

Demography and feeding behavior of *Stenostomum leucops* (Dugés, 1828)

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ABSTRACT

Freshwater turbellarians, despite their mainly benthic habits, interact with pelagic communities of rotifers and cladocerans. However, very little is known about their demographic characteristics, food preference and functional response. To fill that gap we studied one of the most widely spread species, *Stenostomum leucops*. We conducted population growth experiments using abundant food (several rotifer and cladocerans species). To evaluate possible impact of *S. leucops* on planktonic communities, we conducted prey preference experiments at two temperatures: 18 and 23°C. The number of rotifers and cladocerans consumed was calculated by the difference between the initial and final density. We found that diets supplemented with fresh algae achieved higher *S. leucops* densities than those with detritus supplements in their diets. In the case of animal diets, *Euchlanis dilatata* allowed *S. leucops* reach higher densities than the other zooplankton species; *E. dilatata* was positively selected for in the selectivity study at both 18 and 23°C. *Stenostomum leucops* showed a type II functional response on rotifers and the cladoceran *Alona glabra*. Our results suggest that *S. leucops* select their prey according to their vulnerability using different mechanisms, which optimize their food intake.

Key words: *Stenostomum*; functional responses; population growth; prey selection; feeding behavior.

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INTRODUCTION

Freshwater turbellarians can be found in headwater streams to large rivers and in wetlands to large lakes where they are part of the bottom, surface and interstitial benthos (Schwartz and Hebert, 1982; Blaustein and Dumont, 1990). They can also be found in very small streams and brooks or on different types of substrates (e.g., moss, vascular plants, wood snags, detritus, gravel, or sand) in the littoral areas of rivers (Noreña *et al.*, 2005). Littoral species of lotic and lentic environments, inhabitants of plant roots, leaves, and surface sediments (*i.e.*, the first cm) are subject to disturbances during the different seasons due to changes in the water level. This fact causes great oscillations in the abundance dynamics of species (Threlkeld, 1994) and their ability to swim in the water column (Trochine *et al.*, 2006), allow them to interact with the zooplankton community. Their diet includes a wide variety of unicellular organisms and invertebrates especially bacteria, ciliates, rotifers and cladocerans (Nandini *et al.*, 2011; Hammill *et al.*, 2015). Thus, they play an important role in structuring the ecological community and in recycling nutrients to higher trophic levels. Turbellarians have been a neglected group of organisms in ecological research although, it is estimated that there are over 200 species in lotic waters in the world with densities up to 40,000 ind. m⁻² (Kolasa and Tyler, 2010). However, their identification is difficult, especially due to the paucity of trained taxonomists.

Most studies on flat worms are aimed at regeneration (Reddien and Alvarado, 2004; Cebrià and Newmark, 2005; Bely and Sikes, 2010), senescence (Martinez and Levinton, 1992; Mouton *et al.*, 2009) and stem cells subjects (Reuter and Kreshchenko, 2004). Most turbellarians have neoblasts (Newmark and Alvarado, 2000) which allow them to be good models for this type of research. Neoblasts also allow these multicellular organisms to reproduce asexually, primarily by transverse fission, which complicates the study of their demographic characteristics, whereby these studies are scarce and the data are highly variable (Egger *et al.*, 2007; Mouton *et al.*, 2009; Dunkel *et al.*, 2011).

Biotic interactions, especially those related to feeding and competition, are essential in structuring aquatic communities. These interactions depend on several factors: density of organisms (Chesson, 1986), their biological characteristics (Iyer and Rao, 1996; Lampert and Sommer, 2007) and spatial distribution (Dutuilleul, 1993). In predator-prey relationships changes in prey density result in two types of responses of the predator: numerical and functional (Case, 2000). The first involves changes in predator density and the second, adjustments in their feeding rates; both related to prey type and availability (Case, 2000). In the case of some non-generalist predators, life cycles of prey and predator are often linked (Murdoch and Bence, 1987). These responses have been observed in few freshwater flatworms such as

Mesostoma lingua (Dumont and Schorreels, 1990) and *Stenostomum virginianum* (Kratina *et al.*, 2009).

