Trophic structure of free-living nematodes in the Saigon River, Vietnam

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Abstract:

Sub-tidal free-living nematodes in 12 stations along the Saigon River were investigated in both dry and rainy seasons during 2014 and 2015. In total, 157 nematode genera were found to belong to 59 families, and 11 orders of two classes of Enoplea and Chromadorea were identified. The trophic structure of free-living nematode communities was analysed spanning the overall seasons to see that deposit-feeders developed dominantly in the Saigon River indicating their association with food types including particles, bacteria, diatom, etc.. The dominance of deposit-feeders was contributed to a high abundance of genera in each station, including *Theristus, Teschellingia, Monhystera*, *Thalassomonhystera*, *Paraplectonema*, *Daptonema*, *Aphanonchus*, *Sphaerotheristus*, and *Geomonhystera*. Epistrate-feeder types were found as the second most abundant group. The remaining feeding types, such as chewers and suction-feeders, were in rather low proportion at most stations.

Keywords: ***free-living nematodes, Saigon River, Trophic structure, Vietnam.***

Classification number: *3.4*

Introduction

The trophic structures and ecological functioning of free-living nematodes in aquatic environment have been well investigated and documented [1-4]. Nematodes have been found to feed on a diversity of food webs, including bacteria, microalgae, fungi, detritus, suspended organic matter, plants, and animal organisms. The first study of nematode feeding types was conducted by Wieser (1953) [4], and was based on the morphological structure and armature of the buccal cavity. In this study, Wieser differentiated four feeding groups based on lateral optical section of the buccal cavity of the fixed specimens of marine nematodes: 1A) selective deposit-feeders with minute-small stoma without teeth, 1B) Non-selective deposit-feeders with somewhat unarmed stoma, 2A) Epistrate-feeders with medium-sized stoma and small teeth, and 2B) Predators/omnivores with large stoma and large teeth/mandibles. This classification has been widely used in analyses on the trophic structure of free-living nematode [5].

Later some modification and development of Wieser's feeding type classification were proposed by Jensen (1987) [1] and Moens & Vincx (1997) [3], and these were mostly according to nematode cultures and food sources. Specifically, Jensen (1987) [1] distinguished four feeding types of free-living aquatic nematodes: 1) deposit-feeders, 2) epistrate-feeders, 3) scavengers, and 4) predators. However, this scheme does not refer to the nematode with stylet as feeding apparatus. While Moens and Vincx (1997) [3] classified the Westerschelde estuarine nematodes into six major feeding guilds: 1) Microvores, 2) ciliated feeders, 3) deposit-feeders, 4) epigrowth-feeders, 5) facultative predators, and 6) predators.

Additionally, Yeates, et al. (1993) [6], described eight trophic groups based on its source of food, such as 1) plant feeders, 2) hyphal feeders, 3) bacterial feeders, 4) substrate ingesters, 5) predators of animals, 6) unicellular eukaryote feeders, 7) dispersal or infective stage of parasites, and 8) omnivores. This classification was suitable to apply to research on nematode trophic structure and feeding habits in soil ecology. Moens, et al. (2004) [7], also differentiated nematode feeding type structure into eight trophic groups: 1) plant feeders, 2) hyphal feeders, 3) bacterial feeders, 4) substrate ingestion, 5) carnivores, 6) unicellular eukaryote feeders, 7) animal parasite, and 8) omnivores. This trophic structure classification was updated for Yeates' system. Some nematode groups may feed on one or more of a type of food source, depending on their

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feeding habits.

Particularly, in order to facilitate the functional role of nematodes in oligotrophic lake sediment and based on morphological characteristics of the buccal cavity in combination with available food, Traunspurger (1997) [8] grouped free-living nematodes into four feeding types: 1) deposit-feeders: without teeth in the buccal cavity, swallowing food and feeding on bacteria and unicellular eukaryotes; 2) epistrate-feeders: possessing small teeth in the buccal cavity, they tear and swallow the food composed of bacteria, unicellular eukaryotes, diatoms and other algae; 3) chewers: with a voluminous, sclerotised buccal cavity with one or more teeth and denticles which feed on predators on protozoa, other nematodes, rotifers, tardigrades and other small animals; and 4) suction-feeders/omnivores: are characterised by the presence of a stylet and are supposedly omnivorous, they pierce different kinds of food (algae, vascular plants, epidermal cells and root hairs, fungi and animals) by their stylet.

