm in diameter. Vitellogenic oocytes are still present in the germinal layer indicating that vitellogenic activity is not completed.

Stage 6: Partly spawned
Partly spawned ovaries are characterised by the presence of ova that are less densely packed than mature ovaries (Fig. 2G). Vacated spaces are observed in the acini, indicating partial release of ova. In the germinal layer, nutritive tissues are still present as a thin layer surrounding vitellogenic oocytes. These oocytes continue their development and subsequently replace the ova as they are shed.

Testes

Stage 1: Spent
Testes at the spent stage are characterised by the presence of a developing layer of nutritive phagocytes along the acinal walls (Fig. 2H). In early spent testes, relict spermatozoa are still present in the centre of the acini and are being resorbed by the phagocytes. Late spent testes are devoid of any germinal cells and the acini are filled with dense meshwork of nutritive phagocytes. At the spent stage, no spermatogonial proliferation is observed.

Stage 2: Recovery
In recovering testes, clusters of spermatogonia and the first primary spermatocytes are observed lining the acinal walls. In 66% males, this stage occurs at the mid-late spent stage, that is, when the relict

spermatozoa are being resorbed (Fig. 2I). While for the rest, initiation of recovery stage occurs at late spent stage, that is, when all relict spermatozoa have been resorbed (Fig. 2J). The acini are filled with nutritive phagocytes, except in cases where some relict spermatozoa are still present.

Stage 3: Growing
Growing testes are characterised by a developing layer of primary spermatocytes lining the acinal walls (Fig. 2K). The thickness of the spermatocyte layer ranges from 10 to 30 µm. From this layer, columns of spermatocytes project towards the lumen that is still filled with nutritive phagocytes at this stage.

Stage 4: Premature
In premature testes, the layer of nutritive phagocytes is greatly reduced since the tissue is being used up during growth. The resulting lumen is connected to the spermatocyte layer lining the acinal wall by columns of spermatocytes along which, sperm differentiation occurs. After differentiation and maturation, spermatozoa detach from the tips of the columns and accumulate in the lumen (Fig. 2L).

Stage 5: Mature
The lumen of mature testes is filled with densely packed spermatozoa. Most of the nutritive phagocytes have been used up and a thin peripheral spermatocyte layer is observed (Fig. 2M).

Stage 6: Partly spawned
Spermatozoa in the lumen of partly spawned testes are less densely packed. Vacated spaces are observed on the periphery, indicating partial release of spermatozoa. The layer of spermatocyte lining the acinal walls is still present together with columns projecting towards the centre of the acini, indicating that spermatogenesis is not completed (Fig. 2N).

Gametogenic cycle

The relative monthly frequencies of the six maturity stages described in males and females, together with a description of the changes in oocyte diameter are illustrated in figure 3. The gametogenic cycle of *T. gratilla* was annual with peak spawning occurring in July. Yet, differences in its pattern were observed from one year to another and between the sexes. During the first year of study (October 1999 to September 2000), onset of gametogenesis was noted in November in males and February in females while in year two (October 2000 to September 2001), this stage of development was observed in December and January in males and females, respectively. Even though the gametogenic activity started earlier in males, both males and females seemed to reach maturity and start spawning almost at the same time, June in year one and May in year two. The spawning period was spread over 5 to 7 months, as indicated by the proportion of partly spawned stages (see Figs. 3 A and B). The occurrence of vitellogenic oocytes and spermatocytes in mature and partly spawned gonads clearly indicated that gametogenesis was continuous throughout the spawning period. The change in oocyte diameter followed during the study period shows that oocyte size was maximum when maturity and spawning stages were reached (Fig. 3C). The oocyte diameter was minimum when most of the ovaries were in the growing stage (in March).