Some biological characteristics have an influence on predator-prey relationship, for instance: morphology, motion, prey size, and the predation mechanisms (Werner and Hall, 1976; Iyer and Rao, 1996; Lampert and Sommer, 2007). Turbellarians use various mechanisms to capture their prey, including: active search, sit and wait, toxins release or secretion of mucus traps (Blaustein and Dumont, 1990). For example, it has been shown that *Mesostoma lingua* is able to prey on *Daphnia magna* releasing a toxic mucus trap that paralyzes the prey, and then, engulfs it with the ventral area of its body and sucks the prey (Dumont and Carels, 1987). The spatial distribution of predator and prey is important because it controls the probability of encounter with each other (Duttilleul, 1993); organisms sharing the same habitat, have more probabilities of encounters (Gilinsky, 1984). However, it has been reported that *M. ehrenbergii* is able to capture evasive prey that inhabit different habitats, such as the copepods *Boeckella gracilis* and *Acanthocyclops robustus*, using mucus traps (Trochine *et al.*, 2006), and if the worm is very hungry, it can swim very fast, searching for its prey (De Meester and Dumont, 1990). Turbellarians are carnivorous, but their diet also includes algae; maybe to satisfy their eicosapentaenoic acid requirements. Eicosapentaenoic acid is important for growth and reproduction; but most of animals are unable to synthesize it, and must be obtained it from their diet (Wacker and Martin-Creuzburg, 2007); moreover, detritus can be an alternative source, even if quality of fatty acids and nutrients are less than in fresh algae (Perhar and Arhonditsis, 2009), it could be important because in the case of flatworms, this resource would be more available in its preferred benthic habitat. Turbellarians are involved in top-down regulation, being predators; but they are also prey, some of their predators are fishes such as *Poecilia vivipara* and *P. reticulata*; (Dumont *et al.*, 2014) and the predatory ciliate *Dileptus margaritifer* (Buonanno, 2009).

Given the potential growth of *Stenostomum* populations, we aimed at to explore their possible impact on prey by quantifying the growth and consumptions rates of *S. leucops* on selected prey species. In this work, we present data on the population growth on different diets and the feeding behavior of *Stenostomum leucops* (Dugès, 1828) widely distributed in America (Noreña *et al.*, 2005) that can produce changes in morphology, behavior, physiology and life history of its prey. We used littoral prey with different characteristics with respect to morphology, movement, size and habitat; including the rotifers *Euchlanis dilatata*, *Platyonus patulus*, *Lecane bulla*, and the cladocerans *Alona glabra*, *Moina macrocopa* and *Macrothrix triserialis*; the diets were supplemented with fresh algae or detritus.

METHODS

Cultures

Stenostomum leucops was isolated from a pond in the State of Veracruz (Mexico) in 2005 and has been cultured in moderately hard water (EPA; Weber, 1993) on a mixture of zooplankton for several years in our laboratory. We fed *S. leucops* using rotifers (*Platyonus patulus*, *Euchlanis dilatata*) and cladocerans (*Alona glabra* and *Macrothrix triserialis*) *ad libitum*. Cladocerans and rotifers were grown in EPA medium and fed *Scenedesmus acutus*. Cell density was measured using a hemocytometer and the density offered to zooplankton was 1×10^6 cells mL^{-1} . The medium was changed twice a week.

Population growth

We selected similar size worms for all experiments. All the experiments were performed in Petri dishes, where we put 2 worms and 15 mL of EPA medium with *S. acutus*, live or in detrital form. To prepare detritus, we enumerated cell density of *S. acutus* which was kept for four days in darkness at 30°C following Gulati *et al.* (2001).