Traunspurger's 1997 [8] feeding type classification grouped selective and non-selective deposit-feeders into deposit-feeder similar to Jensen (1987) [1], and subdivided the 2B group of Wieser (1953) [4] into chewers and suction-feeders. Applying this feeding type classification is not only quite simple but also provides full information of nematode ecological role in the benthic food webs.

Therefore, the classification from Traunspurger (1997) [8] was selected to apply for studying on the trophic structure of free-living nematodes in the Saigon River. Objectives of this work are to understand how free-living nematode feeding structures and their feeding diversity in the Saigon River of Vietnam.

Materials and methods

Sampling stations

Sampling field trips were conducted during the dry and rainy seasons of 2014 and 2015 (March 2014, September 2014, and March 2015, September 2015, respectively) at 11 ports along the Saigon River and one reference location in Cu Chi District. These were coded as SG (Saigon River), from SG1 to SG12 respectively: SG1 (Cu Chi District), SG2 (Tan Cang Port), SG3 (Ba Son Shipyard), SG4 (Saigon Port), SG5 (Tan Thuan Dong Port), SG6 (Ben Nghe Port), SG7 (Joint Company of Logistic Development No.1 Port - VICT), SG8 (Saigon New Port), SG9 (Bien Dong Port), SG10 (Saigon Shipbuilding Port), SG11 (Lotus Port), and SG12 (Navioil Port) (Fig. 1).

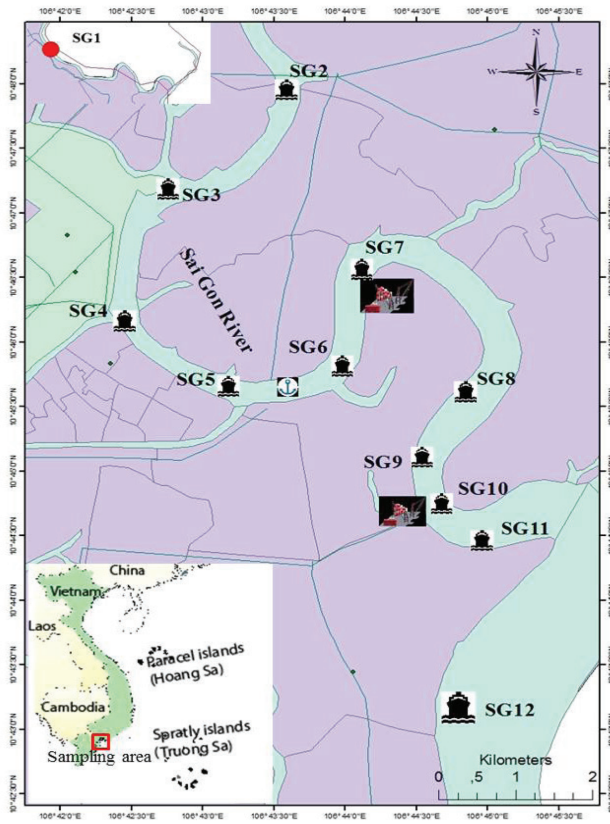

Fig. 1. Sampling stations of free-living nematode communities in study areas.

Sampling collection and laboratory experiments

In each station, sediment samples were collected using a boat with ponar grab, and plastic cores of 3.5 cm diameter (10 cm^2 surface area). The cores were pushed down into the sediment for up to 10 cm deep. At each station, triple samples were taken and put into 150 ml plastic bottles. All samples were then transferred to a laboratory for processing and analysis. In the laboratory sediment samples, the samples were fixed by 7% formalin solution at 60^{o}C temperature, and gently stirred before decantating, extracting, mounting, and making slides according to Smol (2007) [9]. Nematode specimens were identified to a genus level based on classification keys of Warwick, et al. (2005) [10], Zullini (2005) [11], V.T Nguyen (2007) [12], and the NEMYS database of the Ghent University in Belgium [13].

The nematode trophic structure was identified according to Traunspurper (1997) [8], which included four feeding type groups: deposit-feeders, epistrate-feeders, chewers, and suction-feeders based on the buccal cavity structure, and food source, and calculated by percentage. Characteristics of these groups are clearly described in Table 1. This function-based approach offers several advantages: (i) in order to assign a feeding category, it is not necessary to identify specimens to species level, and (ii) it focuses on the ecological role of nematode communities [14].

