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Feeding preferences, seasonal gut repletion indices, and diel feeding patterns of the sea urchin *Tripneustes gratilla* (Echinodermata: Echinoidea) on a coastal habitat off Toliara (Madagascar)

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Abstract The feeding biology of the echinoid *Tripneustes gratilla* from a seagrass bed off Toliara, on the south-west coast of Madagascar, was investigated during the summer and winter seasons. Repletion indices of the gut of *T. gratilla* varied seasonally, in such a way that higher amounts of food occurred in the gut in winter than in other seasons. Conversely, a more detailed analysis of one summer and one winter sample of the gut tissue dry weights revealed higher weights in summer (February) than in winter (August). This was interpreted as a varying capability of nutrient storage and closely related to the feeding activity of the echinoid. This inverse relationship between feeding activity and nutrient storage was suggested to be dependent on gonadal growth. Indeed, higher food consumption was observed during the post-spawning period (August), that is, when most of the energy accumulated was used up for gonadal growth. Low feeding activity occurred when a sufficient amount of energy was accumulated in gut tissues (February), the time corresponding to the initiation of gonadal growth. Investigations on the feeding habits, using Ivlev's electivity index, revealed preferential feeding of *T. gratilla* on *Syringodium isoetifolium*, the dominant seagrass species found in the gut. This was partly related to the higher availability of that particular seagrass species in the field. However, its soft and terete leaf blade's morphology also makes it easier to manipulate and ingest. Studies on the feeding behaviour indicated the presence of a diel feeding cycle in *T. gratilla* individuals in both summer and winter seasons. Yet, its pattern varied between the two studied months (one summer and one winter) as the cycle was less contrasted during period of active feeding, i.e. in winter compared to summer. This study provides novel information on how intrinsic factors, such as physiological state, can govern the feeding activity of *T. gratilla* living in a seagrass habitat.

Introduction

Feeding biology of echinoids have long been a subject of interest. Regular echinoids are known to be generalist herbivores feeding on marine plants and/or algae (Lawrence 1975). They are also known to exhibit peculiar feeding habits and behaviours. Preferential feeding has been demonstrated to occur in echinoids and was often related to food availability, nutritional values of food types and/or the presence of chemical substances in food which attract or repel echinoids (Ogden 1976; Vadas 1977; Ayling 1978; Harrold and Reed 1985; Beddingfield and McClintock 1998). Besides, seasonal variations in feeding activity were also reported in some echinoid species (Fuji 1967; De Ridder and Lawrence 1982; Spirlet et al. 1998) and were explained by changes in physiological states of the individuals, for instance, differential gonadal growth. Such variation in feeding activity usually implies the capability to store nutrient reserves. Various compartments in sea urchins were considered for such storage, the gut and the gonads having been suggested to be the main sites in several species (Lawrence and Lane 1982; Walker 1982). Indeed, like the gonads, the gut also shows annual variation in weight or size, indicating periodical accumulation of nutrient reserves (Lawrence et al. 1965, 1966; Bishop and Watts 1992). In general, the gut is considered to be the first site of nutrient accumulation. These nutrients are then translocated to other body parts such as the gonads (Ferguson 1982; Bishop and Watts 1992; Beddingfield and McClintock 1998). Echinoid species differ in

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foraging behaviours, some feed during both day and night, while others are nocturnal. Occurrence of a diel feeding rhythm was often attributed to responses towards predators (Ogden et al. 1973; Ogden and Lobel 1978; Nelson and Vance 1979; Cowen 1983; Tertschnig 1989). Furthermore, its seems that such behaviour is species specific (McClanahan 1999) and may even vary intraspecifically.

Tripneustes gratilla (Linnaeus, 1758) is a common shallow-water echinoid, ubiquitous in the tropical Indo-West Pacific region, where it constitutes one of the main plant-feeders. Being a primary herbivore, *T. gratilla* plays an important role in maintaining biodiversity in its different habitats, either by direct or indirect recycling of nutrients (Lawrence and Agatsuma 2001). Studies on its feeding habits and behaviours were performed in the Philippines (Klumpp et al. 1993), Papua New Guinea (Nojima and Mukai 1985), northern Madagascar (Maharavo et al. 1994) and Réunion Island (Lison de Loma et al. 1999; Lison de Loma 2000). These studies revealed dietary differences according to the habitats where the echinoid individuals occurred. Yet, investigations relating feeding habits to field observations on abundance of food items are scarce. Likewise, no studies dealing with diel feeding rhythms have taken into account the possible seasonal variation in feeding activities.