Maturity index

The maturity index (MI) provided a quantitative method for documenting the different maturity stages recorded throughout the study period. Since MI is a mean value, it allows better comparisons between sexes, months and years. MI was thus computed each month and is represented on both circular (or polar chart representations), see figure 4, and linear time scales (Fig. 5). Male and female batches were represented separately as their MI values were significantly different from each other (Watson U2 nonparametric test; U2 = 0.42, P < 0.05). Likewise, a significant difference was also observed between the two years. These differences were due to a differential rate of gametogenic activity (RG), that is, change in maturity stage per unit time, observed between sexes and years. During the first five months following spawning (October 1999 to February 2000), RG was 0.095 stage/month in females and 0.279 stage/month in males. This period correspond to the spent and
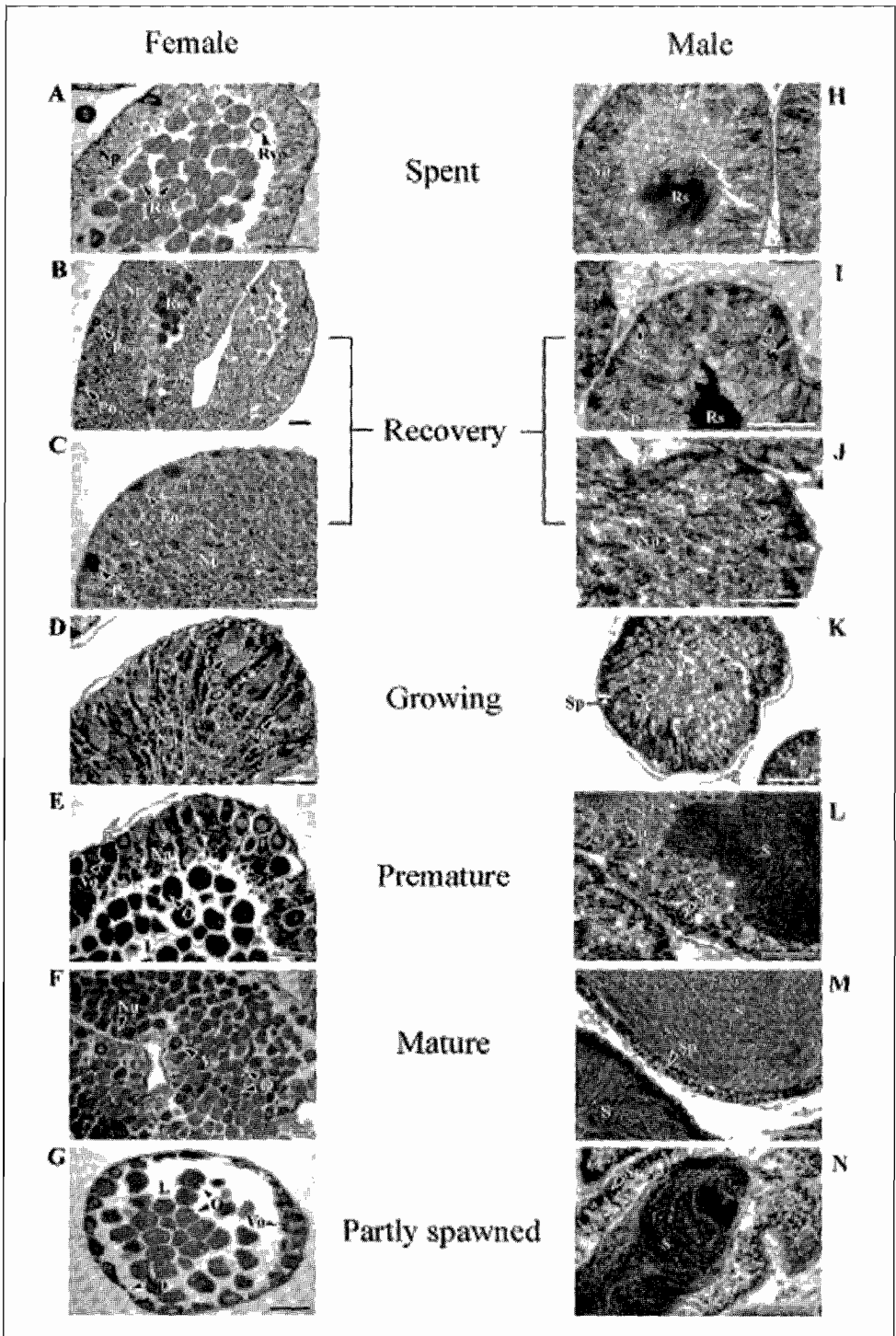
Fig. 2. Histology of ovaries (A-G) and testes (H-N) of *Tripneustes gratilla,* showing the six maturity stages. See text for further description. Abbreviations: Cs, column of spermatocyte; L, lumen; Np, nutritive phagocytes; Nu, nucleus; O, mature ovum; Po, previtellogenic oocyte; Ro, relict ovum; Rs, relict sperm; Rvo, relict vitellogenic oocyte; S, sperm; Sg, spermatogonia; Sp, primary spermatocyte layer; Vo, vitellogenic oocyte. Scale bars = 100 μm
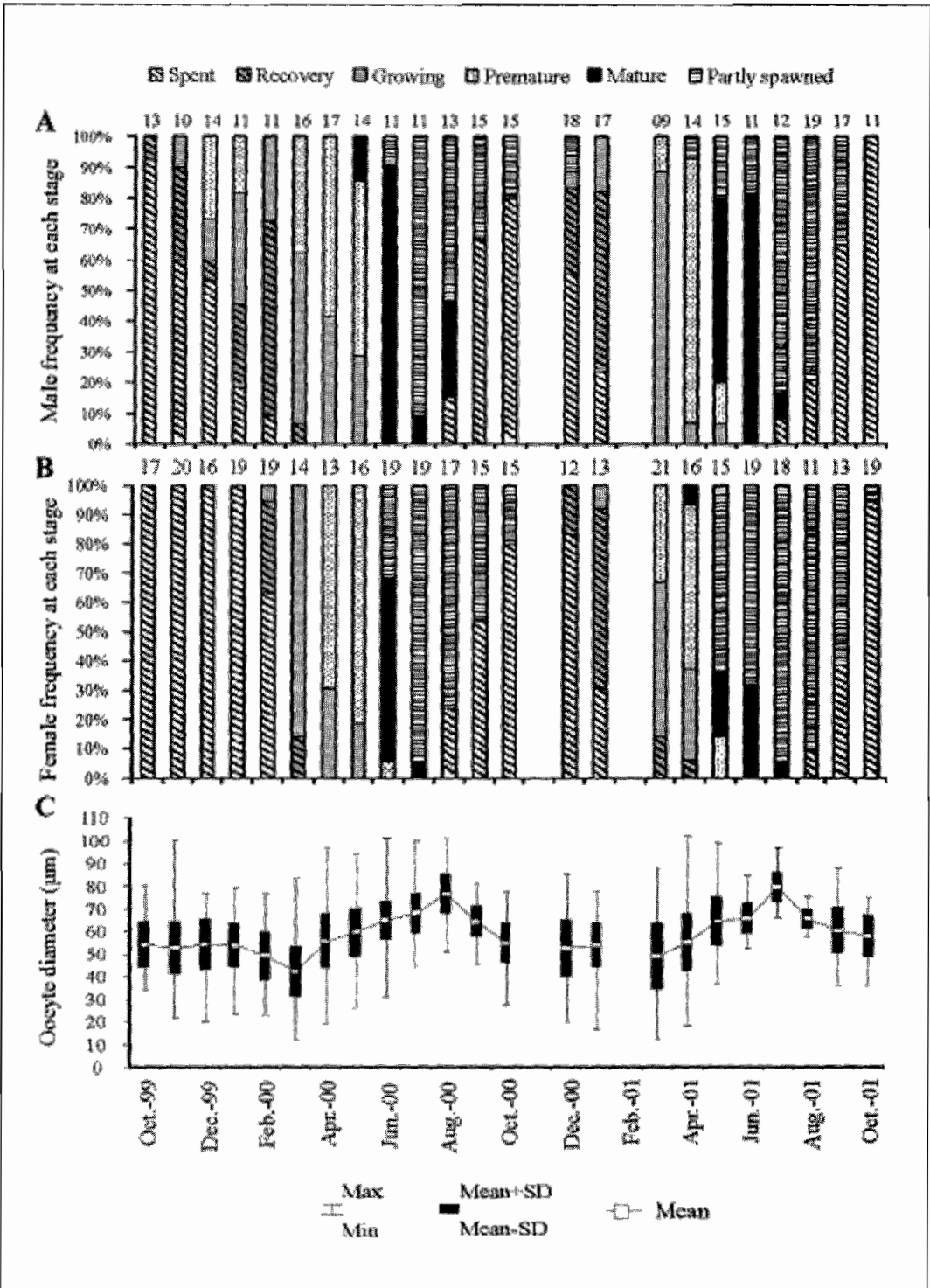
Fig. 3. Relative frequencies of maturity in males testes (A) and female ovaries (B) for *Tripneustes gratilla,* and variation in oocyte diameter (C) over the two years of observation. Data for November 2000 and February 2001 are missing. The number of males or females out of 30 sampled individuals is indicated above each histogram, respectively

