

NOTE

A multiple-choice feeding-preference experiment utilising seagrasses with a natural population of herbivorous fishes

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ABSTRACT: We examined fish food preferences among the 9 most frequent seagrass species from Kenyan lagoonal areas. We tested the hypothesis that grazing fish prefer 'pioneering' seagrass species rather than 'climax' species. The experiment was conducted *in situ* in the Watamu Marine National Park (Kenya), with a natural population of herbivorous fish. The only fish observed eating seagrass leaves was *Calotomus carolinus* (family Scaridae), the second most abundant fish of the family in the park. The 3 variables tested (biomass eaten, % of leaf eaten and leaf marks) revealed that there was a clear preference for 'pioneering', short-lived species such as *Cymodocea rotundata* and *Syringodium isoetifolium* over 'climax', long-lived species such as *Enhalus acoroides* and *Thalassodendron ciliatum* (Friedman's test, $p < 0.005$). The good correlation found between total carbon content and leaf marks indicated that fishes also prefer low 'carbon-fiber' plant species instead of high carbon content species. This study ascertains the feeding behaviour of fish species and suggests new ways of understanding the complicated relationships that bind herbivores and seagrasses, highlighting the role of grazing fish in influencing seagrass abundance and distribution.

KEY WORDS: Food preference · Herbivorous fish · Seagrasses · *Halodule wrightii* · *Halodule uninervis* · *Halophila stipulacea* · *Syringodium isoetifolium* · *Cymodocea rotundata* · *Enhalus acoroides* · *Thalassia hemprichii* · *Thalassodendron ciliatum* · *Cymodocea serrulata* · *Calotomus carolinus*

Seagrass beds are one of the most abundant marine ecosystems in Indian Ocean coastal areas, where they sustain high primary production and provide habitat and food for several fish taxa (Fortes 1995, Vermaat et al. 1995, Wakibya 1995, Agawin et al. 1996). Seagrasses can support high herbivore abundance compared with other aquatic macrophytes (Ogden 1980, Ogden et al. 1980, Cyr & Pace 1993, Cebrián 1996),

with consumption in the tropics ranging from 5 to 90% of the leaf production (Klumpp et al. 1993, Valentine & Heck 1991, Cebrián & Duarte 1998).

Fish herbivory, representing the trophic interactions that take place at the base of the marine food web (Horn 1989), plays an important role in structuring near-shore marine ecosystems. Herbivores have been shown to contribute significantly in determining algal cover in coral reef ecosystems (Hay 1981, Hay et al. 1983, Carpenter 1986, McClanahan 1997), and in influencing seagrass community structure (McClanahan 1994). However, little is known about the consequence of fish herbivory on the relative abundance and composition of tropical seagrasses.

In recent years a large effort has been made to evaluate the relationships that bind fishes and marine plants (algae and seagrasses). The most frequently utilised approaches to the study of herbivory in seagrasses are gut content analysis (Sala 1996, Havelange et al. 1997), clipping assays (Cebrián et al. 1998) and leaf bite observations (Alcoverro et al. 1997, Cebrián & Duarte 1998). A vast majority of these studies have been conducted in relatively simple, mono-specific seagrass habitats. No efforts have been made to evaluate the fish feeding preferences in more complex multispecific seagrass areas.

The Kenyan coast seagrass flora is characterised by 12 species of seagrasses (Wakibya 1995). Nearly all of these species represent 2 contrasting life-history strategies: short-lived, 'pioneering' forms comprising *Halophila ovalis*, *Halophila minor*, *Cymodocea rotundata*, *Halodule uninervis*, *Halodule wrightii*, *Halophila stipulacea*, *Syringodium isoetifolium*, *Zostera capensis*, and long-lived, 'climax' species represented by *Enhalus acoroides*, *Thalassia hemprichii*, *Thalassodendron ciliatum* (Gallegos et al. 1994, Vermaat et al.

