Differential Food Preferences in Three Co-Occurring Species of Tisbe (Copepoda, Harpacticoida)

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ABSTRACT: Killed samples of a monoclonal bacterial strain and an axenic alga were offered in different proportions to Tisbe holothuriae, T. battagliai and T. furcata. Incorporation of carbon ranged from 0.05 to 0.39 µg C (adult male)^-1 d^-1. Using a radiolabelling technique, the ratio in which the two food types were assimilated was compared to the ratio in which they were offered. Neither T. holothuriae nor its sibling species fed selectively under our conditions, but T. furcata showed a clear preference for bacteria. These findings constitute the first evidence for intrageneric dietary differentiation in harpacticoids. The results of the feeding experiments parallel observed differences in mandible structure. They render all the more acute the problem of resource partitioning between T. holothuriae and T. battagliai.

INTRODUCTION

Traditionally, phytal and epibenthic harpacticoids have been collectively considered to feed on 'microalgae, bacteria and detritus' (e.g. Noodt, 1971; Coull, 1973). Such a generalization obviously does not imply that all species are indiscriminate feeders - much as the 'year-round reproduction' of meiofaunal copepods was shown not to imply that all, or even most, species behave as generalists along the time axis of the niche (Coull and Vernberg, 1975). Indeed, recent reviews (Coull, 1973; Lee et al., 1975) surmise that many meiofaunal organisms are specialists with regard to nutritional requirements, nutritional preferences, and ability to utilize specific food species - a point already forcefully made by Provasoli et al. as early as 1959. So even now, 'we still do not know exactly what the meiofauna consume' (Coull and Bell, 1979).

A particularly promising test system to tackle the question of food preferences is provided by the genus Tisbe (Copepoda, Harpacticoida) which occurs as multispecies assemblages in coastal ecosystems all around the world (Fava and Volkman, 1975, 1977; Bergmans, 1979). Table 1 lists published information on food known to be accepted by several Tisbe species (considering only those reports where food sources were adequately checked). The situation is rendered more complicated, as well as more interesting, by the fact that the genus abounds in morphologically similar and even sibling species, which has resulted in scores of misidentifications in both the ancient and recent literature (Volkman-Rocco, 1971). Is Tisbe a guild of generalists or of specialists? In spite of reports of a broad dietary spectrum (Battaglia, 1970) there are some indications in favor of the latter possibility. Lwoff (1927) and Rieper (1978) found that some Tisbe can thrive on a diet of pure bacteria, whereas Betouhim-El and Kahan (1972) reported that T. pori can subsist on any of 9 algae, but none of 6 bacterial strains. Strangely enough, controlled choice experiments involving Tisbe have never been carried out. In this paper we investigate the possibility of (differential) food selection in three sympatric, well-identified (Bergmans, 1979) species. Our preference experiments involved bacteria and 14C-labelled Dunaliella, offered in different relative concentrations.

MATERIAL AND METHODS

Copepods. Tisbe holothuriae Humes, 1957, T. battagliai Volkman-Rocco, 1972 and T. furcata (Baird, 1837) were obtained from the Spuikom, a sluice dock near the Ostend harbor (Flemish Coast). Mass cultures (2,000 ml vessels containing macroalgae and detritus, receiving 50 ml of dense Dunaliella culture every
of the scintillation count was higher when the activity (scintillation counter). A preparatory experiment had shown that the efficiency of the scintillation count was higher when the

**Table 1. Known food sources for Tisbe species.**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Bacteria</th>
<th>Microalgae</th>
<th>Plant detritus</th>
<th>Animal detritus</th>
<th>Living animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controlled*</td>
<td>4, 9, 11</td>
<td>3, 4</td>
<td>13</td>
<td>6, 7, 8, 9</td>
<td>5, 10</td>
</tr>
<tr>
<td>Not controlled</td>
<td>2, 6</td>
<td>1, 2, 6, 8, 9, 12</td>
<td>6, 7, 8, 9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Studies which have unequivocally demonstrated that Tisbe fed on the food source mentioned rather than on the associated microflora.