Table 1. Classification of nematode feeding types according to Traunspurger (1997) [8].

Feeding type	Shape of buccal cavity	Food sources
deposit-feeders (swallowers)	no teeth in buccal cavity	bacteria, unicellular eukaryotes
epistrate-feeders (tear and swallowers)	small teeth present in buccal cavity	bacteria, unicellular eukaryotes, diatoms, other microalgae
chewers	voluminous, sclerotised buccal cavity with one or more teeth and denticles	predators of protozoa, nematodes, rotifers, enchytraeids and tardigrades
suction-feeder	stylet present	omnivorous, algae, plant, fungi and animal

Trophic diversity of Heip, et al. (1985) [15] was applied in order to discover how their diversity:

Trophic index = $\Sigma\theta^2$(total square percentage of feeding types).

Data analysis

Nematode data was processed using Microsoft Excel 2007. Nematode densities were all converted in order to calculate their abundance per 10 cm^2. The significant difference of nematode variables between stations was detected using two-way PERMANOVA analysis. The software PRIMER 6.0 adds on PERMANOVA and STATISTICA 7.0 were applied for significant different test with the number of permutations 9999.

Results

General characteristic of free-living nematode communities

A total of 157 free living nematodes genera were identified over 12 stations within four seasons along the Saigon River. They belonged to 59 families of 11 orders and two classes of Enoplea and Chromadorea. Of these, the highest diversity of nematode genera composition was 61.78% of total belonging to the class Chromadorea. Although the number of taxa in this area was lower in comparison with the adjacent river, the Mekong estuarine system, where 230 nematode genera, 58 families of two classes of Enoplea and Chromadorea were found [16], and were quite higher than the Cua Luc Estuary in North Vietnam [17] with 66 species, 52 genera and 17 families. In a study of Italian contaminated harbours, only 72 genera, and 26 families were reported [18].

In our study, the genera *Parodontophora*, *Terschellingia,* and *Rhabdolaimus* were the most dominant in the river over the seasons. However, some genera showed high densities in only one season: *Daptonema*, *Geomonhystera,* and *Sphaerolaimus* in the 2014 dry season; *Thalassomonhystera* in the 2014 rainy season; *Achromadora*, *Mononchulus* in the 2015 dry season; and *Mesodorylaimus* in the rainy season of 2015. Several genera were abundant in two or the three seasons including *Dorylaimus*, *Diplolaimelloides*, *Ironus*, *Monhystera*, *Mylonchulus*, *Paraplectonema*, *Punctodora*, *Sphaerotheristus, Aphanonchus,* and *Theristus*. This was completely different from the Mekong estuaries where *Halalaimus*, *Rhynchonema*, *Parodontophora*, *Terschellingia*, *Onyx*, *Leptolaimoides*, *Oncholaimellus*, *Omicronema*, *Rhinema*, *Haliplectus*, and *Desmodora* were found to be dominant [5]. According to Heip, et al. (1985) [15], dominant nematode genera in European estuarine rivers such as in Germany, the United Kingdom, Belgium, Finland, South American, the Netherlands, and France were *Adoncholaimus*, *Anoplostoma*, *Axonolaimus*, *Daptonema*, *Leptolaimus*, *Microlaimus*, *Monhystera*, *Metachromadora*, *Ptycholaimellus*, *Sabatieria*, *Theristus*, *Tripyloides*, and *Viscosia*. The difference in the composition and the dominant genera of nematode communities could be explained by different environmental conditions, especially sediment characteristics [16].

Densities of nematode communities ranged from 13.33±2.89 (inds/10 cm^2) at SG4 in the dry season of 2014 to 5863±2396.46 (inds/10 cm^2) at SG8 in the rainy season of 2015. The results indicated that SG8 was occupied the highest densities in 2014 and the rainy season of 2015. The nematode communities at SG8 station during the rainy season of 2015 indicated overall higher densities compared to the other stations (Fig. 2).