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1995, Duarte et al. 1996). The species *Cymodocea serrulata* is peculiar due to differences in its rhizome growth rates (cf. Vermaat et al. 1995, Brouns 1987).

Enhalus acoroides and *Thalassia hemprichii* are defined as 'constant' species (Vermaat et al. 1995), in relation to their capacity for occupying space more permanently. These species are found either in mixed meadows or as monospecific stands. The most common species to occur as monospecific meadows are *Thalassodendron ciliatum* and *T. hemprichii* (Wakibya 1995). This characteristic situation (mixed seagrass meadows) provided, therefore, a unique opportunity to test seagrass preference of herbivorous fish *in situ*. Parrotfishes, apart from being a major component of the herbivorous fish community (Bruggemann et al. 1994), seem to be the most important fish group feeding on seagrasses in Kenya (McClanahan et al. 1994).

The aim of the current experiment was to ascertain herbivorous fish preferences for seagrasses inside a marine park and thereby elucidate possible implications for seagrass species distribution.

Materials and methods. The study was conducted in the Watamu Marine National Park lagoon off the coast of Kenya (03° 22' S, 39° 59' E). The park is characterised by the presence of 12 seagrass species and it has a high biomass of fish. Values of total wet weight of fish are about 10 times higher than in areas outside the park (McClanahan 1997). The experiment was performed in a subtidal seagrass-free area inside the lagoon, between a monospecific *Thalassodendron ciliatum* meadow and the coral and hard-substrate-dominated lagoonal area (McClanahan et al. 1994).

The 9 most frequent seagrass species growing in the park (*Halodule wrightii*, *Halodule uninervis*, *Halophila stipulacea*, *Syringodium isoetifolium*, *Cymodocea serrulata*, *Enhalus acoroides*, *Thalassia hemprichii*, *Thalassodendron ciliatum*, *Cymodocea rotundata*) were chosen for the experiment. Shoots of

each plant species were prepared for the experimental assays considering differences in size and form (Table 1).

We harvested shoots of the 9 seagrass species and transported them to the laboratory in a cool box maintained at 20°C on the day before the field experiment. In the laboratory we selected shoots with leaves without bite marks or, when all the shoots had marks, we cut the tips of the leaves. We tied between 3 and 15 selected shoots of each species together; each group consisted of approximately 15 leaves. The number of shoots in each 'group' differed due to the large differences in the number of leaves per shoot among the species. For instance, while *Halodule uninervis* samples were prepared using 15 shoots, each with a single leaf, *Thalassodendron ciliatum* was prepared using 3 shoots (and the rhizome), each with 5 leaves. We prepared a total of 45 groups of tied leaves and thus 5 replicate groups per species. The initial leaf length of each group was recorded (Table 1). We attempted to retain the original appearance of the species, as normally encountered in the field, disregarding the final obvious differences in size among the plants themselves. This served to balance the number of leaves of each species used in the assay, and reproduced how these species naturally appear in seagrass meadows.

The following day, in the field, 9 groups of shoots (i.e. 1 of each species) were arranged in a circle of 3 m in diameter. Five such circles were set up between the seagrass meadow and the coral patches, assuring that the distance of each replicate circle from the meadow and the corals was the same. A 20 m distance was maintained between each circle of experimental plants.

Two divers recorded fish species feeding and behaviour in the circles during a 2 h period after the experiment was set up. All shoots were removed 24 h after the start of the experiment; no plant deterioration was recorded at this time. The presence or absence of bite marks per leaf and final leaf-length measurements were recorded for all the samples.

Additional shoots (3 per species) representing 'climax' species (*Thalassia hemprichii*, *Cymodocea serrulata* and *Thalassodendron ciliatum*) and 'pioneering' species (*Cymodocea rotundata*, *Syringodium isoetifolium* and *Halodule uninervis*) were collected at random and dried at 70°C until constant weight. Total carbon and nitrogen on seagrass leaves were determined using a Carlo-Erba CHN Analyser.