recovery stages that were achieved at a slower rate in females than males. From March to July 2000, the RG was 0.770 stage/month in females and 0.651 stage/month in males. This increase in rate of gametogenic activity, more pronounced in female than male, corresponded to the growing, maturing and spawning stages. However, during the second year, only a slight difference was noted in the RG between males and females. Overall rate of gametogenic activity was the same for both batches during that year.

The conjunction of the three physiological indices (GI, RI and MI; Figs. 1 and 5) reveals significant relationships. Correlation between RI and MI for both female and male batches were highly significant (linear-circular correlation, $P < 0.01$; Dn = 0.7 for both sexes), while between GI and MI, correlation was significant ($P < 0.05$; Dn = 0.5 and 0.3 for female and male, respectively).
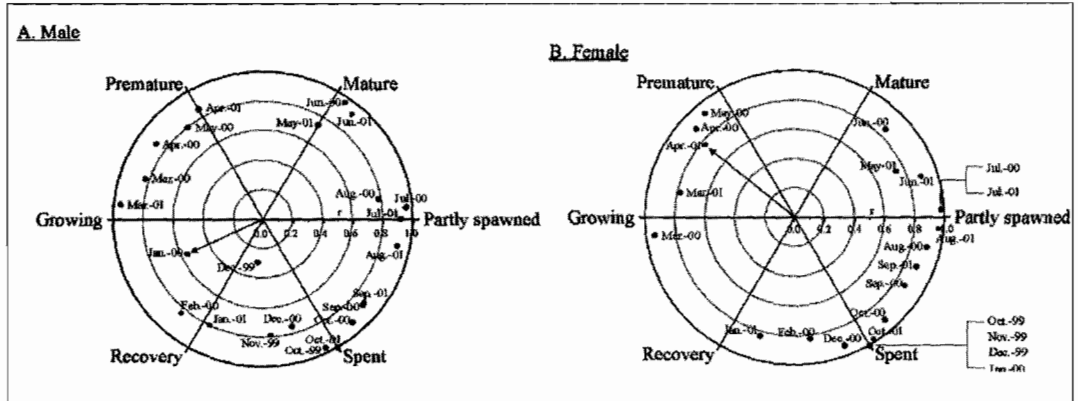

Fig. 4. Polar chart representations of the maturity index for male (A) and female (B) *Tripneustes gratilla*. The chart represents the gametogenic cycle with the six identified maturity stages. A single vector is represented in each chart, its direction indicates the maturity stage and r is the vector length, which is proportional to the homogeneity of the samples
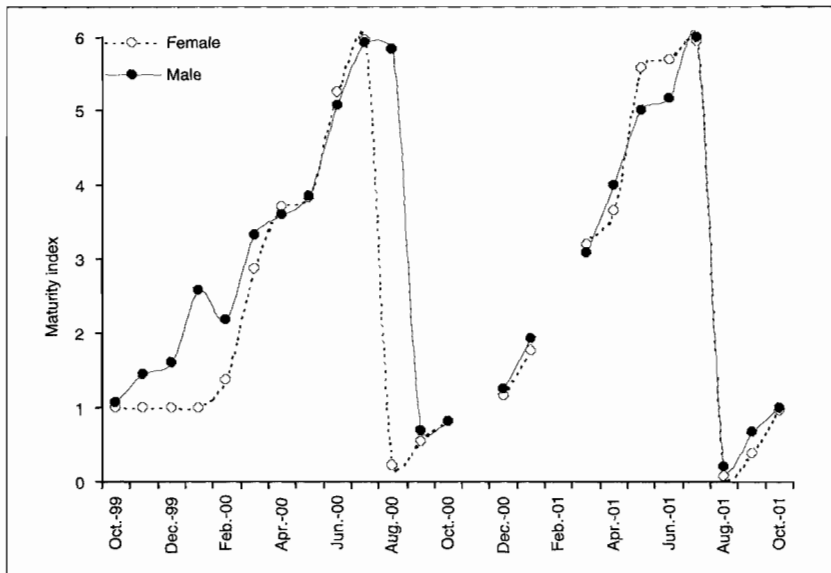

