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Indirect carnivory in *Roridula gorgonias* (Roridulaceae); a carnivorous plant dependent on hemipterans for prey nutrient uptake.

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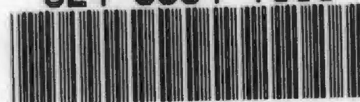
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A. G. Ellis

ABSTRACT

An intricate mutualism between *Roridula gorgonias* (Roridulaceae) and an obligate hemipteran associate, *Pameridea roridulae*, is described. The carnivorous status of *R. gorgonias* is re-evaluated on the basis of the nature of this interaction. *R. gorgonias* effectively traps large numbers of diverse flying invertebrates. However, no detectable proteolytic enzymes are produced by its leaves and *R. gorgonias* is thus unable to directly utilize trapped prey. Instead trapped invertebrates are rapidly consumed by hemipterans resident on the plant. Despite these apparent obstacles to prey nutrient uptake, evidence from ^{15}N labelling experiments suggests that *R. gorgonias* does derive nitrogen from trapped prey. I propose that *R. gorgonias* derives prey nutrients via the faeces of *P. roridulae*. This hypothesis is supported by isotopic evidence from hemipteran exclusion experiments as well as by observations of hemipteran behaviour. Thus the *R. gorgonias-P. roridulae* interaction represents an obligate nutritional mutualism whereby *P. roridulae* utilizes invertebrates trapped by *R. gorgonias* and *R. gorgonias* utilizes processed prey nutrients in *P. roridulae* faeces.

INTRODUCTION

Approximately 535 of the 250 000 recorded angiosperm species are considered to be carnivorous (Givnish et al. 1984). Following the work of Darwin (1875) a disproportionate amount of attention has been devoted to this small group (see Juniper et al. 1989 for review). However, controversy still exists as to the carnivorous status of some plants. Although the question appears simple, i.e. either they utilize nutrients from entrapped invertebrates or they do not, the solution is often not (eg. Bromeliaceae, Givnish et al. 1984). *Roridula gorgonias* (Linnaeus), in particular, has been the source of much confusion and provides an excellent example of the evolutionary complexity which surrounds the carnivorous syndrome in plants.

Both Darwin (1875) and Marloth (1903) considered *Roridula* to be carnivorous; a conclusion based largely on the presence of secretory, tentacular glands on the leaves and its obvious ability to trap insects. In 1910, after investigating the viscid glandular secretion, Marloth reversed his opinion, on the basis that the secretion was balsam-like and apparently contained no digestive enzymes. Lloyd (1934) later confirmed this opinion, describing the viscid

secretion as a resinous substance lacking digestive properties. He also emphasized the differences in glandular structure between *Roridula* and *Drosera* (*Roridula* possesses only one type of gland lacking vasculature and absorptive ability). The opinion of Marloth (1910) and Lloyd (1934) has prevailed and as a result *Roridula* has been excluded from subsequent carnivorous plant texts. Juniper, Robins and Joel (1989), in their recent treatment of carnivorous plants, give *Roridula* cursory mention as a near-carnivore, suggesting that it may derive indirect benefit from trapped insects, possibly through leaf fall.

Furthermore, Marloth (1903) and Lloyd (1934) both noted the presence on *Roridula* plants of a number of arthropod species which are apparently unaffected by the viscid secretion. *R. gorgonias* provides shelter for one species of hemipteran of the genus *Pameridea* (Hemiptera: Miridae). *Pameridea roridulae* (Reuter) is found only on *R. gorgonias* (Dolling and Palmer 1991) and feeds on invertebrates trapped by the plant (Marloth 1903, Lloyd 1934). Lloyd (1934) summed up early opinions of the relationship eloquently when he described it as commensal, "the plant providing a well spread table and the insects being unbidden guests".

Thus *R. gorgonias* represents a well studied , but poorly understood system. Current knowledge is as follows: it traps many insects (Marloth 1903); glandular trichomes on the leaf surface produce a particularly viscid, water insoluble resin (Lloyd 1934); it does not appear to produce digestive enzymes (Marloth 1910, Lloyd 1934) and prey caught by the plant are quickly consumed by "commensals" (Marloth 1903, Lloyd 1934).
This set of conflicting characters supports both carnivorous and non-carnivorous hypotheses. The present study aimed to resolve the resulting contention as to the carnivorous status of *R. gorgonias* and to elucidate the nature of the symbiosis between *R. gorgonias* and *P. roridulae*. These objectives were primarily achieved through the use of stable nitrogen isotope enrichment techniques, which allowed investigation of patterns of nitrogen transfer in the system.

MATERIALS AND METHODS

Study organisms

Roridula gorgonias, an endemic of leached humid mountain seeps in the south western Cape,

South Africa, is one of only two species within the family Roridulaceae (Fig. 6A)(Marloth 1910 & 1925). In the past the genus *Roridula* was placed within the Droseraceae, primarily as a result of its apparent carnivorous habit (eg. Harvey 1868). Like *Drosera* species, *Roridula* leaves bear numerous stalked glands which secrete a very viscid substance (Darwin 1875). Leaves are born in terminal clusters on shrubs up to 60 cm tall (Fig. 6B)(Obermeyer 1970). The root system is poorly developed (Marloth 1925). The inflorescences are born terminally and consist of 10-12 large pink flowers (Fig. 6B)(Obermeyer 1970).

The hemipteran *Pameridea roridulae* (Hemiptera, family Miridae, subfamily Bryocorinae, subtribe Dichyphini) is obligately associated with *R. gorgonias* (Dolling and Palmer 1991). *R. gorgonias* plants support large colonies of hemipterans (Dolling and Palmer 1991). Colonies consist mainly of young hemipterans (instars, Fig. 6C) which cannot fly and are therefore unlikely to move between plants (pers. obs.). Adults are winged (Fig. 6D) and are thus likely to move between plants (pers. obs.). *P. roridulae* has long legs and its body is covered by bristles. It feeds by sucking the fluids of invertebrates trapped on *R. gorgonias*.

Study site

The study was undertaken at Fernkloof nature reserve in Hermanus, South Africa (34°23'30"S, 19°17'30"E) from 5-9 July, 1994. The main study population was situated on a mountain seep alongside a fast flowing stream and had a south east aspect. It consisted of approximately 50 *R. gorgonias* plants (ca. 1m in height) confined to an area of about 100m^2. All plants were in flower during the study period. Also present on the seep were dense populations of *Grubbia rosmarinifolia* and *Osmotopsis asteriscoides*. Although the research was conducted during the height of the wet season the only significant precipitation experienced throughout the duration of the manipulation experiments was mist condensation.

Prey composition and catch rate

Thirteen leaf clusters were cleared of trapped insects (only macro-invertebrate prey was removed i.e. identifiable carcasses, >2mm in length). These leaf clusters were then re-examined after 8 weeks in order to determine catch rates and prey composition. All macro-invertebrate carcasses were removed, identified to order and their body length (excluding

appendages) was measured. Prey length serves as an estimate of the "biomass" of trapped insects, which is otherwise difficult to measure as carcasses remaining on the plant are little more than exoskeletons. The effective trapping area per leaf cluster ($64.04cm^2$) was estimated from the mean number of actively trapping leaves per leaf cluster (24.84, n=19, s=8.08) and the average leaf surface area ($2.578cm^2$, n=19, measured to the full reach of the marginal tentacles of the dorsal and ventral surfaces gravimetrically). Conservative estimates (micro-invertebrate catch not included) of catch rate and catch composition during the July-September period were thus obtained.

Also 48 individual leaves were examined under dissecting microscope in order to determine micro-invertebrate (<2mm) catch composition. Estimates of the magnitude of micro-invertebrate catch were also obtained, whereas rates of micro-invertebrate capture could not be determined as leaves were not previously cleared.

Proteolytic enzymes

The gelatin substrate film method of Heslop-Harrison and Knox (1971) was used to determine whether glands on the leaves of *R. gorgonias* secrete digestive enzymes (proteases). In theory proteases digest the gelatin layers of photographic film. As a result silver halide crystals are released leaving obvious clear patches on the film.

Yeast extract was applied to the *R. gorgonias* leaf surface in order to chemically stimulate production of enzymes. Eight hours after protein application pieces of exposed and developed (yet unfixed) photographic film (Kodalith orthofilm) were attached to stimulated leaves. Film was removed after 24 hours and analyzed for signs of digestion. *Drosera capensis*, a species known to secrete proteases, was also investigated in an identical manner in order to provide a qualitative comparison for the results obtained from *R. gorgonias*.

Hemipteran observations

A number of observations pertaining to the behaviour of the hemipteran, *P. roridulae*, were made. Firstly, the number of hemipterans present per leaf cluster was determined. Secondly, the rate of arrival of hemipterans at trapped prey was investigated. A single anaesthetized *Drosophila* individual was applied to each of 20 leaf clusters at the study population. The initial time of arrival of hemipterans at these flies was recorded. Also, the number of

hemipterans feeding on flies at 15, 30 and 60 minutes after application was recorded. Various observations of hemipteran behaviour were also made, in the field and under dissecting microscope.