The present work deals with the feeding biology of *T. gratilla* on the reef complex of Toliara, on the western coast of Madagascar. Our aims were to study the feeding habits of the species in relation to food availability in a seagrass ecosystem, to investigate the occurrence of a diel feeding cycle and of its variation from one season to another, and to determine whether the stomach and/or the intestine may function as storage organs.

Materials and methods

Sampling site

This study was performed in the Toliara reef complex, which consists of a barrier reef and numerous fringing reefs. Individuals of *T. gratilla* were sampled among the seagrass beds of the fringing reef at Beloza (23°29′.32S; 43°45′.05E; 17 km south of Toliara) (Fig. 1). *T. gratilla* was found in the area at a density of 2–3 individuals m^{-2} (Vaïtilingon, personal observations).

Feeding habits

The feeding habit of *T. gratilla* was studied in February 2002 by comparing the seagrass species in the gut contents to the field abundance of the different species. To do so, ten echinoids of about the same size [mean test diameter (MTD) of 7.8 ± 0.4 cm] were localised randomly and, each time, a quadrat (0.5 m×0.5 m) was placed on the bottom so that the echinoid rested just in the middle of it. Echinoids were then placed in a plastic bag together with all species of seagrasses (fronds and shoots) within the quadrat. Both echinoids and seagrasses were fixed in formaldehyde 5% before being examined. A control experiment was performed which consisted in sampling seagrasses within same-sized quadrats, but in an area where no echinoids were localised. The number of shoots as well as the percentage of frond dry weight (dried to constant weight at 70°C) of different species of seagrasses were determined for each quadrat. Precautions were taken to rinse each frond thoroughly with distilled water, in order to remove the residual formaldehyde and sand particles before being dried. The frond biomass per unit area (expressed as g m^{-2}) was also calculated. After a period of 1 week in the fixative, each echinoid was dissected. The oral region was removed and drained, and the digestive tract was washed thoroughly with distilled water. Gut species composition was analysed from a representative sample of the gut content, namely the content of the first intestinal festoon. This portion of the gut was cut, its content removed, and identification of seagrass species was performed. Each seagrass species identified was separated, and its dry weight was measured (dry at 70°C to constant weight).

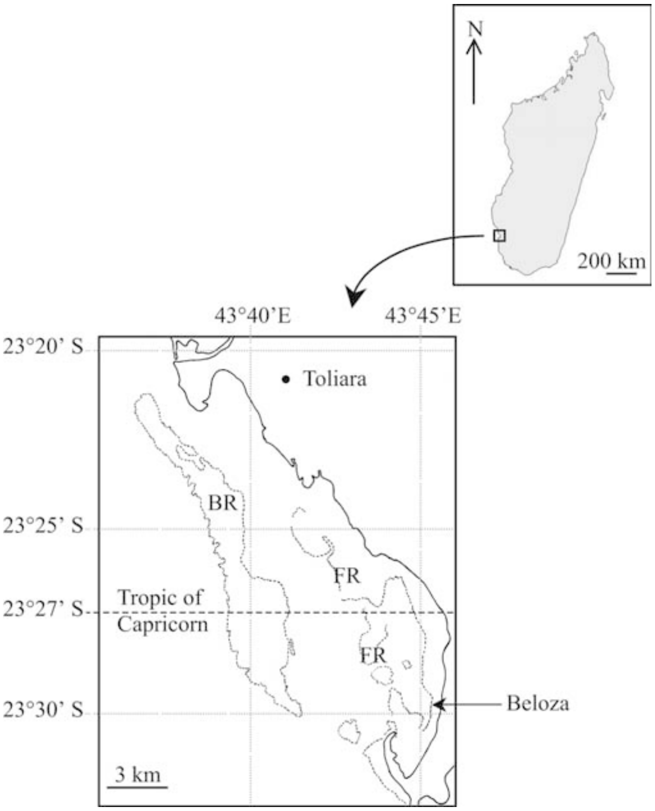

Fig. 1 Location of the seagrass beds at Beloza (*BR* barrier reef; *FR* fringing reef)

To compare abundance of food species, the Ivlev's electivity index (E_i) (Vance 1979) was calculated from the mean percent biomass (based on frond dry weight) of seagrass species found in the first intestinal festoon and in the field . E_i was calculated as follows:

$$E_i = (p_i - P_i)/(p_i + P_i) \quad (1)$$

where p_i is the percentage of seagrass species i in the diet and P_i is the percentage of seagrass species i in the environment. E_i values tending towards +1 indicate seagrass species that are more abundant in the diet, while values tending towards −1 indicate seagrass species that are more abundant in the field. E_i equal to 0 informs us that a species is equally present in the field and in the diet.