3rd d) were set up in September 1976 for T. holothuriae and T. battagliai, and in October 1977 for T. furcata. The experiments were carried out between April and July, 1978. To ensure the availability of animals, single families were subcultured by letting gravid females deposit 1 egg sac per 10-ml Petri dish. Food at this stage was 1 ml of non-axenic Dunaliella added every 2nd or 3rd d. Before each experiment, some 400 adult males were collected from the different dishes and rinsed twice in filtered seawater to remove contaminant ciliates and flagellates.

As a result of our subculturing procedure, the Tisbe males in each experiment constituted a single age group rather than a random sample with respect to age. This circumstance may have affected the absolute quantity of food assimilated (which varied rather much between, though not within, different experiments). It did not influence the selective uptake of algae and bacteria — the object of our investigation — which turned out to be quite reproducible.

**Algae.** Axenic Dunaliella tertiolecta Butcher, strain 19/6b (9.5 µm) of the Cambridge Culture Centre of Algae and Protozoa, was grown on Medium ASP2 (Provasoli et al., 1957). Two d before a feeding experiment took place, 10 µCi of NaH¹⁴CO₃ was added to 250 ml of an exponentially growing culture. Immediately before the experiment, the algae were killed by a temperature shock (10 min at 50 °C), washed twice by centrifugation (1,500 rpm) and resuspended in filtered seawater to remove all traces of dissolved radioactivity. (A control experiment had shown that dead algae do not lose any significant amount of radioactivity over 24 h). The approximate carbon content was then estimated spectrophotometrically at 660 nm, using a previously established calibration curve. Two 1 ml samples were taken for subsequent determination of total organic carbon (T.O.C. analyser) and specific radioactivity (scintillation counter).

**Bacteria.** We used an unidentified rod-shaped bacterium (4 µm) isolated from the Spuikom sediment and grown on liquid medium Marine Broth (Difco). As with the algae, we preferred to use a monoclonal stock to improve reproducibility. Treatment was the same as for the algae, except that here no radioactive marker was added; the washing was done to remove undesirable dissolved organic matter.

**Feeding experiments.** On the basis of the estimated carbon content, bacteria and algae were diluted to a concentration of 50 µg C ml⁻¹. The two cultures were then mixed in different approximate a:b ratios, usually 1:3, 1:1 and 3:1. Using a micropipette, 1 ml of a particular mixture was gently deposited at the bottom of a Petri dish containing 8 ml of filtered seawater (0.8 µm) and sterilized seawater. Food was thus offered in excess, as preference is only expressed when density of preferred food is not limiting (Sih, 1979). Other dishes were prepared with bacteria or algae alone. Of each dish type several replicates (usually 4) were made; to each dish were added 20 adult Tisbe males. Two 1:1 dishes received 20 copepods killed previously by means of a temperature shock (5 to 10 min at 40 °C).

All dishes were left to stand for 24 h at 18 °C. At the end of the experiment, they were emptied over a 50 µm mesh width gauze. After rinsing with 20 ml of seawater, the gauze was reimmersed and the Tisbe transferred onto a cellulose acetate filter. Individuals that had died in the course of the experiment were not used. Overall mortality in the course of our experiments was low (4.5 %) so that stress due to manipulations appears to have been limited. Once dry, each filter was dissolved in 1 ml of 'Lumaseve', with 0.1 ml of H₂O₂ to decolor the whole. The scintillation vials were left to stand for 12 h; 10 ml of 'Lumagel', a standard scintillation liquid, and 40 µl 15 % ascorbic acid were then added. Scintillation counting was done after 1 week. A preparatory experiment had shown that the efficiency of the scintillation count was higher when the
copepods were transferred to the lumasolve on filters rather than in water. The experiment was repeated twice for *T. battagliai* and *T. holothuriae*, three times for *T. furcata*.