Stable isotope labelling experiments

The stable isotope ^{15}N was used to determine whether *R. gorgonias* incorporates nitrogen present in trapped invertebrates. *R. gorgonias* plants were exposed to ^{15}N enriched *Drosophila* flies and in so doing nitrogen movement between trapped insects and the plant could be determined. Isotope labelling rather than natural abundance techniques were used largely in order to allow manipulation of the study system i.e. to allow for manipulation of *P. roridulae* influence on nitrogen uptake by *R. gorgonias*.

1. Isotope labelling procedure

Following Dixon, Pate and Bailey (1980) commercial bakers yeast was raised on a standard Difco-Bacto carbon base medium with $(^{15}NH_4)_2SO_4$ (99 atom % ^{15}N) as the labelled nitrogen source. Yeast cultures were grown in sterile test tubes at 30°C for 48 hours. Resulting yeast was collected by centrifugation and then incorporated into a *Drosophila* feeding medium. *Drosophila melanogaster* were cultured from the egg stage on a standard rearing medium (labelled yeast, glucose, agar, proprionic acid and de-ionized water). Egg laying adult flies were introduced to the media for 5 days and then removed. Resulting labelled offspring emerged after 14 days of incubation at 25°C. An unenriched batch of flies were raised on brown sugar, maize meal, yeast and water.

2. Feeding experiments

Feeding experiments were conducted at the study site described above. Three treatments were used: a) Non-exclusion treatment: Plants fed ^{15}N enriched *Drosophila*. Hemipterans present.

b) Exclusion treatment: Plants fed ^{15}N enriched *Drosophila*. Hemipterans absent.

c) Plants fed unenriched *Drosophila*. Hemipterans present.

Plants from treatment b), the exclusion treatment, were initially cleared of hemipterans by hand after which a formex barrier was established around the stem of each plant in order to

prevent return of hemipteran individuals. Adult hemipterans, which comprise less than 10% of the hemipteran population (see Results, Hemipteran observations), are able to fly. For this reason exclusion plants were examined regularly for the presence of hemipterans. In the event of their return, hemipterans were removed and their presence recorded. This together with microscopic examination of fly carcasses (for signs of hemipteran feeding) following the feeding trials, gave a qualitative idea of the efficiency of hemipteran exclusion for each treatment plant. Although this method did not give complete exclusion efficiency it did have the advantage of not contributing any other variables which might have affected nitrogen uptake in the two treatments distinctly i.e. if exclusion plants had been bagged the resulting decrease in sunlight intensity and moisture condensation on the leaves might have resulted in differences in nitrogen uptake between the exclusion and non-exclusion treatments.

Prior to application of flies all natural prey carcasses were eliminated from the leaf clusters. Slightly anaesthetized *Drosophila* were then applied to recently mature, fully expanded leaves. Six flies were fed to each of the treatment plants, with two flies placed centrally on three leaves per plant (henceforth "fed-leaves"). Flies were only fed to one leaf rosette per treatment plant. Plants were then left for 72 hours before sampling, a period considerably longer than that required by certain other carnivorous plants (*Pinguicula*, *Drosera* and *Dionea*) to effect prey nutrient uptake (Heslop-Harrison and Knox 1971, Clancy and Coffey 1977). This lengthy period was necessary in order to maximize potential nitrogen uptake by *R. gorgonias*. Fed-leaves and the apical bud (with associated young leaves) of each treatment leaf rosette were then collected. Three untreated plants, used to obtain natural ^{15}N abundance, were also sampled, as well as hemipterans from untreated and enriched treatment plants.

3. Excreta route test

An investigation into the direct contribution of *P. roridulae* to the nitrogen nutrition of *R. gorgonias* was considered necessary on the basis of the results of the field experiments described above. A qualitative test was performed using a single plant (from the study population) which had been growing under greenhouse conditions for 2 months. Hemipterans were removed from the plant and then fed six ^{15}N enriched flies. After 24 hours hemipterans were returned to the plant. Over the next 5 days they were allowed to feed on another 10 labelled flies presented on a wire mesh platform attached to the plant. Thus, throughout the

duration of the experiment no labelled flies came into contact with the plant surface. Furthermore, the terminal leaves of the plant were bagged in order to exclude hemipterans and thus eliminate the possibility of contamination of apical buds by hemipteran faeces. After 5 days leaf and apical bud material was harvested for isotope analysis. Leaf material collected prior to the experiment was also subjected to isotope analysis in order to ensure that ^{15}N enrichment observed was due to experimental treatment alone.

4. Sample analysis

After sampling, plant material was examined under dissecting microscope. All invertebrate remains, debris, hemipteran faeces and that portion of leaf in contact with applied flies was removed in order to eliminate the possibility of contamination, by both natural prey and labelled fly residue. After cleaning, samples were soaked in very dilute hydrochloric acid (0.3M) in order to remove halides which react with quartz tubes used during isotope analysis. Samples were then rinsed and freeze-dried. 1-1.5 mg of dried sample was then prepared for automated Dumas combustion in a Carlo-Erba NA 1500 Elemental Analyzer connected to a Finnigan MAT 252 mass spectrometer (Archeometry, University of Cape Town). Samples were analyzed by continuous flow batch run techniques as presented by ISODAT controller software (ISODAT 1994). In order to increase measurement precision it is necessary to analyze samples at similar gas pressures (Hauck 1982). Thus only samples with 28V (voltage at the 28 atomic mass faraday cup) amplitude between 0.5 and 3 V were considered. A standard within the enrichment range required was unavailable during sample analysis and therefore a Merck gelatin standard ($\delta^{15}N=7.0‰$) was used. Precision throughout the runs was found to be 1.46‰ in the natural abundance range. In order to eliminate the possibility of sample contamination during runs blanks and standards were inserted regularly throughout runs. Also samples were run in order of expected enrichment.

Insect samples were treated identically although they were not soaked in HCl. Also, it was impossible to analyze labelled flies as the cup configuration of the mass spectrometer used was only appropriate for enrichments less than 5 atom % ^{15}N i.e. $^{30}N_2$ molecules were not detected and the sensitivity of the 29 atomic mass cup was such that it was flooded at higher enrichments (Dr. A. Hilkert, pers. comm.; Hauck 1982). This configuration was, however, appropriate for analysis of all other samples as enrichments were well below 5 atom %.

Nitrogen yield was obtained from samples run by unautomated Dumas combustion techniques (described in Fiedler and Proksch 1975). The pressure of nitrogen gas present after combustion and purification of samples was determined allowing calculation of nitrogen yield (i.e. conversion factor (0.814) * N_2 pressure/ sample mass).

5. Data analysis

Atom percent ^{15}N values (i.e. $^{15}N/(^{14}N+^{15}N)*100$) were calculated from the ISODAT output as follows:

$$\text{Atom \% } ^{15}N = \frac{100*R_{st}*(\delta_{sa}/1000 + 1)}{1 + R_{st}*(\delta_{sa}/1000 + 1)}$$

where R_{st} = absolute ratio of the standard (i.e. N_{air} = 0.003676 ^{15}N) and δ_{sa} is the $\delta^{15}N$ value of the sample as presented by ISODAT (ISODAT 1994). Atom percent values were further used to calculate the atom percent excess ^{15}N relative to a substrate:

$$APE = at\%_{sample} - at\%_{substrate}$$

where at% represents the atom % ^{15}N. Both the natural ^{15}N abundance of the organism involved and atmospheric ^{15}N abundance were used as substrate values (Hayes 1983). This calculation assumes a constant value for the substrate natural ^{15}N abundance (Hauck 1982). Natural ^{15}N abundance of *R. gorgonias* is, however, likely to be variable due to differences in the nitrogen sources of individual plants. Thus results were expressed relative to the atmosphere which has a constant ^{15}N abundance (i.e. at% = 0.3663).

Atom percent ^{15}N values also allowed calculation of nitrogen budgets (i.e. the amount of nitrogen absorbed) for leaf rosettes with hemipterans present. Two fundamental assumptions were involved in these calculations. The first, that flies were 90 % enriched in ^{15}N, was based on the fact that flies were raised on a medium containing 99 APE ammonium sulphate as the only nitrogen source for their growth. An enrichment of 90 % is thus likely to be a conservative estimate, but this could however not be validated as the facilities necessary to analyze such highly enriched samples were not available. Secondly, in light of the results of

the excreta route test (Fig. 5), it was assumed that other leaves in the leaf cluster show identical ^{15}N enrichment to the fed-leaves. For details of the calculation procedure see Appendix 1.

Alternate means of acquiring insect nitrogen

Preliminary investigations of leaf turnover rate were conducted in order to establish the possible importance of leaf fall, especially of carcass laden leaves, as an indirect means of acquiring insect nitrogen. Young leaves were marked and then examined again after 8 weeks in order to establish a qualitative idea of the rate of leaf development and maturation during this period.

Leaves were also examined for the presence of capsid excretory products as these potentially provide an alternate means by which plants may absorb insect nitrogen. The number of faeces present per leaf was determined under a dissecting microscope. Also, the leaf surface was investigated with the aid of a scanning electron microscope in order to determine stomatal and cuticle characteristics (SEM unit, U.C.T). The leaf undersurface was photographed with a Polaron LT400 Cryoprep coupled to a Leica Stereoscan 440i. Standard cryomicroscopy techniques were used (Robards and Wilson 1993).