Two individuals of *S. leucops* were placed in each Petri dish and were offered seven different diets: a ciliate (*Paramecium* sp.), rotifers (*P. patulus*, *E. dilatata*, *Lecane bulla*) and cladocerans (*A. glabra*, *M. triserialis* and *Moina macrocopa*). The densities were 4 ind. mL^{-1} for *Paramecium* sp.; 2 and 1 ind. mL^{-1} for rotifers and cladocerans, respectively. We estimated daily the density of the organisms using a stereomicroscope. Rotifers and cladocerans were replaced to maintain the initial density. The medium was changed twice a week. The growth rate (day^{-1}) for each population was calculated using the exponential growth equation (Case, 2000):

$$r = \frac{\ln N_t - \ln N_0}{t} \quad (\text{eq. 1})$$

where

r , rate of population increase;

N_0 and N_t , initial and maximum population densities, respectively;

T , is the day on which the maximum density is reached.

We compared the growth rates (r) of *S. leucops* on different diets using one and two-way ANOVA (Sigma-Plot ver. 11).

Feeding preference

The experiments were conducted at two temperatures: 18 and 23°C. Before experiments, each turbellarian was starved for 3 h and acclimated to the respective temperature. Each Petri dish contained 15 mL of EPA medium and *P. patulus*, *E. dilatata*, *Lecane bulla* (15 individuals of each species) and *A. glabra*, *M. triserialis* and *Moina*

macrocopa (5 individuals of each species). The feeding time was 2 h. The consumption of each prey species was calculated by the difference between the initial and final densities. For the selectivity index, α Manly (Krebs, 1999) was used:

$$\hat{\alpha}_i = \frac{\hat{r}_i}{\hat{n}_i} \left(\frac{1}{\sum (\hat{r}_j / \hat{n}_j)} \right) \quad (\text{eq. 2})$$

where

$\hat{\alpha}_i$, Manly's α for prey type i ;

\hat{r}_i, \hat{r}_j , proportion of prey type i or j in the diet ($i, j=1, 2, 3, \dots, m$);

\hat{n}_i, \hat{n}_j , proportion of prey type i or j in the environment;

m , number of types of possible prey.

Functional response

Five densities were used: 0.5, 1, 2, 5 and 10 ind mL⁻¹ in the case of rotifers (*P. patulus*, *E. dilatata*, *Lecane bulla*); and 0.2, 0.5, 1, 2 and 5 ind. mL⁻¹ in the case of cladocerans (*A. glabra*, *M. triserialis* and *Moina macrocopa*). Each Petri dish containing 15 mL of EPA medium, 2 worms (starved for 3 h) and the chosen prey. Feeding time was 2 h after which the prey remained in the Petri dish were fixed using 4% formalin. The number of rotifers and cladocerans consumed was calculated by the difference between the initial and final density. The obtained data were transformed using the Michaelis-Menten equation (Lampert and Sommer, 2007)

$$V_0 = \frac{V_{max}S}{K_m + S} \quad (\text{eq. 3})$$

where

V_0 , consumption rate;

V_{max} , saturation value of the rate of consumption;

S , prey availability;

K_m , prey density at which $V_{max}/2$ was reached.

In order to determine the type of functional response, data was linearized by the equation of the proportion of ingested prey (Trexler *et al.*, 1988): $d=PR/N_0$, where PR =predation rate; and N_0 =initial number of prey.

RESULTS

Stenostomum leucops increased in abundance on diets involving fresh algae but a decreased on diets supplemented with detritus. The experimental duration was 35 days, by which time population densities had reached a peak and then began to decline. The maximum densities were reached between days 11 to 30, but in most treatments they reached maximum densities at day 18. For rotifers as prey, we observed that *S. leucops* reached higher abundances on *E. dilatata* than on *P. patulus* or *Lecane bulla*, in both cases, with fresh algae or detritus. Among cladocerans, population densities were highest on *A. glabra* and *M. macrocopa* supplemented with fresh algae rather than detritus (Fig. 1).

The range for the rate of population increase r varied from -0.69 and 0.141 day⁻¹, depending on the diet. Regardless of the prey species fresh algae in the medium allowed *S. leucops* obtain a higher r than detritus. The population growth rates were negative on diets of *Paramecium* sp.+*S. acutus* and *Paramecium* sp.+detritus (Fig. 2); these diets had a significant effect on the r value ($P<0.05$, F-test, one-way ANOVA, Tab. 1). For diets of algae and rotifer or cladocerans, both type of rotifer ($P<0.01$) and cladoceran species had a significant effect ($P<0.05$) on r values (F-test, two-way ANOVA, Tab. 1).