Investigation on variation in the gut contents

Sampling procedure

A monthly survey of the amount of food in the echinoid gut was followed from October 1999 to October 2001. Each month, 30 echinoids were sampled at low tide and brought to the laboratory for further analysis. During low tides in November 2000 and

February 2001, heavy rains were noted which consequently killed an important number of echinoids. In order not to introduce errors into our results, samplings on these two occasions were not performed. Monthly records of temperature and salinity of seawater at sampling sites were done using a combined salinometer–thermometer apparatus (WTW LF 330, Bioblock Scientific). The sampled echinoids were then measured (ambital diameters) and the fresh weight determined after leaving them on absorbent paper for 3 min to remove traces of residual water on the integument. They were then dissected and the fresh weights of the digestive tract and its contents were determined.

During two seasons, that is summer (warm and humid) and winter (cool and dry), a more detail analysis of the gut content was carried out on one summer (February) and one winter (August) sample using the following protocol. On a 24 h cycle, ten samples of ten adult echinoids were collected every 2–2.5 h in both February 2000 (mean seawater temperature: 29.3 ± 0.8°C) and August 2000 (mean seawater temperature: 23.2 ± 1.4°C). Each individual was immediately measured (height and diameters), weighed (fresh weight), fixed [injection of 5 ml of pure formaldehyde (36%)], and placed in separate plastic bags filled with 100 ml of 5% formaldehyde in seawater for further dissections. Preliminary fixation with pure formaldehyde permitted fine dissection of delicate organs such as the echinoid digestive tract.

Each echinoid was dissected, and its periviseral fluid drained. The digestive tract was washed thoroughly with distilled water to remove all traces of fixative, dissected and separated into 11 different portions: the oesophagus, five stomachal festoons (S1–S5), four intestinal festoons (I1–I4) and the rectum (Anderson 1966; De Ridder and Jangoux 1982). The gut content of each portion was separated from the tissue, placed in separate crucibles and dried at 70°C to constant weight. The tissues were kept for further investigations (see below).

Gut contents

During the 1999–2001 monthly survey, the amount of food in the gut content was evaluated using the repletion index (RI) calculated as follows:

$$\mathrm{RI} = (\mathrm{FW}/\mathrm{TW}) \times 100 \qquad (2)$$

where FW is the fresh weight of the digestive tract and its contents in grams and TW is the fresh weight of the whole animal in grams. This method of calculating RI, which is dependent on the weight of the whole animal was chosen since TW varied significantly (one-way ANOVA, $df=22$, $F=6.300$, $P<0.05$) from one month to another.

In addition, the detailed analyses of the amount of food along the whole gut or along the different festoons were quantified and compared directly in terms of dry weight of gut content. Indeed, as size interferes with feeding rate (De Ridder and Lawrence 1982) and as body weight is influenced by other factors such as volume of coelomic fluid, thickness of the test and gonadal growth, which vary independently of the amount of food in the gut, a method to express the filling of the gut independently of body size and weight was needed. Such a method allowed a more precise estimation of gut content weight, but was applicable only if similarly sized individuals were compared. In fact, in February 2000 the sampled sea urchins had a mean test diameter (MTD) of 7.9 ± 0.5 cm and a mean fresh body weight (MBW) of 238.2 ± 32.5 g. In August 2000 the MTD was 8.1 ± 0.5 cm and MBW was 237.8 ± 52.1 g. Statistical analysis (one-way ANOVA) revealed no significant differences in the MTD ($df=1$, $F=1.889$, $P>0.05$) or in the MBW ($df=1$, $F=0.204$, $P>0.05$) of echinoids sampled between the two seasons.

Diel change in gut content

The occurrence of a diel change in the gut content was investigated by evaluating the amount of food in the different portions of the gut at each sampling time for a 24 h cycle in February and August. Two main portions of the gut were investigated, namely the stomach and intestine, and their filling was expressed by the gut content index (GCI), which was calculated independently of size and weight of the echinoid such that:

$$\mathrm{GCI} = \left(\left[\sum \mathrm{DW}\right]_i / \mathrm{TDW}\right) \times 100 \qquad (3)$$

where DW is the dry weight of the gut content in grams; *i* represents the different portions of the digestive tract, i.e. S1, S2, S3, S4 and S5 (the five stomachal festoons) or I1, I2, I3 and I4 (the four intestinal festoons); and TDW is the dry weight of the total digestive tract content in grams.