RESULTS

Prey composition and catch rates

Macro-invertebrate prey was found to be comprised mainly of dipterans which constituted 77.06% of total catch by abundance and 80.55 % of the total catch by prey length (Table 1). Hymenoptera, Coleoptera and Hemiptera formed the rest of the prey caught during the period July-September. Although not present on sampled plants, lepidopteran, odonate and orthopteran carcasses were observed on other *R. gorgonias* plants. During the period July-September 0.13 insects were caught per cm^2 leaf area, which amounts to 8.38 insects per leaf cluster (Table 1). The insects caught, or rather the macro-invertebrates collected, were on average 3.55mm in length (Table 1). The "biomass" of insects caught during the study period was 0.47mm prey-length per cm^2 leaf area. Thus catch rate of macro-invertebrate prey during

Table 1: Macro-invertebrate (prey>2mm in length) catch composition and catch rates for 13 R.gorgonias leaf rosettes during an 8 week per July-September, 1994. Composition is expressed as the percentage of the total catch belonging to respective insect orders and catch rate and length=std (mm) of prey caught per leaf area.

	DIPTERA	HYMENOPTERA	COLEOPTERA	HEMIPTERA	TOTAL
NUMBER OF SPECIES	19	3	8	2	32
ABUNDANCE (NO. OF INDIVIDUALS)	84	7	11	7	109
NUMBER/ LEAF CLUSTER	6.46±3.73	0.54±1.13	0.85±0.90	0.54±0.54	8.38±4.57
NUMBER/ cm2 LEAF AREA #	0.10	0.01	0.02	0.01	0.13
TOTAL PREY LENGTH (mm)	312.02	17.84	31.99	25.50	387.35
MEAN PREY LENGTH (mm) *	3.71	2.55	2.91	3.64	3.55
PREY LENGTH / LEAF CLUSTER	24.00±15.80	1.37±2.50	2.46±2.68	1.96±0.66	29.80±17.26
PREY LENGTH / cm2 LEAF AREA $	0.37	0.02	0.04	0.03	0.47
% OF CATCH BY ABUNDANCE	77.06	6.42	10.09	6.42	
% OF CATCH BY PREY LENGTH	80.55	4.61	8.26	6.58	

- calculated as number per leaf-cluster / mean leaf-area per leaf-cluster

$ - calculated as prey-length per leaf-cluster / mean leaf-area per leaf-cluster

* - calculated as total prey length / abundance

Table 2: The magnitude and composition of the micro-invertebrate (prey<2mm in length) catch of R. gorgonias as sampled from 48 leaves.

	NO. OF INDIVS.	NO. PER LEAF CLUSTER	NO. PER LEAF	NO. PER cm2 LEAF
THYSANOPTERA	52	26.83	1.08	0.42
DIPTERA	35	18.13	0.73	0.28
HYMENOPTERA	16	8.20	0.33	0.13
COLEOPTERA	9	4.72	0.19	0.07
TOTAL	112	57.88	2.33	0.90

the late winter months was 0.06mm prey-length.cm^{-2}.$week^{-1}$.

The micro-invertebrate catch was largely comprised of Thysanoptera (46.4% of catch) although dipterans did make up a significant portion of the carcasses present (i.e. 31.3%) (Table 2). Coleopterans and hymenopterans were also caught (Table 2). The micro-invertebrate catch during the active trapping life of the 48 leaves sampled was 0.91 prey individuals per cm^2 leaf area. Thus, by extrapolation, 57.88 micro-invertebrate carcasses were present on each leaf cluster at the time of sampling.

Proteolytic enzymes

Photographic film placed on the leaves of *R. gorgonias* showed no evidence of digestion, suggesting that no proteolytic enzymes are produced by *R. gorgonias*. *Drosera capensis* on the other hand showed significant production of proteases (Fig. 6E).

Hemipteran observations

A mean of 7.19 hemipterans were present per leaf cluster ($s=3.91$, $n=21$), although this is a relatively arbitrary figure as the number of hemipterans present correlates with leaf cluster size (Fig. 1, $r^2=0.74$, $p<0.01$). Adults comprised only 6.6% of the hemipteran population at the study site.

All but one of the 20 *Drosophila* fed to individual plants were located by hemipterans within 60 minutes of application. The average time of initial location of flies (for the 19 that were located) was 11:09 minutes (s=12:06 minutes, n=19) after application. Peak hemipteran presence at flies was observed at 15 minutes after application (Fig. 2). After this the proportion of applied flies with hemipterans present was found to decrease (Fig. 2). Also, differences existed in the feeding patterns of adult and instar hemipterans (Fig. 2). Adult presence at applied flies decreased significantly during the period of observation whereas instar presence was found to increase slightly.

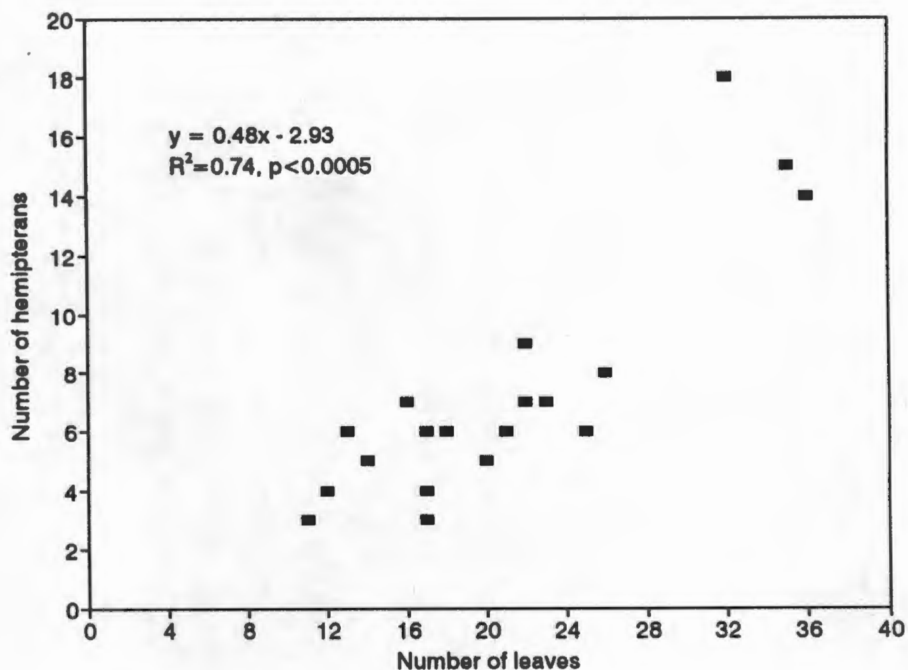

Fig. 1: The relationship between the number of hemipterans present and the size of *R. gorgonias* leaf clusters. Data displayed are for individual leaf clusters.

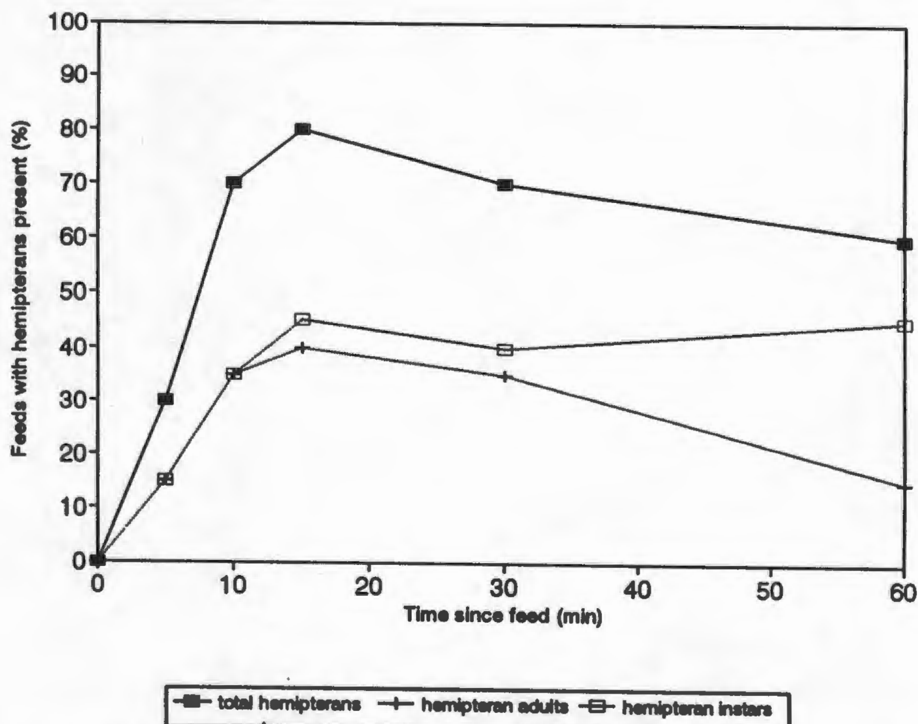

Fig. 2: The rate of arrival of hemipterans at flies applied to *R. gorgonias* leaves, displayed as the proportion of applied flies (total=20) with hemipterans present as a function of time since application of fly individuals. Total hemipterans refers to those flies with any hemipteran present, adult hemipterans to those flies with adults hemipterans present and instar hemipterans to those flies with non-adult hemipterans present.