The use of dead bacteria and algae in the preference experiments had a three-fold advantage. First, it excluded colonisation of the copepod cuticles by bacteria and algae. (To use living food and a control with dead *Tisbe* would have been no solution, as the cuticle of a dead organism appears to be colonised much more quickly than that of a living one). Second, this procedure ruled out the occurrence of changes in specific radioactivity as a consequence of algal and bacterial metabolism during the experiment. Third, the difference in attainability between algae and bacteria was minimized. *Tisbe* is a raptorial bottom dweller (Marcotte, 1977). The bacterial strain used in the experiments showed the tendency to settle to the bottom, while living *Dunaliella* remain in suspension. The use of freshly killed rather than living food organisms is hardly likely to affect the significance of our conclusions for the natural situation; in particular, if *Tisbe* feeds on phytoplankton *a: all in situ*, it will almost certainly be on senescent and dead cells settled to the bottom. Also, Lwoff (1927) and Rieper (1978) successfully used dead bacteria as a food source for *Tisbe*.

**Calculations.** Quenching corrections were made according to the external standard method. In each experiment, the mean CPM (counts-per-minute) value of the series without radioactivity (i.e. the series where only bacteria were added) served as a blank to be subtracted from the other measurements. Next, all

<table>
<thead>
<tr>
<th>Species</th>
<th>Carbon added</th>
<th>Carbon assimilated</th>
<th>Average Estimated % algal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Algal (μg)</td>
<td>Total (μg)</td>
<td>% algal</td>
</tr>
<tr>
<td><em>T. holothuriae</em>,</td>
<td>31</td>
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<td>30</td>
</tr>
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<td>88</td>
<td>50</td>
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<td></td>
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<td>100</td>
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<td></td>
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<tr>
<td><em>T. battagliai</em>,</td>
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</table>

* Value excluded from calculations
values were converted to 24 h and 1 individual. All uptake of radioactivity not resulting from ingestion will be measured in dead animals also. A preliminary experiment had shown this uptake to be approximately proportional to the amount of radioactivity added and to the number of animals in the dish. Therefore the mean CPM value of the dishes with killed animals was weighted by the different concentrations of radioactive algae offered and subtracted from the respective measurements. Mean uptake (in CPM) in the 'pure algae' replicates was then calculated.

The CPM value for each dish was divided by the mean uptake of algae in the series where only algae were offered to obtain a measure of the proportion in which algae and bacteria had been assimilated. In doing so, we assumed that the total amount of carbon ingested was the same for all animals in one experiment, i.e. we assumed the uptake of unlabeled (bacterial) carbon to make up for the difference between measured uptake in the 'pure algae' and 'mixed food' treatments. The exact proportion in which the two food sources were offered was calculated from the total organic carbon contents as measured with the T.O.C. analyser.

In an approach inspired by Emlen (1966), we plotted percentage algal carbon offered along the abscissa and percentage algal carbon assimilated along the ordinate. Experimental points near the bisector of this graph indicate no selection, i.e. the proportion in which algal carbon was ingested equals the proportion in which it was offered. Points below or above the bisector indicate preferential uptake of bacteria and algae, respectively.

Finally, using the T.O.C. and scintillation data for the algal culture, the amount of algal carbon ingested was calculated.

RESULTS

Results are presented in Figures 1–3 and Table 2. We observed a range of assimilation values of 0.18–0.39 µg C copepod⁻¹ d⁻¹ for *Tisbe holothuriae* and *T. battagliai*, 0.05–0.12 µg C copepod⁻¹ d⁻¹ for *T. furcata*. This range narrowly overlaps that measured by Rieper (1978) on *T. holothuriae* (both sexes) in a
similar short-term radiolabeling experiment, as she observed a maximum of 0.07 µg C copepod⁻¹ d⁻¹.

Whereas the sibling species Tisbe holothuriae and T. battagliai behaved as indiscriminate feeders under our experimental conditions, we found T. furcata to show a clear preference for bacteria. This is evident from the strongly nonlinear relationship between the proportion of algae offered and assimilated, respectively (compare Emlen, 1966). Tisbe is able to actively discriminate between food items, as shown by the 'rejection reaction' observed by Marcotte (1977).