Fig. 5. Linear representation of the maturity index (MI) for female and male batches of *Tripneustes gratilla* followed during the two years study period. Each value is a mean computed from 30 individuals

Temperature, day length and salinity

A marked annual variation was observed in seawater temperature and day length (photoperiod) while seawater salinity varied to a much lesser extent (Fig. 6). As expected, the annual variation observed in the measured abiotic parameters defines the two seasons that prevail in the region of Toliara, austral summer (warm and humid: from October to March) and austral winter (cool and dry: from April to September). Table 2 shows the relationship between the physiological indices and the measured abiotic parameters. Significant correlations (Pearson product moment correlation, $P < 0.05$) were observed between the main reproductive parameters (GI and MI) and temperature and day length while GI was not correlated to changes in seawater salinity. GI was negatively correlated to temperature and day length, indicating that higher values of GI were noted when temperature and day length were low. This information could not be derived for MI (from such analysis) since the value of the linear-circular correlation coefficient, Dn, is an absolute one. The repletion index (RI) also showed similar association with temperature and salinity but not to day length where its correlation was not significant (Pearson product moment correlation, $R = -0.37, P > 0.05$).

Table 2. Correlation between the physiological indices of reproduction determined for *Tripneustes gratilla* and abiotic parameters. The significance of the association between repletion index (RI), gonad index (GI) and the abiotic parameters was checked using Pearson product moment correlation method. Linear-circular correlation was used when maturity index (MI) was considered. D_n and R, are correlation coefficients. The correlation was not significant when $P > 0.05$, significant when $P < 0.05$ and very significant when $P < 0.01$

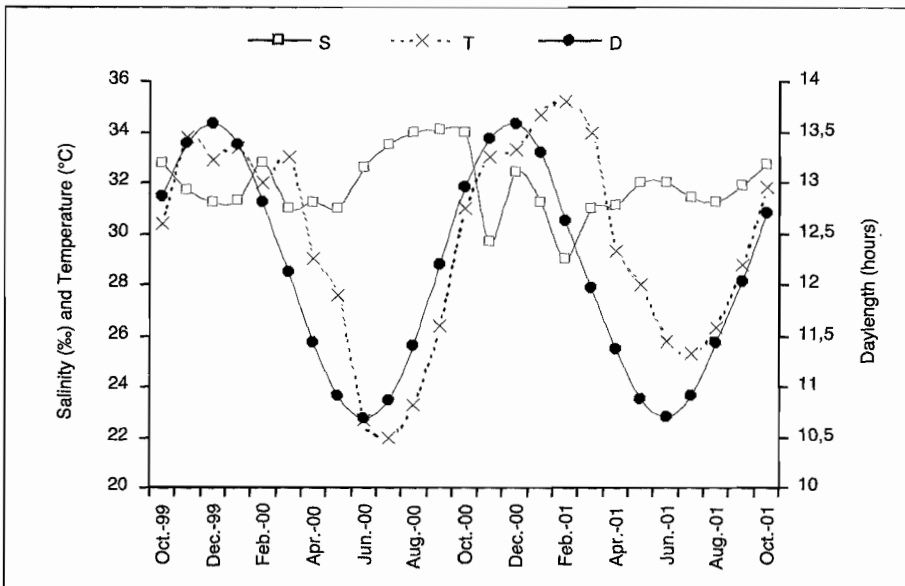| Physiological indices	Abiotic parameters		
	Temperature	Day length	Salinity
RI	$R = -0.71, P < 0.01$	$R = -0.37, P > 0.05$	$R = 0.71, P < 0.01$
GI	$R = -0.44, P < 0.05$	$R = -0.59, P < 0.01$	$R = -0.14, P > 0.05$
MI-male	$D_n = 0.87, P < 0.01$	$D_n = 0.92, P < 0.01$	$D_n = 0.37, P < 0.05$
MI-female	$D_n = 0.84, P < 0.01$	$D_n = 0.93, P < 0.01$	$D_n = 0.41, P < 0.05$

Fig. 6. Salinity (S), temperature (T) and day length (D) recorded during the two years study period