Data from the prey selectivity experiments showed that among the zooplankton species, at prey density tested, *E. dilatata* and *L. bulla* were positively selected in both temperatures but *P. patulus* was avoided. At 18°C *S. leucops* also consumed *M. triserialis* but at 23°C none of the cladocerans was selected and preference for *E. dilatata* increased (Fig. 3).

Tab. 1. Results of one and two-way analysis of variance performed on the r values of *S. leucops* feeding with different algal and animal diets.

	Source of variation	DF	SS	MS	F	P
One-way ANOVA <i>S. acutus</i> - <i>Paramecium</i> sp.	Between groups	1	0.0220	0.0220	37.349	0.002
	Residual	5	0.00294	0.000589		
	Total	6	0.0249			
Two-way ANOVA algal diet-rotifer	Algae type (fresh or detritus)	1	0.0507	0.0507	7.268	0.015
	Rotifer species	2	1.850	0.925	132.675	<0.001
	Algae type x Rotifer	2	0.0843	0.0422	6.049	0.010
	Residual	17	0.119	0.00697		
	Total	22	2.239	0.102		
Two-way ANOVA algal diet - Cladocera	Algae type (fresh or detritus)	1	0.201	0.201	0.036	
	Cladocera	2	0.289	0.144	0.045	
	Algae type x Cladocera	2	0.304	0.152	0.040	
	Residual	16	0.612	0.0383		
	Total	21	1.493	0.0711		

DF, degrees of freedom; SS, sum of squares; MS, mean square; F, F-ratio.