We analysed all data with a non-parametric Friedman's test as is recommended in Roa

Table 1 Initial number of shoots and leaves and leaf lengths in the experimental plots for each species. Due to the particular features of *H. stipulacea*, pieces of horizontal rhizome taken. Numbers of leaves for each rhizome are shown

Plant	Leaf length (cm)	No. of leaves per shoot	No. of shoots per sample
<i>Enhalus acoroides</i>	30	3	3
<i>Thalassodendron ciliatum</i>	8	5	3
<i>Cymodocea serrulata</i>	12	3	4
<i>Syringodium isoetifolium</i>	15	1	10
<i>Halodule uninervis</i>	12	2	7
<i>Halophila stipulacea</i>	–	17, 23, 25, 17, 19	–
<i>Thalassia hemprichii</i>	10	2	5
<i>Cymodocea rotundata</i>	15	2	6
<i>Halodule wrightii</i>	10	1	15

(1992) for the cases in which parametric statistics cannot be applied and the number of variables is ≥ 5 .

Results and discussion. *Calotomus carolinus* (family Scaridae) was the only fish observed eating plant leaves from the plots in our 2 h observation (a total of 20 observations in the 5 plots). In the field, this species was regularly identified as mainly responsible for the fish bite marks observed on plant leaves. *C. carolinus* is the second most abundant fish of the family in the park (McClanahan 1997), and is recognised as an algae and seagrass grazing parrotfish (Smith 1986, Lieske & Myers 1997). *Leptoscarus vaigensis*, another well-known seagrass eating parrotfish, was not seen around the plots while they were being prepared or in subsequent direct observations.

Most of the plant samples collected after 24 h from the field showed signs of herbivory. The bite marks observed were ring-shaped and very cleanly cut compared with the clearly jagged marks of sea urchin herbivory (see Fig. 2 in McClanahan et al. 1994). Herbivorous sea urchins are almost absent in this area (McClanahan et al. 1994, unpubl. data). For these reasons we assume that all the analysed shoots were bitten by *Calotomus carolinus* or other seagrass-eating parrotfish.

Our experiment offered several seagrass species simultaneously to herbivorous fish, to avoid the difficulties inherent in separate-offer designs. Separate-offer designs have been criticised for not addressing the subject of food feeding preference, since no food choice is offered to the consumer (Peterson & Renaud 1989). Furthermore, it has been demonstrated by several authors that when offered no choice consumers may utilise less preferred food at higher rates to compensate for the low nutritional value of the offered species (Roa 1992).

We used the leaf bite marks (presence or absence), cm of leaf eaten and percentage of leaf consumed (relative to the initial) to test fish preferences among seagrass species. There was a significant difference in the presence of bite marks per leaf among the 9 plant species ($p < 0.0043$, $n = 5$, Friedman's test). *Enhalus acoroides*, *Thalassodendron ciliatum* and *Cymodocea serrulata* were poorly bitten seagrass species, while the other species showed bite frequency higher than 50% (Fig. 1). None of the plants offered were ignored by fishes (Fig. 1). This comparison can only inform us about plant choice, but gives us no clue about the biomass eaten. This can, however, be estimated by analysing the cm of leaf eaten. We also found highly significant differences in cm of leaf eaten ($p < 0.0008$, $n = 5$, Friedman's test, Fig. 1). It is important to highlight that the tallest, long-lived plants, such as *E. acoroides* and *T. ciliatum*, were almost untouched, while small, short-lived plants, such as *C. rotundata* and *Syringodium isoetifolium*, were completely eaten,