Stable isotope labelling experiments

1. Natural ^{15}N abundance

The natural ^{15}N abundance of mature *R. gorgonias* leaves was found to be 14.96 ‰ (n=2, s=2.23) (Table 3). Young leaves of the apical bud had δ = 11.61 ‰ (n=2, s=4.26). The natural abundance δ15N value for adult hemipterans was 6.56 ‰. Plants fed unenriched flies (δflies = -3.24 ‰, n=1) had delta values lower than natural abundance i.e. δ = 2.54 ‰ (n=2, s=0.08) for mature (fed) leaves and δ = 1.71 ‰ (n=2, s=2.10) for new leaves (Table 3).

2. Labelling experiments

All plants fed labelled flies showed ^{15}N enrichment (Fig. 3). Although a lot of variation between individuals within each treatment does exist (range for non-exclusion fed-leaves was 0.24-0.64 atom % excess and for exclusion fed-leaves was 0.02-0.11 atom % excess), plants with hemipterans present were consistently more enriched than those from which hemipterans had been excluded (Fig. 3).

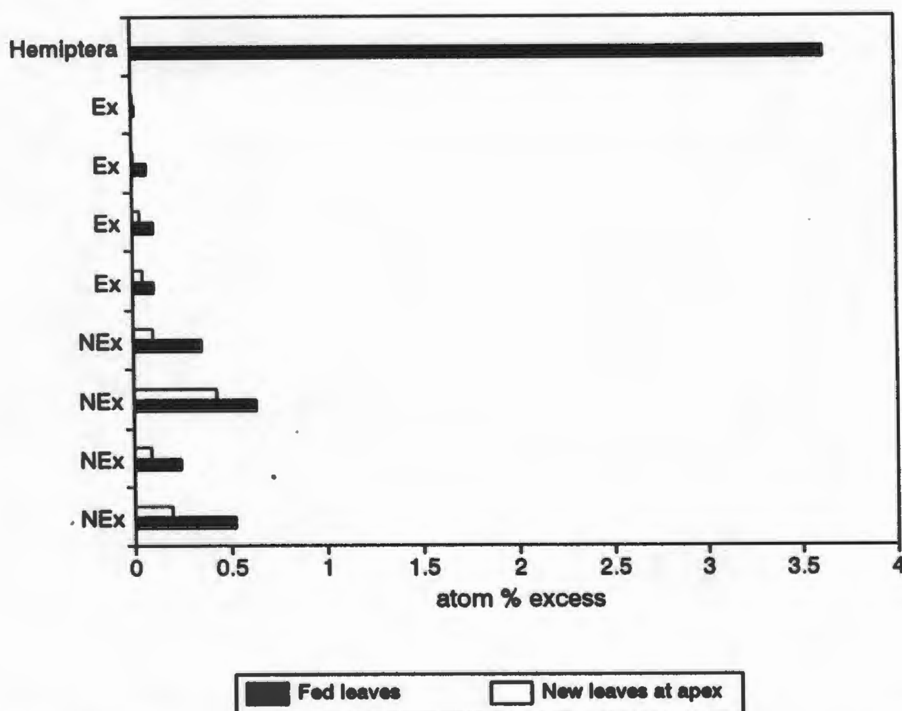

Fig. 3: ^{15}N enrichment (APE relative to atmospheric ^{15}N abundance) of *P. roridulae* and *R. gorgonias* individuals due to application of labelled flies showing variation within treatments, between exclusion (Ex) and non-exclusion (NEx) treatments and between fed and new leaves.

Table 3: The results of ^{15}N labelling experiments displayed as $\delta^{15}N$ (‰), atom % and atom % excess (relative to natural abundance and relative to the atmosphere). For *R. gorgonias* enrichment of fed-leaves (F) appears above and new (young) leaves (Y) of the apical bud below in italics. Sample standard deviations (s) appear in parentheses.

			Delta		Atom %		Atom % excess #		Atom % excess *	
R.gorgonias	Natural abundance (n=2)	F	14.96	(2.23)	0.37171	(0.00081)			0.00541	(0.00081)
		Y	*11.61*	*(4.26)*	*0.37049*	*(0.00155)*			*0.00419*	*(0.00155)*
	Unenriched treatment (n=2)	F	2.54	(0.08)	0.36718	(0.00003)	-0.00453	(0.00003)	0.00088	(0.00003)
		Y	*1.71*	*(2.10)*	*0.36688*	*(0.00077)*	*-0.00361*	*(0.00077)*	*0.00058*	*(0.00077)*
	Hemipteran exclusion treatment (n=4)	F	221.68	(116.67)	0.44707	(0.04252)	0.07536	(0.04252)	0.08077	(0.04252)
		Y	*73.72*	*(59.93)*	*0.39314*	*(0.02186)*	*0.02265*	*(0.02186)*	*0.02684*	*(0.02186)*
	Non-exclusion treatment (n=4)	F	1208.87	(483.86)	0.80521	(0.17502)	0.43350	(0.17502)	0.43891	(0.17502)
		Y	*560.67*	*(438.73)*	*0.57024*	*(0.15925)*	*0.19975*	*(0.15925)*	*0.20394*	*(0.15925)*
R.gorgonias (excreta route test)	Before treatment (n=1)		-3.87		0.36484				-0.00146	
	After treatment (n=1)	F	4497.62		1.98089		1.61605		1.61459	
	(n=2)	Y	*4337.41*	*(192.48)*	*1.92425*	*(0.06806)*	*1.55941*	*(0.06806)*	*1.55795*	*(0.06806)*
P.roridulae	Natural abundance (n=1)		6.56		0.36865				0.00235	
	Enriched (n=1)		10317.12		3.99402		3.62537		3.62772	
D.melanogaster	Unenriched (n=1)		-3.24		0.36507				-0.00123	

- APE measured relative to the natural abundance of the organism involved in each category.
* - APE measured relative to atmospheric ^{15}N abundance i.e. 0.3663 atom %.

When hemipterans were present fed leaves were 0.44% (n=4, s=0.18) enriched in ^{15}N relative to atmospheric ^{15}N abundance (ie 0.3663 atom %), whereas in the absence of hemipterans fed-leaves had an APE value of 0.08% (n=4, s=0.04, Fig. 4). The difference between non-exclusion and exclusion treatments is significant (no overlap in standard deviation bars, Fig. 4; Mann-Whitney U=-2.17, $p<0.05$ for both fed and new leaves). Also, although new leaves did display some ^{15}N enrichment they were consistently less enriched than fed-leaves i.e. 0.03 vs. 0.08 APE in the hemipteran exclusion treatment and 0.20 vs. 0.44 APE when hemipterans were present (Fig. 4).

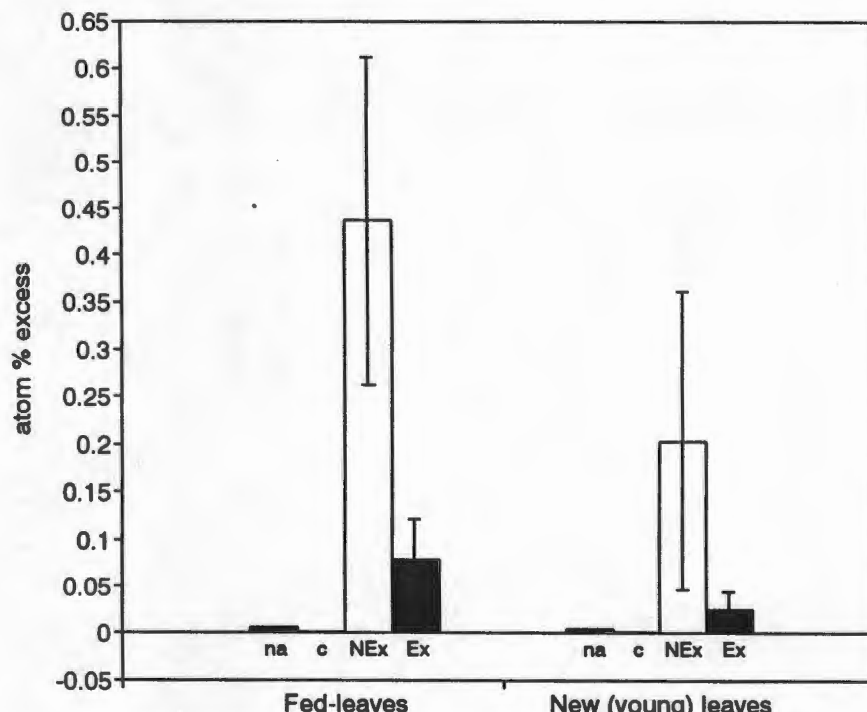

Fig. 4: Comparison of ^{15}N abundance (expressed as APE relative to the atmosphere) in fed-leaves and young leaves of plants from the four treatments i.e. untreated plants or natural abundance (**na**), plants fed unenriched flies (**c**), hemipteran exclusion treatment (**Ex**) and non-exclusion treatment plants (**NEx**). Bars indicate sample standard deviation.

Hemipterans present on plants fed labelled flies also showed substantial enrichment i.e. 3.62 APE (Fig. 3). They were therefore ca. 8 times more enriched than the fed-leaves of the plants on which they were caught.