Relationship between the maturity stages, seasons and physiological indices

Combining the above results, Table 3 shows the relationship between the described maturity stages, seasons and the variability of the studied physiological indices. Here, the six maturity stages observed were grouped in three main reproductive phases: spent/recovery, growing/ premature and mature/partly spawned. Spawning clearly happened during winter season and may extend up to early summer. Spawning occurred intermittently as suggested by the predominance of partly spawned individuals during that period. Spent and recovery stages started after spawning and lasted for most of the summer season. Onset of gametogenesis occurred by the late summer and the first mature individuals were observed from early to mid-winter.

additional quantitative information that is the rate of gametogenesis, which is the speed of change from one maturity level to another. Although the timing of reproduction was more or less synchronised between males and females, the rate of gametogenesis within and between sexes was variable throughout the gametogenic cycle. Lastly, representation of MI values on a circular scale provides a better visual estimate of the gametogenic cycle since both years are superimposed. These advantages of MI analysis over GI analysis have also been pointed out and discussed by Spirlet *et al.* (1998) in a study on the reproductive cycle of a temperate echinoid species. Although the correlation between GI and MI was significant, in our study, the low values of correlation coefficient Dn (0.3-0.5) indicate poor relationship between the two indices. Furthermore, the poor correlation between GI and maturity state observed in other echinoid species (Laegdsgaard

Table 3. Relationship between the maturity stages of *Tripneustes gratilla,* seasons, and the variability of the three studied physiological indices. Note that winter extends from April to September and summer from October to March

Maturity stages	Seasons	Physiological indices		
		Repletion Index (RI)	Gonad Index (GI)	Maturity Index (MI)
Spent and recovering	Late winter and summer	Decreasing and low	Low	Low and increasing
Growing and premature	Late summer and early winter	Low	Increasing	Increasing
Maturing and partly spawned	Winter and early summer	High	High and decresing	High and decreasing

DISCUSSION AND CONCLUSION

Between the two physiological indices used to characterise the reproductive cycle, the maturity index is more reliable than gonad index. Monthly maturity indices were less variable than the corresponding gonad indices as suggested by the values of the vector length (r), which were close to one, with the exception of male samples in December 1999 and January 2000. This low variability of MI values allows detection of slight differences between male and female batches and even between yearly reproductive patterns, information that could not be derived from the analysis of GI. Besides, MI analysis provides

et al., 1991; King *et al.*, 1994), confirms the reliability of MI analysis in characterising reproductive cycle of echinoids. From the above-cited studies, it emerges that the annual variability in GI is due to factors other than maturity state such as, food availability and gonad composition in nutrient reserves and this largely explains its poor correlation with gametogenic condition.

Tripneustes gratilla is widely distributed in the Indo-West Pacific regions, particularly in the tropical zones. However, in the West Pacific, it can be found in latitudes as high as 35°N and as low as 30°S that constitute its distribution limits. In these regions, *T. gratilla* was observed to exhibit an annual reproductive cycle with variable intensities.

At Seto bay in Japan (35°N), where temperature ranges from 14°C to 28°C, breeding season was observed from July to September, that is, in summer (Kobayashi, 1969). At the Solitary Islands, New South Wales, Australia (30°S), temperature variation was observed to a much lesser extent, minimum of 20°C in winter and maximum of 26°C in summer. In this region, although mature individuals were observed year round but reproductive synchronicity was poor between males and females such that breeding was seasonal and occurred in autumn (O'Connor *et al.*, 1978). Throughout the tropical regions, a general tendency was noted, that annual periodicity in reproductive cycle, if present, was less defined towards the equator (see Chen & Chang, 1981). The present study together with one study done in the north of Madagascar (Maharavo, 1993) can be used to illustrate this decrease in amplitude of the reproductive cycle as echinoid populations are localised nearer to the equator. Maharavo indicated that populations of *T. gratilla* in different localities at Nosy-Be (13°20'S; 48°20'E, northwest coast of Madagascar) had mature individuals all the year round, with a peak spawning intensity observed at the end of winter (September). Our study shows that the population of *T. gratilla* at Beloza (latitude 23°S) has a more defined reproductive cycle, with breeding season extending from mid to late winter, than conspecific individuals in Nosy-Be (latitude 13°S).