**DISCUSSION**

Food preferences. Preferential uptake of heterotrophic carbon was mentioned by Brown and Sibert (1977) for mixtures of harpacticoids, including 'Tisbe furcata'. Our results indicate that it would be unwise to extrapolate this to all Tisbe, let alone to all harpacticoids. Apart from our own study, however, there exists but a single hint at species-specific food preferences pertaining to harpacticoids: the beautiful food-patch choice experiment of Lee et al. (1977) on Nitocra typica and Leptocaris brevicornis, offered 8 species of algae. Different food preferences are probably related to different nutritional values, a topic on which some more information is available (Provasoli et al., 1959; Shiraishi and Provasoli, 1959; Betouhim-El and Kahan, 1972; Lee et al., 1976; Sellner, 1976; Rieper, 1978), though some of the evidence is open to serious criticism. (Provasoli et al.'s experiment entailed massive inbreeding and sampling drift. Lee et al. do not state the number of replicates per food source, which seems to have been unity: so the observed differences could very well not reflect nutritional phenomena but extreme sampling error, especially with regard to age-dependent reproductive value (sensu Fisher, 1958) of the females used to start each culture).

Our findings constitute the first evidence for intrageneric dietary differentiation in harpacticoids. The possibility of such differentiation was adumbrated by Provasoli et al.’s (1959) observation that the same axenic strain of Platynonas could support 8 generations of Tigriopus japonicus, but only 2 of T. californicus. Analysing gut contents, Fryer (1957) had demonstrated dietary differentiation among closely related freshwater cyclooids. In his study, as in ours, similarity in diet was correlated with phylogenetic affinity (albeit at a different level of relatedness).

Mandible structure. Differences in the structure of mouthparts, especially concerning form and number of teeth on the mandibles, have often been linked to dietary differences in the Copepoda (Anraku and Omori, 1963; Maly and Maly, 1974; Sullivan et al., 1975; Ivester and Coull, 1977; Turner, 1978). Marcotte (1977), studying the functional morphology of the oral appendages of T. furcata by means of slow-motion videotape, concluded that the mandibles serve to crush the food between them and against the labrum.

One of us (W.V.) studied mandible morphology by light and scanning electron microscopy. Adult males were dissected in glycerine under a stereomicroscope. For SEM, mandibles were transferred to alcohol which could support 8 genera of Sepia eggs (Richard, 1976). Both species may nevertheless be mass-cultured using the same methods as for the other Tisbe.

Ecology of Tisbe spp. Six species of Tisbe occur in the Spuikom (Bergmans, 1979) including 1 recently discovered and as yet unidentified species. T. bulbisetosa and T. gracilis appear to be rare residents. Our preliminary food selection investigations with these species have not yielded unequivocal results. Though it is premature to draw conclusions from this, it may be that our test system is inappropriate for them. Lang (1948) considers that T. gracilis may be a preferential carrion-feeder, a view supported also by the more recent observation that it eats dead Sepia eggs (Richard, 1976). Both species may nevertheless be mass-cultured using the same methods as for the other Tisbe.

One of us (M.B.) has studied the phenology of Tisbe spp. in the Sluice dock during 3 consecutive years, 1975–77. With minor year-to-year variations, T. furcata was observed from December through July, with a March-May spring bloom. This bloom possibly relates to the fact that the sluices connecting the Dock to the Ostend harbor remain closed from March to
October. Planktonic marine bacteria introduced with the last charge of harbor water suffer heavy mortality in the Dock (Podamo, 1972). In the light of our findings, this may provide a rich food source for T. furcata, to which also remains of larger planktonic organisms may contribute (either directly or as a substrate for bacteria). Warwick (unpubl.) has recently observed a similar relationship between the bloom of certain harpacticoid species and mass mortality of specific phytoplanktonic populations.

The sibling species *Tisbe holothuriae* and *T. battagliai* attain their greatest population densities in fall (August—November) and can be observed through February. Data collected so far indicate some measure of spatial segregation over the Sluice Dock as a whole; nevertheless the temporal and spatial niche dimensions of these two species appear exceedingly similar. The situation is certainly reminiscent of the *Enhydrosoma propinquum* – *E. baruchi* coexistence observed by Ivester and Coull (1977) and that of the *Eucyclops* spp. observed by Fryer (1957). *T. holothuriae* and *T. battagliai* in the Sluice Dock are often encountered in the same small-surface sample, a point also made by Fava and Volkman (1975) working on the lagoon of Venice. With respect to these sibling species, our present findings regarding food preference and mandible morphology raise more problems than they resolve.


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