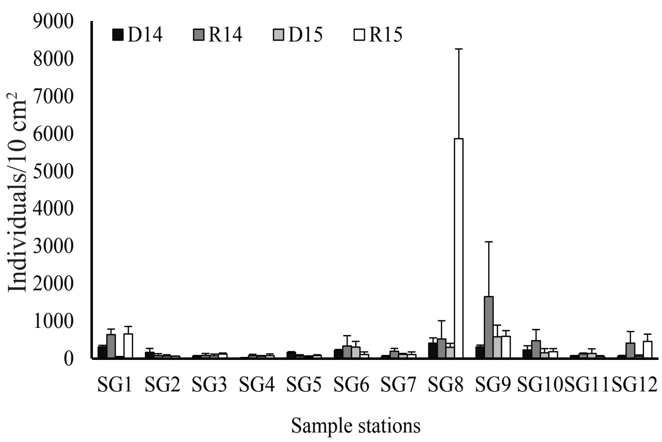

Fig. 2. Densities of nematode communities in the Saigon River.
D: Dry season, R: Rainy season.

The trophic structure of free-living nematode communities

A total of 157 free living nematode genera were identified in Saigon River harbours, and they were classified into four main feeding types according to Traunspurger (1997) [8], which are: deposit-feeders, epistrate-feeders, chewers, and suction-feeders. In which, deposit-feeders prevail in whole communities, with 50 genera (31.85% total), and they also occupied dominantly in all seasons. Particularly, this group prevailed high percentage from 35.56% (SG4) to 95.93% (SG8) in total individuals during the dry season of 2014; from 46.03% (SG1) to 90.94% (SG8) in the rainy season of 2014; from 36.96% (SG2) to 79.95% (SG8) in the dry season of 2015; and from 45.33% (SG4) to 92.67% (SG12) in the rainy season of 2015. Specifically, they were highly dominant over stations during the rainy season of 2014 (46.03% at SG5-90.94% at SG8) (Fig. 3). The highest percentage of deposit-feeders nematode group overall stations indicates that particles, bacteria, diatom, etc., was copious in the sediment [8]. The results of a two-way PERMANOVA analysis for deposit-feeders group showed a significant difference between stations ($p = 0.0007$), but there were no indication in seasonal effect ($p = 0.062$) as well as interaction of seasons and stations ($p = 0.34$).

The dominant trophic groups in the Saigon River related to its genera richness. This was demonstrated by a contributory rate of the dominant genera. During the rainy season of 2014, *Theristus* (occupied 31.7-84.8% total individuals in the stations from SG2 to SG12), *Teschellingia* (42.49% at SG1), and more *Monhystera*, *Thalssomonhystera*, and *Paraplectonema* were the abundant genera. The genera of *Daptonema* (70.38% in total of number individuals at SG6, 88.09% at SG8, and 97.35% at SG7), *Monhystera* (62.98%, 38.66%, 34.99%, and 23.67% correspond to SG12, SG9, SG10, and SG3, respectively), *Terschellingia* (80.55% at SG11, 23.365% at SG9, and 20.45% at SG1), *Aphanonchus* and *Sphaerotheristus* (both equal to 23.9% at SG4), *Geomonhystera* (17.8% at SG2) were found to be high in the dry season of 2014. *Theristus* (at SG1, SG2, and from SG6 to SG12 with 10.55-68.71%), *Terschellingia* (10.24-28.65% at SG4, SG7, SG8, SG11, and SG12), *Sphaerotheristus* (21.16-26.87% at SG3, SG4, and SG10), *Paraplectonema* (20% at SG1), *Aphanonchus* (10.24-22.51% at SG1, SG2, and SG4) were more present in the dry season of 2015. *Theristus* (from SG2 to SG12 with 21.2-87.37%), *Terschellingia* (78.3% at SG1, and 66.65% at SG12), *Aphanonchus* (at SG2 with 12.22%) were dominant in the wet season of 2015. These genera belong to the deposit-feeders group. The high percentage of genera richness lead to the dominated extremely of deposit-feeders group which are without teeth in the buccal cavity, swallowers the foods, feed on bacteria, and unicellular eukaryotes.