This difference in reproductive pattern noted at the two latitudes is associated with environmental factors. Factors that govern the intensity of reproductive cycle seem to be both abiotic and biotic. In our study, seawater temperature and day length (photoperiod) were highly correlated with maturity index, while salinity shows poorer correlation. The abiotic cues influencing gametogenic cycle of *T. gratilla* are principally temperature and photoperiod, which is clearly demonstrated by the Madagascar echinoid populations. Regions of Nosy-Be have temperature differences between summer and winter from 4°C to 6°C while the difference in photoperiod is 1.4 hours (Maharavo, 1993). These differences are more pronounced in Toliara lagoon: temperature differs by 10°C and photoperiod by 2.9 hours. Therefore, echinoid populations from regions characterised by greater seasonal temperature and photoperiodic variations show more defined reproductive cycles. Such a relationship between exogenous factors and reproductive cycle has been noted in temperate echinoids e.g. *Paracentrotus lividus,* that shows a definite single annual spawning period in Ireland (Byrne, 1990) and Brittany (Spirlet *et al.*, 1998). However, populations located further south, in the Mediterranean sea, show two spawning periods each year (Fenaux, 1968; Pedrotti, 1993; Fernandez, 1996). While the exact influence of exogenous factors on gonadal growth and gametogenesis are difficult to determine it seems from our study, that gametogenesis (growing stage) starts when temperature begin to drop. Gonads were still in recovery phase as photoperiod shortened in December. Therefore, gonadal growth in *T. gratilla* can be associated with decreasing water temperature as it was observed in the Irish and French populations of P. lividus (Byrne, 1990; Spirlet *et al.*, 1998). As suggested by Lane & Lawrence (1979), factors influencing gonadal growth differ from those triggering spawning. In the studied population of *T. gratilla,* it appears that rising temperature and photoperiod serves as cues for inducing spawning. Rise in temperature has been described to favour gamete release in P. lividus (Fenaux, 1968; Byrne, 1990, Spirlet *et al.* 1998).

Among the biotic factors influencing reproductive cycle, food plays a predominant role. Many invertebrate species time their spawning period to phytoplankton blooms so that the larvae benefit from a nutrient rich environment (Starr *et al.,* 1990) including some echinoids *Strongylocentrotus droebachiensis* (Himmelman, 1978) and *P. lividus* (Lopez *et al.*, 1998) where spawning is synchronised to late winter and spring phytoplancton blooms respectively. In a review on the annual cycle of phytoplankton and primary production in tropical seas, Sournia (1969) pointed out that in regions of Nosy-Be two periods were recognised: minimum values from September to January (first half of austral summer) and maximum but variable values for the second half of austral summer to austral winter. In addition, a qualitative difference was observed during these two periods characterised by a predominance of

diatoms in austral winter and a mixture of diatoms, dinoflagellates and cyanobacteria in the austral summer. Optimal conditions for phytoplankton proliferation appear during these rainy, low temperature seasons which allow mixing of surface and deep waters. While detailed plankton studies are lacking, we expect a similar situation, as the climatic conditions, while of different magnitude are almost the same. Therefore winter spawning observed in populations of *T. gratilla* at Beloza is probably synchronised with winter phytoplankton blooms and the predominance of diatoms, which had been recognised as food requirements for the larvae (Lawrence *et al.*, 1977).

At the adult level, Pearse (1969) suggested that gametogenesis in echinoids could not be initiated until a critical level of nutrients was available within the storage tissues. In this study, repletion index (RI) was highly correlated to maturity index (MI). Similarly in the *T. gratilla* population at Beloza it was demonstrated that RI was related to gonadal growth and to the nutrient reserves in the gut tissues (Vaïtilingon *et al.*, 2003). Indeed, nutrient reserves in gut tissues were higher during late summer (February) and this period coincides with onset of gametogenesis. As the increase in oocyte size starts in March there is evidence that gametogenesis is initiated only when sufficient reserve nutrients are accumulated in the gut tissues and in the nutritive phagocytes of the gonads. All these indicate that, although abiotic factors do influence gametogenic cycle, food (nutrient reserve) plays a predominant role in reproductive cycle of *T. gratilla*.

Knowing that the required stage of the reproductive cycle for gonad consumption is the pre-gametogenesis stage, we recommend harvest of *T. gratilla* from November to February where gonads are in recovery to growing stages.

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