Seasonal variation in gut tissue weight

The gut tissues left after dissection (see section "Sampling procedure") were separated, placed in crucibles and dried at 70°C to constant weight. The dry weight of the whole gut tissue in February was compared to that in August in order to detect any possible seasonal variability. The gut tissue weights of the different stomachal and intestinal festoons were also compared to each other. Note that even though the stomach has been separated into five festoons and the intestine into four only, both organs are more or less of the same length and equivalent to the circumference of the sea urchin test. This is important when comparing the dry weight of stomachal and intestinal tissues together.

Statistical analysis

Both parametric and non-parametric tests were used to compare different groups. Data were tested for normality using the Kolmogorov–Smirnov test, and Levene's test was used to check for homogeneity of variances. Analysis of the diel change in gut content was made after transformation of the GCI values by the Freeman and Tukey method (Zar 1996). The transformed values were then compared using one-way ANOVA. Comparison of dry weights of gut contents between festoons were done using non-parametric Mann–Whitney *U*-test, since values deviated from normality. Values of dry weight of digestive tract tissues were first checked for normality using Levene's test (homogeneity of variances) before being compared using one-way ANOVA followed by post hoc multiple comparison (Tukey test). For both parametric and non-parametric tests, the significance level used was $P<0.05$.

Results

Feeding habits

Six species of seagrasses coexist in the seagrass beds of Beloza, namely *Cymodocea serrulata*, *Syringodium isoetifolium*, *Halodule uninervis*, *Halodule wrightii*, *Thalassia hemprichii* and *Thalassiodendron ciliatum*. Only the first five species were included in this study. *T. ciliatum* was rarely found at the sampling sites and its distribution is patchy. *C. serrulata* was the dominant seagrass species found in the field in terms of mean percent frond biomass (58.8%; see Table 1). However, in terms of availability, *S. isoetifolium* accounted for the highest number of shoots per square metre (up to 2040). These two species were noted in all ten sampled quadrats, together with *H. uninervis*. The seagrass composition within the control quadrat did not differ from those above. Indeed, the two dominant species were *C. serrulata* (percentage of frond biomass = 54.4%; number of shoots = 210) and

Table 1 Seagrass composition in the field and in the gut of *Tripneustes gratilla*. Data in the field are expressed as field biomass (g m^{-2}), number of shoots per square metre and mean percent biomass. In the gut, data are expressed as mean percent biomass only (*Nobs* number of quadrats in which the different seagrass species were observed). Total number of quadrats observed (*n*) was 10

Taxon	Nobs	Field biomass (dry weight in g m^{-2})			No. of shoots m^{-2}			Mean percent abundance (±SD)	
		Min.	Median	Max.	Min.	Median	Max.	Field	Gut
Syringodium isoetifolium	10	10.4	17.8	39.2	372	694	2040	24.1±7.1	72.7±16.5
Cymodocea serrulata	10	28.5	58.7	61.2	476	566	784	58.8±6.7	11.4±8.0
Halodule uninervis	10	3.3	5	20.9	136	314	796	9.8±6.5	12.7±11.2
Halodule wrightii	1	0	–	10.8	0	–	280	1.7±4.1	1.8±4.7
Thalassia hemprichii	4	0	0.2	14.2	0	2	112	5.6±8.8	1.3±2.9

S. isoetifolium (percentage of frond biomass = 22.4%; number of shoots = 470). No species of macroalgae were found in our sampled quadrats.

The gut contents of individuals of *Tripneustes gratilla* at Beloza were exclusively composed of seagrass species. Food fragments were not organised into pellets, but were found loosely agglomerated together by a mucus substance. The main seagrass species found in the gut of the echinoid was *S. isoetifolium* (see Table 1). It accounted for 24.1% of field abundance (mean percent frond biomass), but 72.7% of the dry weight of the gut content. Electivity indices calculated from frond biomass revealed similar results: *S. isoetifolium* showed the most positive E_i value (+0.5) and was the most abundant species in the diet (Fig. 2); *H. uninervis* was the second most abundant seagrass species found in the diet; *C. serrulata* and *T. hemprichii* were clearly not favoured. *H. wrightii* was found in one quadrat only, so the obtained value was not significant. As expected, *T. ciliatum* was never observed in the diet.