In non-exclusion plants 17.6% of the ^{15}N present in labelled flies was absorbed by the fed leaf cluster (i.e. 0.0227 mg of 0.1287 mg ^{15}N applied, Appendix 1). A further 19.58% of fly nitrogen was present in the hemipteran population of the fed leaf cluster.

3. Excreta route test

Substantial ^{15}N enrichment (APE=1.61% for mature leaves and APE=1.56% for young leaves) also occurred in the plant which was only indirectly subjected to enriched flies. Enrichment still occurred although labelled flies were not directly applied to the plant, but were instead fed to hemipterans which were then allowed to move back onto the plant (Fig. 5). Enrichment was ca. 4 times higher in this plant than in those plants fed directly i.e. 1.61 APE for the indirectly fed plant versus 0.44 APE for the directly fed plants.

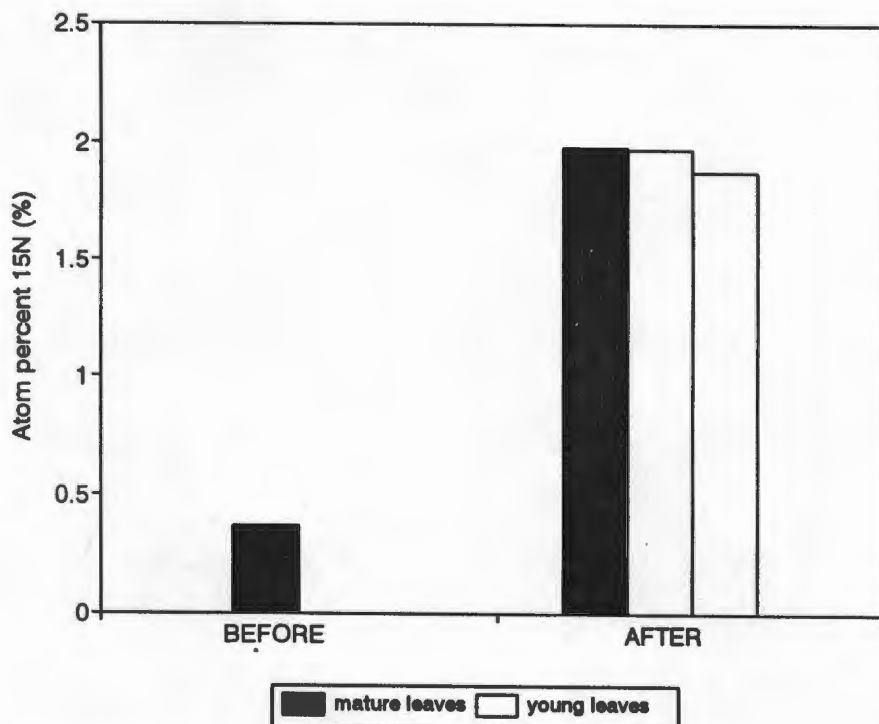

Fig. 5: ^{15}N abundance (atom % ^{15}N) of a *R. gorgonias* plant before and after manipulation of the isotopic composition of its hemipteran population. Only abundance of mature leaves before manipulation is displayed, whereas abundance of both mature and young leaves (from which hemipterans were excluded) after manipulation is displayed.

Alternate nitrogen sources

Marked leaves moved from the terminal position X to position Y, close to the leaf rosette base, (i.e. through approximately 120°) during the 2 months study period (Fig. 6B). Older leaves, near the base of the leaf cluster, appear not to be actively involved in insect capture and are soon abscised (pers. obs.). The active trapping life of each leaf is therefore likely

Figure 6: A, *R. gorgonias* plants in typical moist, mountainous habitat. **B**, A terminal leaf cluster of *R. gorgonias*. X represents initial position of marked leaves and Y represents their position 2 months later. **C**, Young hemipteran instar feeding on Drosophila. **D**, Adult hemipteran feeding. **E**, Photographic film subjected to digestion by enzymes from the leaves of *R. gorgonias* (Rg) and *D. capensis* (Dc), showing the lack of enzyme production in *R. gorgonias*. **F**, Hemipteran faeces (f) on the undersurface of a *R. gorgonias* leaf. Also, visible are the glandular leaf trichomes (t). **G**, SEM of leaf undersurface showing hemipteran faeces (f), leaf trichomes (t) and stomata (s). **H**, Glandular trichomes (t) on the leaf undersurface.

to be less than 2 months.

Hemipteran faeces were detected on 18 of the 27 leaves examined (Fig 6F and 6G), especially on the leaf under-surface. The number of fąeces present was higher for fed-leaves from the non-exclusion treatment (1.93 faeces per leaf, s=1.49, n=15) than from the exclusion treatment (1.08 faeces per leaf, s=1.68, n=12). Besides the solid faeces displayed in Fig. 6F and Fig. 6G hemipterans were also observed to produce a liquid excreta soon after feeding on trapped prey.
A thin cuticle and many stomata were found to be present on the leaf under-surface (Fig. 6G).

DISCUSSION

Is *R. gorgonias* carnivorous?

Juniper et al. (1989) defined carnivorous plants as those plants which use entrapped animal tissues for their nutrition. This amounts to a simplification of the definition of Givnish et al. (1984). They proposed that a plant must fulfil two requirements to be classified as carnivorous i.e. it must be able to absorb nutrients from dead animals juxtaposed to its surface, thereby increasing fitness and it must have some unequivocal adaptation or resource allocation whose primary result is the attraction, capture or digestion of prey.

Isotopic evidence for carnivory

1. Natural ^{15}N abundance

On the basis of these definitions and the results presented above, *R. gorgonias* is carnivorous. Evidence comes mainly from stable isotope labelling experiments. Firstly, the ^{15}N natural abundance of *R. gorgonias* was found to be 14.96 ‰ (Table 3), a value which can be considered high for plants obtaining nitrogen only from the soil (usually between -8 and 10 ‰, Shearer and Kohl 1986). It is more typical of higher trophic level carnivores, which have higher $\delta^{15}N$ values due to various trophic level effects, notably the tendency of catabolic processes (urine production) to eliminate the lighter ^{14}N isotope (Peterson and Fry 1987,

Handley and Raven 1992). This is further evidenced by the fact that *R. gorgonias* has a significantly higher $\delta^{15}N$ value than *P. roridulae*, a known carnivore (Table 3). Thus, the high ^{15}N natural abundance value of *R. gorgonias* suggests that it may derive nitrogen from a source other than the soil i.e. invertebrates. The $\delta^{15}N$ value for *R. gorgonias* is considerably higher than those reported for other carnivorous plant species i.e. 0.819-3.302 ‰ for Australian *Drosera* spp. (Schulze et al. 1991) and 0.37 ‰ for *Uticularia vulgaris* (Friday and Quarmby 1994). This possibly indicates that *R. gorgonias* is strongly reliant on invertebrates for its nitrogen requirements, or on the other hand could simply be the result of differences in background ^{15}N levels of the soil, vegetation and invertebrate fauna.

2. Unenriched treatment

The second line of evidence comes from the unenriched treatment plants. These plants had significantly lower $\delta^{15}N$ values than untreated plants (Table 3). This suggests that plants absorbed unenriched fly nitrogen, which was strongly depleted in ^{15}N ($\delta^{15}N$ = -3.24 ‰), causing dilution of the ^{15}N within the plant and a resulting decrease in the ^{15}N abundance of the treatment plants.

3. Labelling experiments

Similarly, plants fed labelled flies showed significant ^{15}N enrichment, providing the third line of evidence for the carnivorous status of *R. gorgonias* (Fig. 3). Although enrichment values for *R. gorgonias* treatment plants were relatively low (eg. 0.44 % for fed-leaves from non-exclusion treatment plants, Fig. 4), they are comparable to results obtained from other similar studies. For example, Friday and Quarmby (1994) found ^{15}N enrichment of between 1.12 and 1.97% in fed-leaves of *Utricularia vulgaris*. Dixon et al. (1980) on the other hand showed enrichment of 21.8 atom % excess ^{15}N in *Drosera erythrorhiza* individuals. The high enrichment obtained in this study may be due to the long duration of the experiment ie 4 weeks of continual weekly feeding. *D. erythrorhiza* was shown to absorb 76.1% of applied nitrogen (Dixon et al. 1980). Only 17.6% of *Drosophila* nitrogen applied to *R. gorgonias* plants was present in the leaves of the fed leaf cluster and a further 19.6% was present in the hemipteran population of the fed plant (Appendix 1). It would thus appear that the efficiency of nitrogen uptake is lower in *R. gorgonias* than in *D. erythrorhiza* although

without information from other plant parts (i.e. inflorescences, stems, roots and other leaf clusters) this statement remains speculative.