The second high percentage of feeding type was epistrate-feeders with 47 genera (29.94%). They also presented quite high percentages in each station. For instance, the station SG5 (in the dry season 2014 with 35.81%, the dry season 2015 with 39.32% and 36.50% in the rainy season 2015), SG1 (40.29% in the dry 2015), and SG7 (32.78% in the rainy season 2015) had the numerous presence of the genera *Parodontophora*, *Rhabdolaimus*, *Punctodora*, *Achromadora*, *Udonchus*, and *Simanema*. The epistrate-feeding nematodes had a small tooth in the buccal cavity, they tear and swallow the foods, feed on feed on bacteria, unicellular eukaryotes, diatoms and other algae. There was a significant difference between stations for feeding epistrate-feeders (Two-way PERMANOVA of $p = 0.48$). Nevertheless, it was not found in season ($p = 0.501$) and interaction between seasons and stations ($p = 0.7$)

For chewers and suction-feeders, they contributed a very low percentage to the communities (Fig. 3). However, the results of the two-way PERMANOVA analysis showed some interesting features in these groups. The relative abundance of chewers were found significant differences in both stations ($p = 0.0034$) and seasons ($p = 0.0003$). In contrast, only suction-feeders were found to be significantly different between seasons ($p = 0.0036$), but not in stations ($p = 0.073$) and two factors interaction ($p = 0.078$).

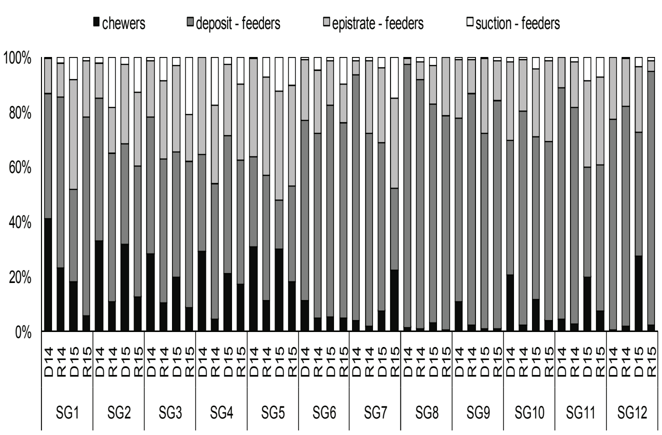

Fig. 3. A percentage of free-living nematode communities in the Saigon River in dry (D) and rainy (R) season from 2014-2015.

The trophic index of free-living nematode communities in the Saigon River during four sampling time ranged from 0.32±0.03 (lowest in SG5) to 0.77±0.13 (highest in SG8) (Fig. 4). From station SG1 to SG5, trophic value index was quite low and showed high from station SG6 to SG12. The diversity of nematode communities in the Saigon River from Ben Nghe Port (SG6) to downstream present a tendency higher in comparison to the upper part.

The two-way PERMANOVA analysis for the trophic index showed a significant difference between stations along the river ($p = 0.0003$), and between dry and rainy season ($p = 0.006$), but there was no difference in interaction effect ($p = 0.387$).

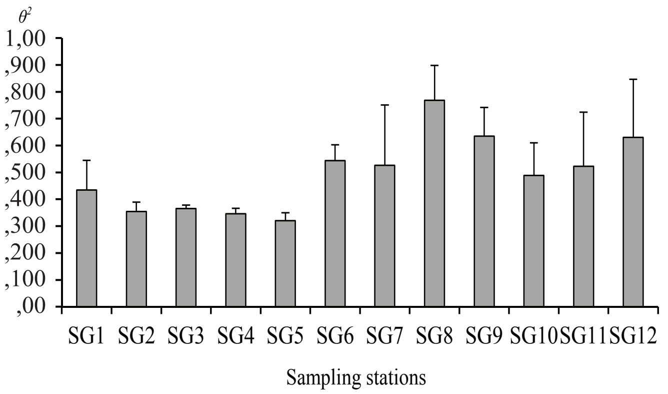

Fig. 4. The average and standard deviation of trophic index of nematode communities in four sampling times.

Discussion

The nematode community's density in our study was shown with a larger deviation in comparison to other research. Most specifically, from 168.7 to 1602.6 inds/10 cm² in harbor stations in Italy [18]; 454.0±289.9 - 3137.7±337.1 inds/10 cm² in the Mekong estuaries [5]; and 67-1666 inds/10 cm² in the Westerschelde [19]; 317-1002 inds/10 cm² in Shin River, Kasuga River, and Tsumeta River in Takamatsu, Japan [20]. However, nematode densities in the Saigon River were lower than that in the Oosterschelde [21] with values of 100-7100 inds/10 cm² or five European estuaries [22] with values from 130-14500 inds/10 cm².