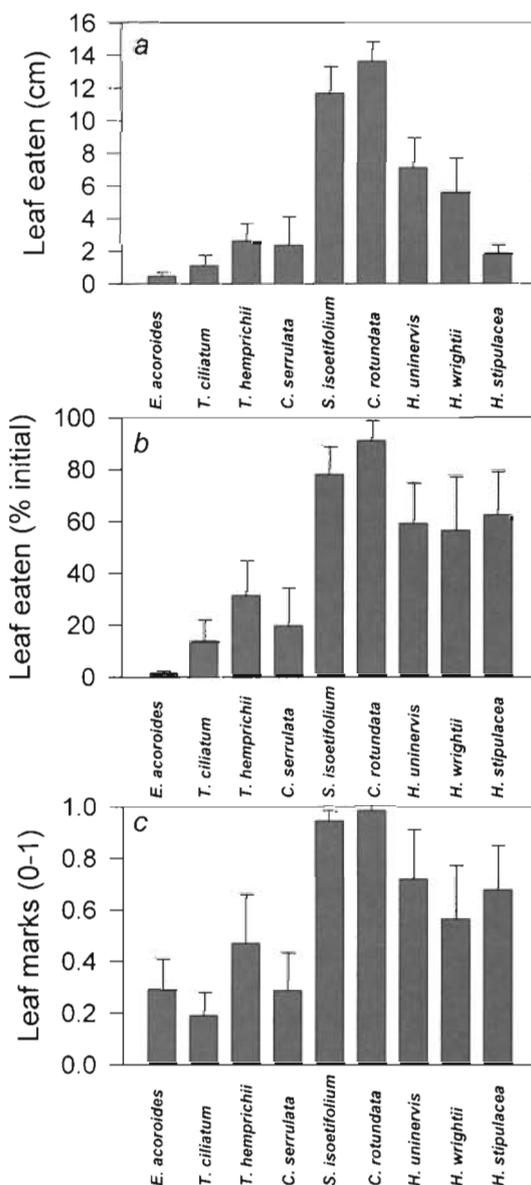


Fig. 1. (a) Amount of leaf eaten (cm), (b) percentage of initial leaf length eaten, and (c) average of the leaf marks, for the 9 seagrass species utilised. Mean and standard errors are shown

indicating that fishes do not choose the biggest and most evident plants. The case of *Halophila stipulacea*, for which we found few cm eaten, is unusual because leaves do not exceed lengths of 4 cm (Fig. 1).

In order to avoid the problem of leaf length discrepancy between different species, we calculated the percentage of leaf eaten. Once again, we found significant differences ($p < 0.0027$, $n = 5$, Friedman's test). Using this approach, small-leaf plants such as *Halophila stipulacea* and *Halodule uninervis* emerge as the most preferred species together with *Cymodocea rotundata* and *Syringodium isoetifolium*, while *Enhalus acoroides*

Table 2. Percentage of nitrogen and carbon (%N, %C) and carbon-nitrogen ratio (C:N) in the leaves of 6 selected plants representing 'pioneering' and 'climax' forms. Data represent average values (and SD) of 3 samples

Plant	%N	%C	C:N
<i>Cymodocea rotundata</i>	3.1 (0.08)	39.5 (0.15)	128
<i>Thalassia hemprichii</i>	2.8 (0.06)	39.1 (0.3)	14.1
<i>Cymodocea serrulata</i>	3 (0.09)	41 (0.51)	15.7
<i>Thalassodendron ciliatum</i>	3 (0.06)	42.1 (0.03)	16.4
<i>Syringodium isoetifolium</i>	2.1 (0.08)	36.6 (0.28)	17.5
<i>Halodule uninervis</i>	2 (0.17)	39 (0.71)	19.3

and *Thalassodendron ciliatum* were the least preferred of the species offered (Fig. 1).