Enrichment of young leaves associated with the apical bud indicated translocation of nitrogen within the plant. Friday and Quarmby (1994), found one third of the total ^{15}N fed to *Utricularia vulgaris* in the growing tips. In *R. gorgonias* enrichment of young leaves was considerably lower than fed-leaves (Fig. 3 and 4). The possibility therefore exists that the quantity of ^{15}N applied (i.e. 0.1287mg) was too small to allow significant enrichment of growing tips, or more likely that the duration of the feeding experiments was too short to allow equilibration of ^{15}N in the system, a problem commonly encountered in high enrichment studies (Deleens et al. 1994). This is supported by the fact that the excreta route test, which had higher ^{15}N dosage and longer feeding period, gave similar enrichments for fed and young leaves both of which were more enriched than plants from other treatments (Fig. 5). Differences in ^{15}N dosage could also contribute to the large variation in enrichment observed between treatment plants (i.e. 0.40 atom % for fed-leaves of the non-exclusion treatment). This variation seems typical of labelling experiments and is in fact lower than the variation observed by Friday and Quarmby (1994) in fed leaves of *U. vulgaris* i.e. 0.85 atom %. They attribute variation in *U. vulgaris* to differences in leaf size and not to ^{15}N dosage effects. Similarly, variation in enrichment between treated *R. gorgonias* individuals can also be explained in terms of differences in leaf and leaf cluster size i.e. the ^{15}N label is likely to be diluted in larger leaves and leaf clusters. Furthermore, variation is especially likely if one considers the excreta route hypothesis (see Discussion, Excreta route hypothesis) in which case hemipteran number, position of hemipteran faeces and various other factors would also lead to variation in enrichment of a particular leaf.

Non-isotopic evidence for carnivory

From the stable isotope studies reported above it can be concluded that *R. gorgonias* does derive nitrogen from entrapped invertebrates. Support for this hypothesis also comes from sources other than isotopes. Firstly, *R. gorgonias* traps large numbers of invertebrates (Table 1 and 2). Verbeek and Boasson (1993) report total life-time catches of between 1.8 and 3.9 mm of prey per cm^2 leaf area for Australian Droseraceae with mean prey lengths of between 0.6 and 1.8mm. *R. gorgonias* in comparison trapped 0.47mm of macro-invertebrate (i.e.

>2mm) prey per cm^2 leaf area in only 2 months during late winter, a period when abundance of adult winged insects is not high. Also, 0.9 micro-invertebrate (i.e. <2mm) prey individuals were trapped per cm^2 leaf area (Table 2). The mean length of *R. gorgonias* prey was 3.5 mm which is substantially larger than that reported for *Drosera* species (Verbeek and Boasson 1993) and for *Pinguicula nevadense* (Zamora 1990a), suggesting that constraints on the capture of large prey are weaker in *R. gorgonias*. Thus *R. gorgonias* very effectively traps large numbers of flying invertebrates which lends support to its status as a carnivore.

Secondly, the leaf morphology of *R. gorgonias* is clearly adapted for insect capture (Fig. 6F and 6H). The suggestion that the glandular leaf trichomes function primarily as a defense against herbivory is unlikely. A large number of diverse invertebrates, including many non-herbivorous species, are trapped by *R. gorgonias*. This suggests that the effectiveness of the trichomes has advanced beyond the simple repulsion of potential herbivores. Thus leaf trichomes represent an unequivocal adaptation to prey capture which fulfills the second of Givnish et al.'s (1984) requirements for carnivorous plants. Givnish et al. (1984) further propose that carnivorous plants should largely be confined to nutrient-poor, sunny, moist habitats, a description which perfectly fits the habitat of *R. gorgonias*, lending further support to its carnivorous status.

Direct carnivory, an impossibility?

On the basis of the evidence presented above the views of Marloth (1910), Lloyd (1934) and all subsequent workers can be rejected. Surprisingly though, their main arguments which suggest that *R. gorgonias* is not carnivorous cannot be refuted and were in fact supported by this study. Firstly, the suggestion of both Marloth (1910) and Lloyd (1934) that *R. gorgonias* does not produce digestive enzymes (proteases) was confirmed by the results of the gelatin substrate film experiment (Fig. 6E). Secondly, SEM analysis of leaf structure (Fig. 6G and 6H) confirms early views that *R. gorgonias* lacks the sessile glands involved in enzyme production and nutrient uptake in many other carnivorous genera eg. *Drosera* (Juniper et al. 1989), *Drosophyllum* (Green et al. 1979) and *Pinguicula* (Heslop-Harrison and Knox 1971). Thirdly, the impact of the hemipteran, *P. roridulae*, which was considered by Lloyd (1934) to be commensal on *R. gorgonias*, was emphasized by the results of this study. Hemipterans were found to occur in large numbers on all *R. gorgonias* plants and to feed on trapped

invertebrates (Fig. 1, Fig. 6C and 6D), which they rapidly locate and devour (Fig. 2). The phenomenon of kleptoparasitism of "fly-paper trap" carnivorous plants, particularly by ants, has been reported in the literature (Thum 1989, Zamora 1990b). However, it has never involved an obligate associate which occurs in such large numbers and has such obvious impact on trapped prey as is the case in the *P. roridulae-R. gorgonias* interaction. This interaction is best compared to those reported between pitcher traps and their obligate commensals. For example, chironomid midge larvae are reported to consume more than 50% of prey trapped by Darlingtonia pitchers (Juniper et al. 1989 and references therein).

These three observations suggest firstly that *R. gorgonias* has no means of digesting trapped invertebrates and secondly, that even if it were capable of digesting prey it is unlikely that it would derive much benefit as prey are rapidly consumed by *P. roridulae*. Direct carnivory is therefore essentially an impossibility for *R. gorgonias*, which means that the nitrogen uptake demonstrated by isotope analyses must occur through some indirect route. Carlquist (1976) suggested that *R. gorgonias* might benefit from prey nitrogen when prey laden leaves fall to the ground. Leaf turnover was found to be high (Fig. 6B) and leaf fall does, therefore, provide a possible yet indiscriminate nitrogen source. However, the majority of nitrogen in carcasses attached to falling leaves is likely to have been consumed by hemipterans (Fig. 2). Thus the benefit derived from prey *per se*, via leaf fall, is likely to be low. Another possibility is that *R. gorgonias* plants obtain nutrients indirectly after bacterial or fungal digestion of trapped invertebrates. This mechanism has been suggested for the majority of carnivorous plants, notably the pitchers *Sarracenia* and *Darlingtonia* (Lloyd 1942) and *Byblis*, a plant very similar in gross morphology to *Roridula* (Juniper et al. 1989). However, prey must remain on the plant for some time in order for bacterial and fungal digestion to take place. In *R. gorgonias* the rapid consumption of trapped prey by *P. roridulae* renders this route unlikely, which leads to the suggestion of the direct involvement of *P. roridulae* in the nitrogen nutrition of *R. gorgonias*.

The excreta route hypothesis

I propose that *R. gorgonias* acquires prey nitrogen indirectly through the faeces of *P. roridulae*. Invertebrates trapped on the *R. gorgonias* leaves are consumed by *P. roridulae*. Prey tissue then passes through the hemipteran digestive system and in so doing is converted

into a form which *R. gorgonias* can utilize. A portion of the prey nutrients are deposited on the leaf surface in the form of hemipteran faeces, which are then absorbed by *R. gorgonias*. I am therefore effectively proposing that *P. roridulae* functions as the "digestive system" of *R. gorgonias*.

This hypothesis is supported by the results of the isotope labelling experiments. Significant differences in enrichment existed between exclusion and non-exclusion treatment plants i.e. the absorption of prey nitrogen by *R. gorgonias* plants decreased significantly when hemipterans were absent (Fig. 4). This suggests the direct involvement of hemipterans in nutrient uptake. Furthermore, the results of the excreta route test demonstrate conclusively the involvement of hemipterans and more particularly the absorption of nitrogen from hemipteran faeces by *R. gorgonias* (Fig. 5). The excreta route test was designed such that the only means by which *R. gorgonias* could have acquired enriched nitrogen was through hemipteran excretory products.

Hemipterans were found to produce two types of excreta; a solid, relatively dry faeces (Fig. 6F and 6G) and a liquid excreta not unlike the "honeydew" produced by fluid-feeding herbivorous hemipterans (Chapman 1969, Woodward et al. 1970). Both could potentially provide a nitrogen source for *R. gorgonias*. The latter, which is produced soon after or even during feeding (pers. obs.), seems the more likely source as its liquid form makes absorption by the leaves feasible. However, in the moist, misty climate characteristic of *R. gorgonias* habitats it is possible that solid faeces would be dissolved during moisture condensation on the leaves thus also allowing foliar absorption of their nutrient load. Both faeces types appear to be preferentially deposited on the undersurface of the leaves, probably because of the relative sparcity of mucilage secreting trichomes on this surface (pers. obs., Fig 6F and 6G). The leaf undersurface lacks sessile absorptive glands (Lloyd 1934) and a cuticle and numerous stomata are present (Fig 6G and 6H). Thus faecal nutrient uptake must occur either through stomata or through the more resistant cuticle. Both these nutrient uptake routes are known to occur in many plants eg. phyllosphere nitrogen fixers (Dobereiner 1983). It is therefore feasible that foliar uptake of nutrients from animal faeces may not be restricted to *R. gorgonias*, but may represent a means of supplementing nutrient uptake in many plants.