Analysed results of the trophic structure of free-living nematode communities in this study were also found in the same line with the study of Gheskiere, et al. (2004) [23], in a tourists impacted sandy beach of the De Panne, Belgium. The authors reported that non-selective deposit feeders dominated all of the zones, except in the drift line, where epistratum feeders were dominant on sandy beaches. According to Moreno, et al. (2008) [18], the dominant trophic group of nematode communities at Marina Degli Aregai Port in Italy was represented by the non-selective deposit-feeders (1B: 61%), and followed by the epistrate-feeders (2A: 23%). In addition, at Station O of the Genoa-Voltri Port, the trophic groups had a high presence of deposit-feeders (59%) when we incorporate selective deposit-feeders (1A: 31%), and non-selective deposit-feeders (1B: 28%) together, followed by epistrate-feeders (2A: 38%).

Contrasting deposit-feeders to epistrate-feeders, suction-feeders, and chewers had a lower presence across almost all stations over the seasons. They contributed from 19.75% and 18.47% in total (with 31 and 29 genera), respectively. In all probability, the genera belong to these groups were a small percentage of the nematode communities. Whereas Nicholas, et al. (1992) [24], recorded that the predaceous nematode species were highest in number (>86%) in the sediments of the shore of Lake Alexandrina, Bogut & Vidakovic (2002) [25] reported that chewers were the major group of nematofauna at the eulittoral of Lake Sakadas: 68.28% of total nematofauna at submerged site A, 70.13% at site B (at the land-water interface), and 54.16% at emerged site C. Suction-feeders were the next important nematofauna group: 19.98% at site A, 23.60% at site B, and 36.97% at the site C, followed by deposit-feeders (5.98-11.78%). They also supported irregularities in the water level and the quantity of food available, and had a major influence on the changes in the distribution of nematode feeding-types. The chewers group had voluminous, sclerotised buccal cavities with one or more teeth and denticles, and feed on predators including protozoa, other nematodes, rotifers, tardigrades, and other small animals. While suction-feeders, which are omnivores, are characterised by the presence of a stylet and are supposedly omnivorous, they piercing different kinds of food (algae, vascular plants, epidermal cells and root hairs, fungi and animals) by using their stylets.

Furthermore, it was interesting that the trophic structure of free-living nematode communities also can indicate the environmental situation. Moreno, et al. (2008) [18] found the nematode trophic structure in three stations with different environmental conditions in the Genoa-Voltri Ports: highly polluted station M, polluted station I, and less polluted in station O. The trophic structure of nematode communities was characterised by a dominance of epistrate-feeders (2A: 33% at station M, 38% at station O and 46% at station I), following by selective deposit-feeders (1A: 33% at station I), and non-selective deposit-feeders (1B: 31% at station M). At Portosole Port, the dominant trophic group was found to be epistrate-feeders (2A: 59%), followed by non-selective deposit-feeders (1B: 24%). This was in line with results from the Saigon River where found mainly deposit-feeders and epistrate-feeders meanwhile very few chewers and suction-feeders which feed protozoa, nematodes, rotifers, enchytraeids, and tardigrades as well as omnivorous, algae, plant, fungi, and small animals.

In addition, Dražina, et al. (2014) [14] informed the community that specific trophic nematode groups occupied differently depending on freshwater substrate and available food sources, different parts of river beds and lakes basin. For instance, in sandy mud sediment, deposit-feeders prevail. In the periphyton habitats harbour, where was rich and diverse nematode communities, epistrate-feeding nematodes dominated. These authors established the domination of suction-feeding nematodes in stream bryophytes; a high percentage dorylaimid nematodes in terrestrial mosses across Europe and in bryophytes and in the deeper layers of the tufa substrates.

Conclusions

Feeding type structures of free-living nematode communities in the Saigon River were characterised by a dominance of nematode feeding type deposit-feeders and epistrate-feeders. The other feeding types, such as chewers and suction-feeders, were rather low proportion in this river. Dominant feeding types were influenced by high abundant genera occupied in the habitat and food available.

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