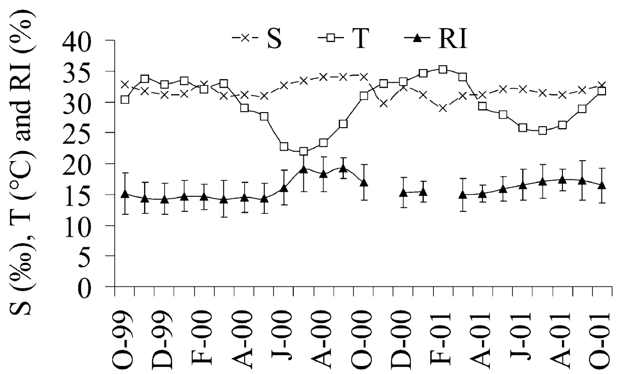

Fig. 3 *Tripneustes gratilla*. Salinity (*S*), temperature (*T*) and the corresponding repletion index (*RI*) during the two years of observation. Values of RI represent means ± SD of 30 echinoids

Gut contents

The repletion index showed an annual variation, with a more defined winter peak in 2000 (Fig. 3). RI was around 14% throughout the summer, that is from November 1999 to May 2000, and then increased to 19% by the end of winter (July–September 2000). The differences between summer and winter values were statistically significant (one-way ANOVA; $P < 0.05$). The same, but less marked pattern, was observed in 2001, i.e. an increase from 15% to 17%.

The mean dry weight (MDW) of the gut contents differed according to seasons (Fig. 4). The MDW of the gut contents were significantly higher in August than in February (Mann–Whitney *U*-test; $P < 0.05$); a difference of 40% was observed. A detailed analysis of the different festoons of the digestive tract revealed an accumulation of food in the intestine (Fig. 5). About 80% of the food

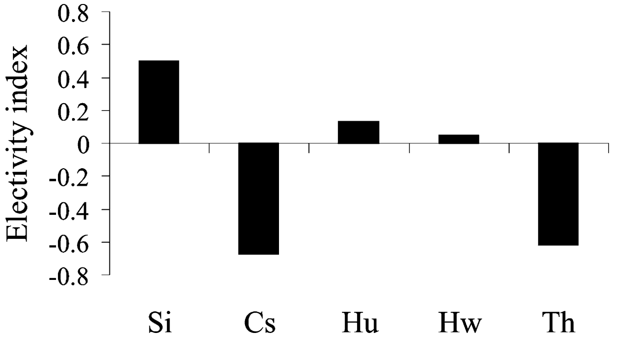

Fig. 2 Electivity indices calculated from the mean percent biomass of the different seagrass species (Si, *Syringodium isoetifolium*; Cs, *Cymodocea serrulata*; Hu, *Halodule uninervis*; Hw, *Halodule wrightii*; Th, *Thalassia hemprichii*)

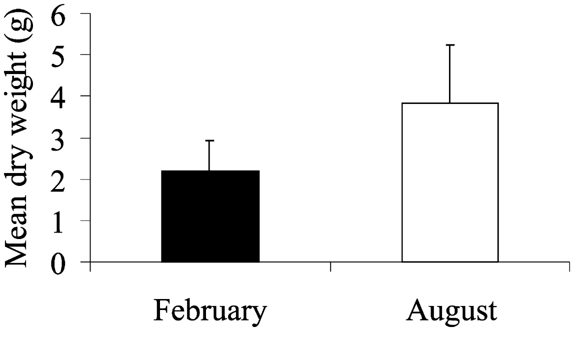

Fig. 4 *Tripneustes gratilla*. Mean dry weight of the gut contents of pooled individuals sampled within a 24 h cycle. *Each bar* represents the mean dry weight (+SD) of the digestive content of 100 individuals. The two histograms are significantly different from each other (Mann–Whitney *U*-test; $P < 0.05$)

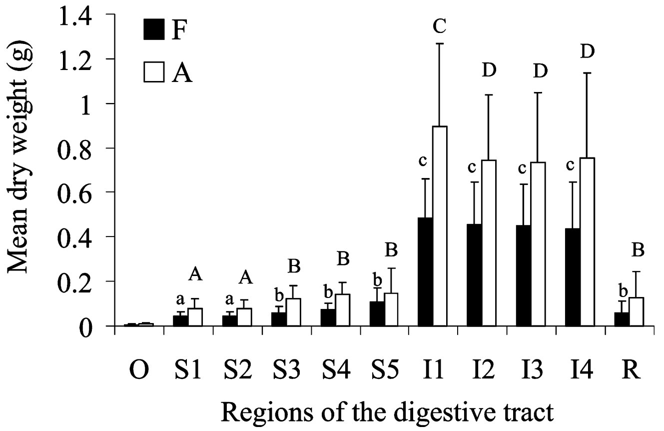

Fig. 5 *Tripneustes gratilla*. Dry weight of the gut contents of the different festoons of the digestive tract. Each bar represents the mean (+SD) of pooled individuals sampled within a 24 h cycle ($n=100$) in February (*F*) and August (*A*) (*O* oesophagus; *S1–S5* five stomachal festoons; *I1–I4* four intestinal festoons; *R* rectum). Histograms with different letters above bars are significantly different from each other (Mann–Whitney *U*-test; $P<0.05$)