The *R. gorgonias-P. roridulae* interaction represents an intricate nutritional mutualism. *P.*

roridulae benefits from prey captured by *R. gorgonias* and *R. gorgonias* benefits from the digestion of prey by *P. roridulae*. It constitutes a processing chain interaction i.e. an interaction which involves the passage of a resource through a sequence of condition changes over time (Heard 1994a and 1994b). Each consumer is specialized to the resource in a particular condition i.e. *P. roridulae* to trapped invertebrates and *R. gorgonias* to processed prey or faeces. Their dynamics are intricately linked even though they do not compete directly. Heard (1994a) suggests that interactions of this nature must be either amensal (-,0) or commensal (+,0). Although the nutritional interaction between *R. gorgonias* and *P. roridulae* is mutualistic, each link in the chain is commensal, supporting Heard's (1994a) view (eg. *P. roridulae* benefits directly from prey capture, *R. gorgonias* is unaffected).

Heard (1994b) reports a processing chain commensalism between midge and mosquito associates of *Sarracenia* pitchers. The interaction between pitchers and their associated fauna represents the closest reported parallel to the *R. gorgonias-P. roridulae* interaction. Both *Sarracenia* and *Darlingtonia* pitchers appear not to secrete enzymes, but are instead primarily reliant on bacterial enzymes for prey digestion (Juniper et al. 1989). Also, macro-invertebrate associates (chironomid midges) are known to aid in the digestion of prey. They are, however, considered to be commensal and are not essential to the plants survival (Juniper et al. 1989). The fact that exclusion treatment plants showed some ^{15}N enrichment suggests that *R. gorgonias* may not be dependent on *P. roridulae* for prey nutrient uptake (Fig. 3 and 4). I, however, suggest that it is. Firstly, exclusions were not 100% efficient (see Methods, Feeding experiments) which would explain why some enrichment did occur. Secondly, no other feasible routes of prey nutrient uptake are apparent. Finally, *R. gorgonias* seedlings are difficult to grow in captivity (Kirstenbosch Bot. Gardens; J. Jameson, pers. comm.) which I suggest is due to the absence of hemipterans on these plants. Further investigation is required to resolve this important aspect of the *R. gorgonias-P. roridulae* interaction.

Extent of the mutualism

The interaction between *R. gorgonias* and *P. roridulae* extends beyond the nutritional aspects described above. The most striking adaptation of *P. roridulae* to life on the resin coated *R. gorgonias* leaves is the ease with which it moves about. The ability to live on densely hairy and viscid-hairy plants is characteristic of Miridae of the subtribe Dicyphini to which *P.*

roridulae belongs (Dolling and Palmer 1991). Southwood (1986) attributes this ability to various factors: the pretarsal configuration of this group allows them to grasp trichomes by their non-viscid stalks; they are in the habit of holding their body well clear of the plant; they possess behavioural methods of removing trapped tarsi from the resinous trichomes and they groom frequently. Lloyd (1934) additionally suggests that the numerous bristles (setae) covering *P. roridulae* and the sharp tips of its limbs present a small surface area to the resinous leaf surface, decreasing the chances of them becoming trapped and making escape possible. Also, hemipteran instars appear to use their probosces to free entrapped limbs (pers. obs.) which supports the notion that they may produce substances capable of degrading the resins of *R. gorgonias* (Lloyd 1934).

Adult hemipterans were observed to probe the leaf surface with their probosces. The possibility therefore exists that they may utilize plant material directly, possibly as a source of water and carbon compounds. This is contrary to the view of Lloyd (1934) who believed that the only food acquired directly from the plant comes from the flower. All flowers observed were frequented by young hemipterans which did appear to be feeding on the anthers as previously suggested by Marloth (1910). Furthermore, a feeding heirachy appears to exist in *P. roridulae* colonies, young hemipterans only feeding on trapped prey once adults and later instars had vacated it (Fig. 2). Thus, plant material may serve as an important food supplement especially for young hemipterans.

The presence of hemipterans in the flowers of *R. gorgonias* prompted Marloth (1903) to suggest that they function as pollinators. Although young hemipterans do carry *R. gorgonias* pollen (pers. obs.), their apparent inability to move between plants would make them very inefficient pollinators. Also, the large conspicuous flowers of *R. gorgonias* (Fig. 6B) seem redundant in the event that *P. roridulae* is the primary pollinator (Obermeyer 1970). Yet despite their apparent attractiveness, no insects other than *P. roridulae* were observed on the flowers. Thus it appears likely that *P. roridulae* is a pollinator of *R. gorgonias*. Whether it is an efficient primary pollinator has yet to be resolved.

Evolution of the interaction

P. roridulae benefits from the association with *R. gorgonias* in two main ways: *R. gorgonias*

provides both a predator free environment in which to live and reproduce and a regular food source which can be efficiently acquired at low energetic cost (Dolling and Palmer 1991). Thus the selective forces driving the hemipteran adaptations described above (i.e. the ability to move unhindered on the *R. gorgonias* leaf surface) are obvious. The selective pressures causing *R. gorgonias* to maintain the interaction are, however, less clear. It would seem that the "excreta route" is a less efficient means of acquiring prey nutrients than through the conventional use of digestive enzymes (see Discussion, Is *R. gorgonias* carnivorous?, Isotopic evidence for carnivory, Labelling experiments). However, the use of *P. roridulae* as a "digestive system" does eliminate the nutrient and metabolic costs of producing digestive enzymes and associated glands. Whether this outweighs the losses to the "excreta route" is unknown.

This apparent discrepancy in selective pressures may be a product of the evolutionary pathway which led to the modern-day interaction. I envisage the following scenario for the evolution of the *R. gorgonias*-*P. roridulae* interaction. The ancestral lineage of *R. gorgonias* presumably consisted of plants clothed in glandular trichomes as a defense against herbivores, a strategy not uncommon in modern plants (Southwood 1986). As these defences became better developed insects were trapped and even killed, a situation which Juniper et al. (1989) consider to be an evolutionary prelude to carnivory. Ancestors of the hemipteran subtribe Dicyphina were concurrently evolving the ability to live on viscid, hairy plants, a characteristic of all extant members of this group (Dolling and Palmer 1991). As insect capture by the *R. gorgonias* stock became more regular a switch from herbivory to carnivory in early *P. roridulae* stock seems likely. Such diet changes are known to be widespread (Mattson 1980). The utilization of trapped insects by hemipterans would effectively block the pathway to the evolution of carnivory in *Roridula*. The selective pressure to evolve a means of digesting and absorbing animal tissue is non-existent if all trapped invertebrates are rapidly consumed by hemipterans. Also, no pressure would exist to evolve improved insect trapping ability. However, faeces of the recently evolved carnivorous hemipterans are likely to have resembled that of their herbivorous counterparts i.e. liquid "honeydew" (Woodward et al. 1970). Faeces of this type could be easily utilized by the plants. This, together with the possibility that hemipterans may have been involved in pollination, provides the selective pressure necessary for *R. gorgonias* to maintain the symbiosis. Adaptation would have resulted, firstly to ensure the permanent residence of the hemipterans on the plant and

secondly, to increase trapping ability in order to nourish both members of the mutualism, resulting in the extant situation described above.

P. roridulae is known to be obligately associated with *R. gorgonias* possibly because it has not evolved the ability to trap and kill prey independently of the plant (Dolling and Palmer 1991). I have previously speculated as to whether *R. gorgonias* is obligately dependent on *P. roridulae*, and suggest dependence on *P. roridulae* for carnivory i.e. prey nutrient uptake and possibly survival (see Discussion, Excreta route test). The *R. gorgonias-P. roridulae* interaction is therefore an intricately evolved obligate nutritional mutualism and not a commensalism as was previously the belief (Marloth 1903, Lloyd 1934).

This remarkable mutualism may not be unique. The closely related *R. dentata* (Planchon)-*P. marlothi* (Poppius) pair are likely to display similar nutritional dynamics, although this case is further complicated by the presence of several crab spider species (*Synaema*) on the plant (Marloth 1903), which are most likely parasitic on the mutualism. Also, the nutrition of the Australian Byblidaceae may be similar. The genus *Byblis* resembles *Roridula* in that it does not manufacture digestive enzymes (Juniper et al. 1989) and supports obligate hemipteran colonies (Lloyd 1942, Lamont 1994). *Byblis* is currently regarded as truly carnivorous and is thought to derive prey nutrients indirectly through fungal activity (Juniper et al. 1989). I, however, suspect that the nutritional dynamics of *Byblis* may resemble those of *R. gorgonias* closely i.e. carnivorous, primarily deriving prey nutrients indirectly through its mutual hemipteran symbionts.

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REFERENCES

Carlquist, S. 1976. Wood anatomy of Roridulaceae: Ecological and phylogenetic implications. Am.J.Bot. 63(7): 1003-1008.

Chapman, R.F. 1969. The Insects: Structure and function. The English Univ. Press Ltd. p56-70.

Clancy, F.G. & M.D. Coffey. 1977. Acid phosphatase and protease release by the insectivorous plant *Drosera rotundifolia*. Can.J.Bot. 55: 480-488.

Darwin, C. 1875. Insectivorous plants. John Murray, London.

Deleens, E., J.-B. Cliquet & J.-L. Prioul. 1994. Use of ^{13}C and ^{15}N plant label near natural abundance for monitoring carbon and nitrogen partitioning. Aust. J. Plant Physiol. 21: 133-146.

Dixon, K.W., J.S. Pate & W.J. Bailey. 1980. Nitrogen nutrition of the tuberous sundew *Drosera erythrorhiza* with special reference to catch of arthropod fauna by its glandular leaves. Aust. J. Bot.: 283-297.