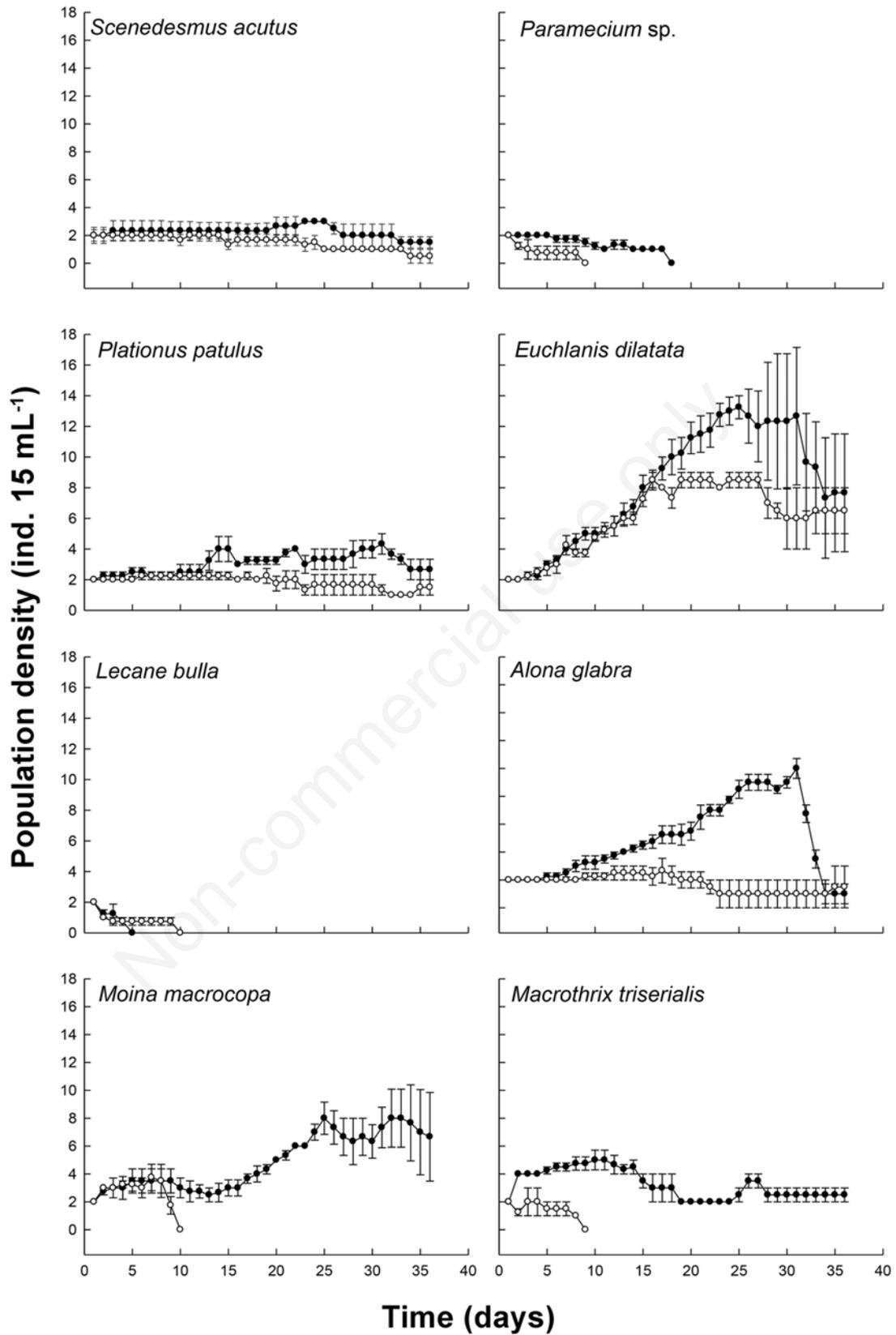


Fig. 1. Population growth of *S. leucops* offered: a) *S. acutus*; b) detritus; c) *P. patulus* with *S. acutus*; d) *P. patulus* with detritus; e) *E. dilatata* with *S. acutus*; f) *E. dilatata* with detritus; g) *A. glabra* with *S. acutus*; h) *A. glabra* with detritus. Shown are mean±SE based on four replicate observations.

Functional response curves showed increased prey consumption with increasing prey density in the medium (Fig. 4). In general, changes in temperature did not have a significant impact with respect to prey consumption. The maximum number (\pm SD) of rotifers prey consumed by *S. leucops* during 2 h feeding, were 8.75 ± 3.8 at 23°C for *E. dilatata*, 10.87 ± 2.4 at 18°C for *P. patulus* and 5.75 ± 0.9 at 18°C for *L. bulla*. When *A. glabra* as prey, the maximum prey consumed was 3.33 ± 0.29 at 18°C .

DISCUSSION

Turbellarians are involved in structuring zooplankton communities through modifying directly and/or indirectly the morpho-physiological or demographic characteristics of organisms (Hammill *et al.*, 2015). They could be a link in channelizing energy between the pelagic and benthic zones but there are few studies on the interaction of organisms from both zones (Threlkeld, 1994). Published data suggest that an increase in density of flatworms leads to decreased zooplankton such as *Daphnia* and *Bosmina* (Caramujo and Boavida, 2000), and *Moina* (Blaustein and Dumont, 1990). In this study we observed highest densities of *S. leucops* on *E. dilatata* and *A. glabra*, both species share a similar habitat of littoral regions of water bodies as the worm (Kolasa and Tyler, 2010). It appears that the spines of the rotifer *P. patulus* and the cladoceran *M. triserialis* are effective defenses since the population growth rate of the worms was considerably lower on these prey. *Stenostomum leucops* has a simple pharynx without much capacity for expansion. In some other invertebrate predators such as *Asplanchnopus multiceps* fed *P. patulus* and *M. triserialis*, the growth rates were low (Nandini and