found in the gut was distributed along the four intestinal festoons, and this was true in both February and August. In August, food distribution was not uniform along the intestinal festoons. MDW of food in the first intestinal festoon was significantly higher (Mann–Whitney *U*-test; $P<0.05$) than in the other parts of the intestine. That difference was not observed in February. Along the stomachal festoons, an increasing amount of food was found from S1 to S5, so that dry weight of gut content was evenly distributed in S1 and S2, but was significantly less than in S3, S4 and S5. This pattern of food distribution was observed in both seasons. No empty intestinal festoons were noted in any of the dissected echinoids. However, 7–8% of those dissected had at least one empty stomachal festoon. In February 50% of the dissected echinoids had no food particles in the oesophagus, while in August this value was 33% only.

Diel change in gut content

Distinct diel periodicity was observed in the gut content of *T. gratilla* during the two studied months. Analysis of the GCI of the stomach (Fig. 6) and intestine (Fig. 7) for February and August revealed significant variations (Table 2). In August, the GCI of the stomach showed two maxima, a small one around dawn (sunrise at 0600 hours) and a large one towards dusk (sunset at 1730 hours). In February, only one maximum was observed just after dusk (sunset at 1845 hours). The GCI of the intestine followed the opposite periodic cycle, with maximal values around midday and minimal values towards dusk in February and August. The extreme values of the gut content indices of both stomach and intestine were more contrasted in February than in August, and the minimum and maximum values were both obtained in February.

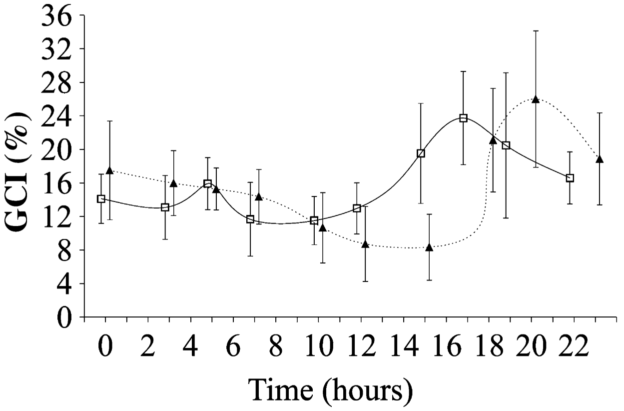

Fig. 6 *Tripneustes gratilla*. Stomach: diel variations in the gut content index (*GCI*) in February (*triangle*) and August (*square*); means ± SD, $n=10$ individuals

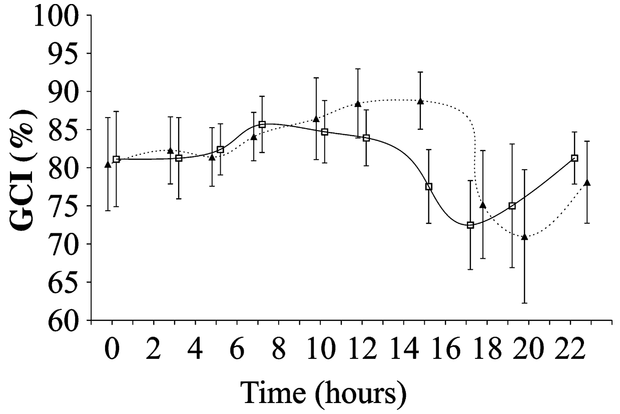

Fig. 7 *Tripneustes gratilla*. Intestine: diel variations in the gut content index (*GCI*) in February (*triangle*) and August (*square*); means ± SD, $n=10$ individuals

Table 2 *Tripneustes gratilla*. Statistical analysis of the diel variations in the dry weight of the stomachal (Σ S1 to S5) and intestinal (Σ I1 to I4) contents using one-way ANOVA for the two studied seasons, August and February. Values for stomach and intestine, see Figs. 6 and 7, respectively

Effect	*df*	MS	*F*	*P*
Stomach				
Aug	9	27.673	8	<0.001
Feb	9	59.496	12.528	<0.001
Intestine				
Aug	9	26.787	7.240	<0.001
Feb	9	50.651	10.768	<0.001

Analysis of gut tissue weight

Greater values of the mean dry weight of total gut tissues were observed in February than in August. A difference of 25% was observed, which was significant (one-way ANOVA; $P<0.05$) (Fig. 8). This seasonal difference was also true when considering the pooled festoons of the stomach and of the intestine (Fig. 8).