We found no correlation between leaf marks, percentage of leaf eaten or cm of leaf eaten and nitrogen content (Table 2, $p > 0.05$). The carbon:nitrogen or total nitrogen ratio, a widely used index of nutritional quality, has been described as not always being appropriate when comparing seagrasses. Part of this nitrogen may be inorganic or associated with non-protein amino acids and protein complexes of no known nutritional value (Klumpp et al. 1989). In contrast, good correlation was found when we compare leaf marks and total carbon content (Fig. 2). These results can be explained by a preference for low 'carbon-fiber' plant species instead of high carbon content species. These organoleptic properties could explain fish preference

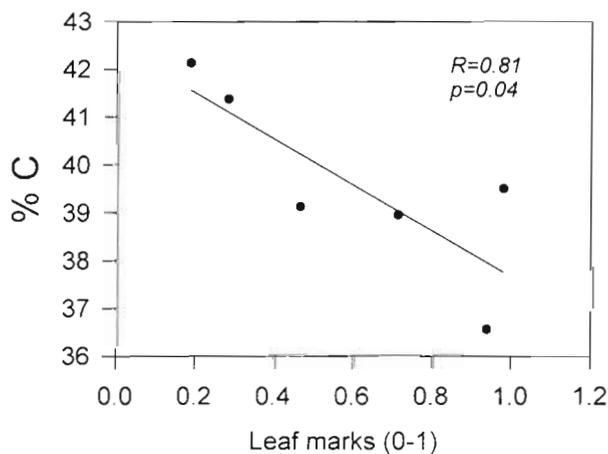


Fig. 2. Average of the leaf marks ($n \geq 45$) in relation to the average of total carbon content ($n = 3$) for the 6 species analysed

for 'pioneering' seagrasses such as *Syringodium isoetifolium* and *Cymodocea rotundata*. In contrast, sea urchins have been described to control the abundance of some long-lived, 'climax' plants such as *Thalassia testudinum* (Tertschnig 1989, Valentine & Heck 1991) and *T. hemprichii* (Klumpp et al. 1993).

Our results indicate that grazing fishes like *Calotomus carolinus* may not have the same impact on the biomass and on the abundance of 'climax' seagrasses like *Thalassodendron ciliatum* and *Thalassia hemprichii* as observed for sea urchins. However, they could play a key role in controlling the proliferation of new 'pioneering' species. We are tempted to interpret these results as representing an ecological equilibrium in which herbivorous fish control short-lived seagrass species, eliminating them from the community, thus allowing long-lived species to establish and proliferate. Multispecific seagrass communities are dependent on several factors (light irradiance, nutrient concentration, tide-dependent air exposure, etc.) and it is difficult and naïve to implicate a single structuring force as responsible for the observed seagrass community. However, as we illustrate in this paper, herbivorous fish can play a significant role in structuring seagrass communities, influencing both the plant biomass and species composition.

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LITERATURE CITED

- Agawin NSR, Duarte CM, Fortes MD (1996) Nutrient limitation of Philippine seagrasses (Cape Boliano, NW Philippines): *in situ* experimental evidence. *Mar Ecol Prog Ser* 138:233–243
- Alcoverro T, Duarte CM, Romero J (1997) The influence of herbivores on *Posidonia oceanica* epiphytes. *Aquat Bot* 56:93–104
- Brouns JJWM (1987) Growth patterns of some Indo-West-Pacific seagrasses. *Aquat Bot* 28:39–61
- Bruggemann JH, Begeman J, Bosma EM, Verburg P, Bree-man AM (1994) Foraging by the stoplight parrotfish *Sparisoma viride*. II. Intake and assimilation of food, protein and energy. *Mar Ecol Prog Ser* 106:57–71
- Carpenter RC (1986) Partitioning herbivory and its effects on coral reef algal communities. *Ecol Monogr* 56:345–363
- Cebrián J (1996) The fate of autotrophic production with special reference to herbivory: from seagrasses to some general patterns among plant communities. PhD thesis, UPC Universitat Politècnica de Catalunya, Barcelona
- Cebrián J, Duarte CM (1998) Patterns in leaf herbivory on seagrasses. *Aquat Bot* 60:67–82
- Cebrián J, Duarte CM, Agawin N, Merino M (1998) Leaf