Dobereiner, J. 1983. Dinitrogen fixation in rhizosphere and phyllosphere associations. In: Pirson, A. & M.H. Zimmerman (Eds). Encyclopaedia of Plant Physiology. New series Vol. 15A. Springer-Verlag, Berlin. p 330-351.

Dolling, W.R. & J.M. Palmer. 1991. *Pameridea* (Hemiptera: Miridae): predaceous bugs specific to the highly viscid plant genus *Roridula*. Syst.Entom. 16: 319-328.

Fiedler, R. & G. Proksch. 1975. The determination of nitrogen-15 by emmission and mass spectrometry in biochemical analysis: A review. Anal.Chem.Acta 78: 1-62.

Friday, L. & C. Quarmby. 1994. Uptake and translocation of prey derived ^{15}N and ^{32}P in *Utricularia vulgaris*. New Phytol. 126: 273-281.

Givnish, T.J., E.L. Burkhardt, R.E. Happel & J.D. Weintraub. 1984. Carnivory in the bromeliad *Brocchinia reducta*, with a cost/benefit model for the general restriction of carnivorous plants to sunny, moist, nutrient-poor habitats. Am.Nat. 124(4): 479-497.

Green, S., T.L. Green & Y. Heslop-Harrison. 1979. Seasonal heterophylly and leaf gland features in *Triphyophyllum* (Dioncophyllaceae) a new carnivorous plant genus. Bot.J. Linn.Soc 78: 99-116.

Handley, L.L. & Raven J.A. 1992. The use of natural abundance of nitrogen isotopes in plant physiology and ecology. Plant, Cell & Environ. 15(9): 965-985.

Harvey, W.H. 1868. The genera of South African plants, ed. 2. Juta, Cape Town.

Hauck, R.D. 1982. Nitrogen-isotope-ratio analysis. In: A.L. Page (Ed). Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties-Agronomy monograph no. 9 (2nd edition). p735-779.

Heard, S.B. 1994a. Processing chain ecology: resource condition and interspecific interactions. J.Animal.Ecol 63: 451-464.

Heard, S.B. 1994b. Pitcher-plant midges and mosquitoes: a processing chain commensalism. Ecology 75(6): 1647-1660.

Heslop-Harrison, Y. & R.B. Knox. 1971. A cytochemical study of the leaf-gland enzymes of insectivorous plants of the genus *Pinguicula*. Planta 96: 183-211.

ISODAT. 1994. Isodat operating manual version 5.1. Finnigan MAT.

Juniper, B.E., R.J. Robins & D.M. Joel. 1989. The carnivorous plants. Academic Press, London.

Lamont, B.B. 1994. Triangular trophic relationships in Mediterranean-climate Western Australia. In: M. Arianoutsou & R.H. Groves (Eds). Plant-Animal Interactions in

Mediterranean-type Ecosystems. Kluwer Academic Publishers, Netherlands. p83-89.

Lloyd, F.E. 1934. Is *Roridula* a carnivorous plant? Can.J.Res 10: 780-786.

Lloyd, F.E. 1942. The carnivorous plants. The Ronald Press Co., New York.

Marloth, R. 1903. Some recent observations on the biology of *Roridula*. Ann.Bot 17: 151-157.

Marloth, R. 1910. Further observations on the biology of *Roridula*. Trans.Roy.Soc.S.A. 2: 59-61.

Marloth, R. 1925. The flora of South Africa. Vol. 2. Darter Bros & Co., Cape Town.

Mattson, W.J. 1980. Herbivory in relation to plant nitrogen content. Ann.Rev.Ecol.Syst. 11: 119-161.

Obermeyer, A.A. 1970. Roridulaceae. In: Codd, L.E., B. DeWinter, D.J.B. Killick & H.B. Rycroft (Eds). Flora of Southern Africa 13. Kirstenbosch. p201-204.

Peterson B.J. & B. Fry. 1987. Stable isotopes in ecosystem studies. Ann.Rev.Ecol.Syst. 18: 293-320.

Robards, A.W. & A.J. Wilson (Eds). 1993. Procedures in Electron Microscopy, Basic Biological Preparation techniques for SEM. John Wiley and Sons Ltd. p11:2.1-11:4.1.

Schulze E.-D, G. Gebauer, W. Schulze & J.S. Pate. 1991. The utilization of nitrogen from insect capture by different growth forms of *Drosera* from Southeast Australia. Oecologia 87: 240-246.

Shearer, G. & D.H. Kohl. 1986. N_2 fixation in field settings: estimations based on natural ^{15}N abundance. Aust.J.Plant Physiol. 13: 699-756.

Southwood, T.R.E. 1986. Plant surfaces and insects - an overview. In: Juniper, B. & T.R.E. Southwood. Insects and the plant surface. London. p1-22.

Thum, M. 1989. The significance of oppurtunistic predators for the sympatric carnivorous plant species *Drosera intermedia* and *Drosera rotundifolia*. Oecologia 81: 397-400.

Verbeek, N.A.M. & R. Boasson. 1993. Relationship between types of prey captured and growth form in *Drosera* in southwestern Australia. Aust.J.Ecol. 18: 203-207.

Woodward, T.E., J.W. Evans & V.F. Eastop. 1970. Hemiptera. In: CSIRO. The Insects of Australia. Melbourne Univ. Press. p387-458.

Zamora, R. 1990a. The feeding ecology of a carnivorous plant (*Pinguicula nevadense*): prey analysis and capture constraints. Oecologia 84: 376-379.

Zamora, R. 1990b. Observational and experimental study of a carnivorous plant - ant kleptobiotic interaction. OIKOS 59: 368-372.

Appendix 1: Calculations of the amount of insect nitrogen entering the vegetative tissue of fed leaf clusters of *R. gorgonias*. Data for inflorescences, stems, roots and leaf clusters other than that fed labelled flies were unavailable and therefore total nitrogen budgets could not be calculated. Weights are dry weights based on few samples (n=1 for both flies and hemipterans). Also yield data are based on few samples (n=1 for flies and hemipterans). Thus the extrapolations involved in these calculations are likely to lead to inaccuracy and results should thus only be regarded in their broadest sense.

^{15}N present in applied flies

- fly enrichment = 90 atom % (assumed)
- fly yield = 9.2 μmol $N.mg^{-1}$ (from N_2 gas pressure)
  = 128.8 $\mu gN.mg^{-1}$ (9.2 * N atomic mass)
  = 0.1288 $mgN.mg^{-1}$Fly
- fly weight = 0.185 mg

But, 6 flies were fed to each leaf rosette, therefore:

- fed fly weight = 1.11 mg (6 * fly weight)
- total fed N = 0.1430 mgN (fed fly weight * fly yield)
- ^{15}N per feed = 0.1287 $mg^{15}N$ (total fed N * fly enrichment)

^{15}N present in a leaf cluster from the non-exclusion treatment

1. Mature leaves:

- leaf enrichment = 0.43350 APE (Table 3)
- leaf yield = 0.8 μmol $N.mg^{-1}$ (from N_2 gas pressure)
  = 11.2 $\mu gN.mg^{-1}$ (0.8 * N atomic mass)
  = 0.0112 $mgN.mg^{-1}$leaf
- leaf weight = 18.0 mg

The mean number of mature (actively trapping) leaves per leaf cluster is 24.84 (see Methods,

Prey composition and catch rate). Therefore:

- total leaf weight = 447.12 mg (24.84 * leaf weight)
- total leaf N = 5.0077 mgN (total leaf weight * leaf yield)
- ^{15}N excess = 0.0217 mg^{15}N (total leaf N * leaf enrichment)

2. Apical bud and associated new leaves:

- bud enrichment = 0.19975 APE (Table 3)
- bud yield = 0.0112 mgN.mg^{-1}leaf (as for mature leaves)
- bud weight = 44 mg
- total bud N = 0.4928 mgN (total bud weight * bud yield)
- ^{15}N excess = 0.0010 mg^{15}N (total bud N * bud enrichment)

- Total ^{15}N excess = 0.0227 mg ^{15}N (mature leaves + apical bud)

^{15}N present in hemipterans from fed leaf cluster

- hemipteran enrichment = 3.62537 APE (Table 3)
- hemipteran yield = 8.2 μmol N.mg^{-1} (from N_2 gas pressure)
  = 114.8 μgN.mg^{-1} (8.2 * N atomic mass)
  = 0.1148 mgN.mg^{-1}hemipteran
- hemipteran weight = 0.84 mg
- total hemipteran N = 0.0964 mgN (hemipteran weight * yield)
- ^{15}N excess = 0.0035 mg^{15}N (total hemipteran N * enrichment)

On average 7.19 hemipterans are present on each leaf cluster (Results, Hemipteran observations).
Therefore, total hemipteran ^{15}N excess = 0.0252 mg^{15}N

Summary

Total ^{15}N in flies = 0.1287 mg

^{15}N excess in leaf cluster = 0.0227 mg

^{15}N excess in hemipterans = 0.0252 mg

Therefore,

% of fly N in leaf cluster = 17.64% (^{15}N excess / fly ^{15}N)

% of fly N in hemipterans = 19.58%

Thus 3 days after feeding 17.64% of applied nitrogen was present in the leaves of the fed leaf clusters of non-exclusion treatment plants.