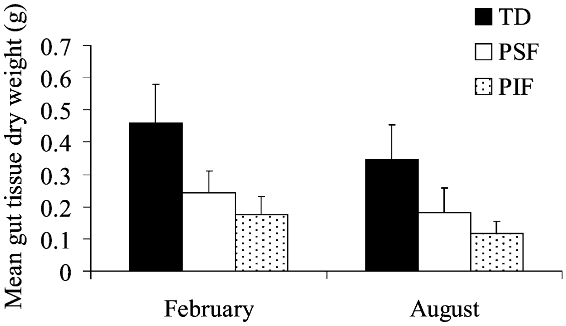

Fig. 8 *Tripneustes gratilla*. Dry weight of gut tissues of the total digestive tract (*TD*), pooled stomachal festoons (*PSF*) and pooled intestinal festoons (*PIF*) during the two studied months, February and August. *Each bar* represents the mean dry weight (+SD) of the gut tissue of pooled individuals sampled within a 24 h cycle ($n = 100$). All histograms are significantly different from each other (one-way ANOVA; $P < 0.05$)

Furthermore, the weight of the stomachal tissue was always significantly higher than that of the intestinal one (one-way ANOVA; $P < 0.05$).

Discussion

Individuals of *Tripneustes gratilla* in the seagrass bed of Beloza were demonstrated to have differences in gut wall dry weight according to seasons. Furthermore, they appeared to be food selective, and their gut contents showed diel and seasonal variations.

Lawrence et al. (1965) provided clear evidence of an annual cycle of the gut tissue weight in the echinoid *Strongylocentrotus purpuratus*. Further studies revealed that the gut acts as a storage organ and that this storing capacity varies seasonally (Lawrence et al. 1966). Such observations were also made in other echinoid species such as *Arbacia lixula* (Fenaux et al. 1977) and *Lytechinus variegatus* (Klinger et al. 1988; Bishop and Watts 1992). Moreover, Bishop and Watts (1992) demonstrated that nutrient storage occurred in both stomachal and intestinal epithelia, though the stomach epithelium was always thicker than the intestinal one. In *T. gratilla* there was also a weight increase in gut tissues according to season. Although one winter and one summer sample each of gut tissue weights were studied in detail, our observations predict an accumulation of nutrients from August (winter) to February (summer). This prediction is supported by the similar nutrient storage patterns observed in the above-mentioned echinoid species. The varying degree of gut weight in February and August could be related to gonadal growth. Like other echinoids, *T. gratilla* is known to show an annual reproductive periodicity. Such observations have been made for Taiwan's *T. gratilla* population (Chen and Chang 1981) and for northern Madagascar's population (Maharavo et al. 1994) as well as for Beloza's (Vaïtilingon, personal observations). In the latter, growth of gonads started in February and culminated in July, which corresponds with the spawning period. It seems thus that when gonadal growth starts, i.e. in February, a sufficient amount of energy has been accumulated in the individual's gut wall. Similar findings regarding the close relationship between nutrient storage in the gut and growth of gonads have been reported for *A. lixula* (Fenaux et al. 1977) and *L. variegatus* (Bishop and Watts 1992).