- growth response to simulated herbivory: a comparison among seagrass species. *J Exp Mar Biol Ecol* 220:67–81
- Cyr H, Pace M (1993) Magnitude and patterns of herbivory in aquatic and terrestrial ecosystems. *Nature* 361:148–150
- Duarte CM, Hemminga MA, Marbà N (1996) Growth and population dynamics of *Thalassodendron ciliatum* in a Kenyan back-reef lagoon. *Aquat Bot* 55:1–11
- Fortes MD (1995) Seagrasses of East Asia: environmental and management perspectives. RCU/EAS Technical Report Series, United Nations Environment Programme 6, Bangkok
- Gallegos ME, Merino M, Rodriguez A, Marbà N, Duarte CM (1994) Growth patterns and demography of pioneer seagrasses *Halodule wrightii* and *Syringodium filiforme*. *Mar Ecol Prog Ser* 109:99–104
- Havelange S, Lepoint G, Dauby P, Bouquegneau JM (1997) Feeding of the sparid fish *Sarpa salpa* in a seagrass ecosystem: diet and carbon flux. *PSZN I: Mar Ecol* 18: 289–297
- Hay ME (1981) Spatial patterns of grazing intensity on a Caribbean barrier reef: herbivory and algal distributions. *Aquat Bot* 11:97–109
- Hay ME, Colburn T, Dowing D (1983) Spatial and temporal patterns in herbivory on a Caribbean fringing reef: the effects on plant distribution. *Oecologia* 58:299–308
- Horn MH (1989) Biology of marine herbivorous fishes. *Oceanogr Mar Biol Annu Rev* 27:167–272
- Klumpp DW, Howard RT, Pollard D (1989) Trophodynamics and nutritional ecology of seagrass communities. In: Larkum AWD, McComb AJ, Shepherd SA (eds) *Biology of seagrasses: a treatise of the biology of seagrasses with special reference to the Australian region*. Elsevier, Amsterdam, p 394–457
- Klumpp DW, Salita-Espinosa JT, Fortes MD (1993) Feeding ecology and trophic role of sea urchins in a tropical seagrass community. *Aquat Bot* 45:205–229
- Lieske E, Myers R (1997) *Coral reef fishes*. Princeton University Press, Princeton, NJ
- McClanahan TR (1997) Primary succession of coral-reef algae: differing patterns on fished versus unfished reefs. *J Exp Mar Biol Ecol* 218:77–102
- McClanahan TR, Nugues M, Mwachireya S (1994) Fish and sea urchin herbivory and competition in Kenyan coral reef lagoons: role of reef management. *J Exp Mar Biol Ecol* 184:237–254
- Ogden JC (1980) *Faunal relationships in Caribbean seagrass beds*. Garland STPM, New York
- Ogden JC, Tighe S, Miller S (1980) Grazing of seagrasses by large herbivores in the Caribbean. *Am Zool* 20: 949–950
- Peterson CH, Renaud PE (1989) Analysis of feeding preference experiments. *Oecologia* 80:82–86
- Roa R (1992) Design and analysis of multiple-choice feeding-preference experiments. *Oecologia* 89:509–515
- Sala E (1996) The role of fishes in the organization of a Mediterranean sublittoral community. PhD thesis, University of Marseille
- Smith M (1986) *Smith's sea fish*. Springer Verlag, Berlin
- Tertschnig WP (1989) Diel activity patterns and foraging dynamics of the sea urchin *Tripneustes ventricosus* in a tropical seagrass community and a reef environment (Virgin Islands). *Mar Biol* 10:3–21
- Valentine J, Heck K Jr (1991) The role of sea urchin grazing in regulating subtropical seagrass meadows: evidence from field manipulations in the northern Gulf of Mexico. *J Exp Mar Biol Ecol* 154:215–230
- Vermaat JN, Agawin N, Duarte CM, Fortes MD, Marbà N (1995) Meadow maintenance, growth and productivity of a mixed Philippine seagrass bed. *Mar Ecol Prog Ser* 124: 215–225
- Wakibya JG (1995) The potential human-induced impacts on the Kenya seagrasses. *UNESCO Rep Mar Sci* 66:176–187

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