The seagrass composition at Beloza differs from that which has been reported in other tropical seagrass ecosystems where *Thalassia hemprichii* is the dominant species, e.g. in the Philippines and in Papua New Guinea (Klumpp et al. 1993). At Beloza, the two most dominant species are *Cymodocea serrulata* (in terms of biomass) and *Syringodium isoetifolium* (in terms of shoot density), and *T. hemprichii* is rarely found. This is consistent with the fact that *C. serrulata* and *S. isoetifolium* are species commonly found in regions with low disturbances, while *T. hemprichii* is usually located in disrupted ecosystems (Clarke and Kirkmann 1989). Indeed, the south-west coast of Madagascar is rarely subjected to perturbation such as cyclones, and the seagrass bed at Beloza may consequently be considered rather stable. Among the three species occurring commonly, *S. isoetifolium* is the most frequently found in the gut of *T. gratilla* and accounted for 72% of the biomass of the gut content. Is this a consequence of preferential feeding on *S. isoetifolium* or is this simply due to higher accessibility of that particular seagrass species? Although rather variable, the shoot density of *S. isoetifolium* per quadrat is higher (up to twofold higher) than that of *C. serrulata*, thus suggesting that the former is more available to the echinoids. However, although the density of *S. isoetifolium* is twice that of *C. serrulata*, it is seven times more favoured than the latter, which indicates that it is not only a question of availability. Thus, a greater palatability of *S. isoetifolium* cannot be excluded. (Note: marine plants are known to produce substances which either repel or attract echinoids; Ogden 1976; Vadas 1977; Ayling 1978; Harrold and Reed 1985; Beddingfield and McClintock 1998.) However, one should recall that *S. isoetifolium* differs from the other co-occurring seagrasses by the morphology of its leaves. The leaf blades of *S. isoetifolium* are long, terete and soft, while in *C. serrulata* and the other species from Beloza, they are flat, strap-shaped and tough (Kuo and McComb 1989). As has already been proposed by Lowe (1974), it is probable that echinoids have a greater ability to feed on terete plants than on large or flattened ones. Indeed, manipulation of food items by the different appendages of the echinoid is an important step in the feeding process, and plant species that are easily caught and transferred to the mouth would be favoured. Consequently, the mechanical aspect of plant collecting by echinoids could be of major importance here.

T. gratilla at Beloza showed seasonal change in gut content, which followed the opposite pattern to that of gut tissue weight. Indeed, gut content of *T. gratilla* was higher in August (winter) than in February (summer).

Seasonal variations in food consumption have also been recorded in other echinoids, such as *Strongylocentrotus purpuratus* (Ebert 1968; Gonor 1973), *Allocentrotus fragilis* (Sumich and McCaulery 1973) and *Paracentrotus lividus* (Spirlet et al. 1998). Such variations in gut content were considered to result from differential feeding activity and were related to individual gonadal cycles (Fuji 1967; Gonor 1973; Spirlet et al. 1998). Observations made with *T. gratilla* support this idea, indeed, as both the gut tissue weight and the change in gut content–that is, in feeding activity–highly correlates, though conversely, with the reproductive cycle of the species. Higher food consumption was observed in August, which corresponds to the period when most of the energy accumulated has been used up for gonadal growth (August is the post-spawning period). During that period, *T. gratilla* can be considered to have a high energy demand, thus resulting in higher feeding activity and allowing nutrients to be stored in the gut. This accumulation increases towards the warmer season, i.e. in February. Consequently, the decrease in feeding activity could be related to the accumulation of a sufficient amount of energy in echinoid tissues to start the growth of gonads.

A clear diel change in gut content was observed during the two studied seasons in *T. gratilla* individuals at Beloza. This diel change resulted from a feeding cycle consisting of accumulation of food by feeding and rejection by egestion, throughout a 24 h cycle. Such a feeding cycle has previously been observed in *Diadema setosum* (Lawrence and Hughes-Games 1972) and *Paracentrotus lividus* (Cam 1997) as well as *T. gratilla* (Lison de Loma et al. 1999; present study). In these species, food was found to accumulate almost exclusively in the intestine, an organ whose high retention capacity was attributed to its spacious organisation and absorption properties (De Ridder and Jangoux 1982). Although such a diel feeding cycle was observed in *T. gratilla's* population at La Réunion Island (Lison de Loma et al. 1999), no indication was given about its variability throughout the year. Yet our results show that diel feeding cycle in *T. gratilla* varied during the two studied months. During the period of active feeding (August), *T. gratilla* appeared to feed twice a day (early in the morning and at dusk), while in February feeding happened only at dusk. Moreover, the gut content index for both the stomach and intestine revealed more extreme values in February. All this suggests that the diel feeding cycle is more obvious when echinoids are less actively feeding and vice versa. Studies aiming at the detection of diel feeding cycles in echinoid populations clearly should take such seasonal variability into consideration. This could be one of the reasons to explain why no diel feeding cycle was found in the Philippine population of *T. gratilla* (Klumpp et al. 1993). Indeed, the latter authors made only a single observation during the post-hot season, when the echinoids were presumably actively feeding; thus the diel feeding cycle may have been masked. The same interpretation could be drawn from the single observation made by Nojima and Mukai (1985), who did not notice a diel feeding cycle in a population of *T. gratilla* from Papua New Guinea.

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