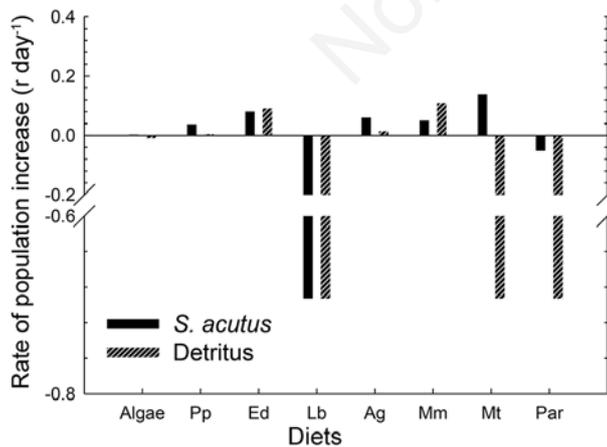


Fig. 2. r values of *S. leucops* offered six diets. Sa, *S. acutus*; D, detritus; Pp/Sa, *P. patulus*/*S. acutus*; Pp/D, *P. patulus*/detritus; Ed/Sa, *E. dilatata*/*S. acutus*; Ed/D, *E. dilatata*/detritus; Ag/Sa, *A. glabra*/*S. acutus*; Ag/D, *A. glabra*/detritus. Mean values \pm SE based on four replicate observations are shown.

Sarma, 2005). Although it is assumed that turbellarians grow well on detritus and/or protozoan diets, we did not find this trend in this study. This could be due to the low carbon content of the diet and the lack of nutrients; in nature the turbellarians need be exposed to a richer and more varied detrital diet.

The peaks of maximum density achieved by *Stenostomum* were low compared with other smaller worms (Kolasa and Tyler, 2010) most probably due to its mode of reproduction which is transverse paratomy. The highest densities of *S. leucops* were reached when their diet was supplemented with fresh algae but not detritus, probably because of the poor nutritional quality of the latter (Sterner and Hessen, 1994). Nutritional value of these resources is reflected in the somatic phosphorus content and composition of fatty acids (DeMott and Pape, 2005; Arndt and Sommer, 2014). *Paramecium* sp. allowed *S. leucops* survive just less than 20 days, even though these heterotrophic flagellates include in their natural diet (Vera *et al.*, 2001), participating in the transfer of energy. However, it appears that they are not a good source of nutrients. It has been shown that ingesting

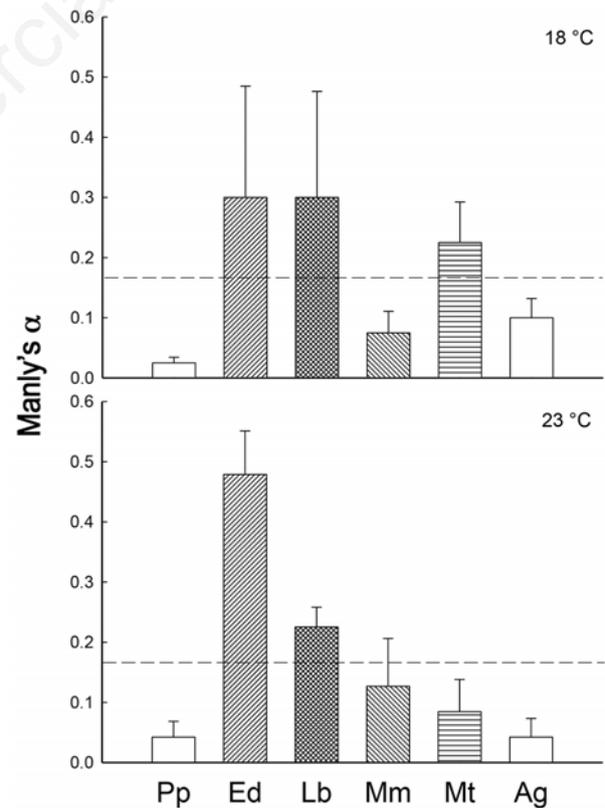


Fig. 3. Feeding preference of *S. leucops* offered six prey types at two temperatures: a) 18°C ; and b) 23°C . Pp, *P. patulus*; Ed, *E. dilatata*; Lb, *L. bulla*; Mm, *M. macrocopa*; Mt, *M. triserialis*; Ag, *A. glabra*. The Manly's α -values (mean \pm SE) based on four replicate observations are shown.

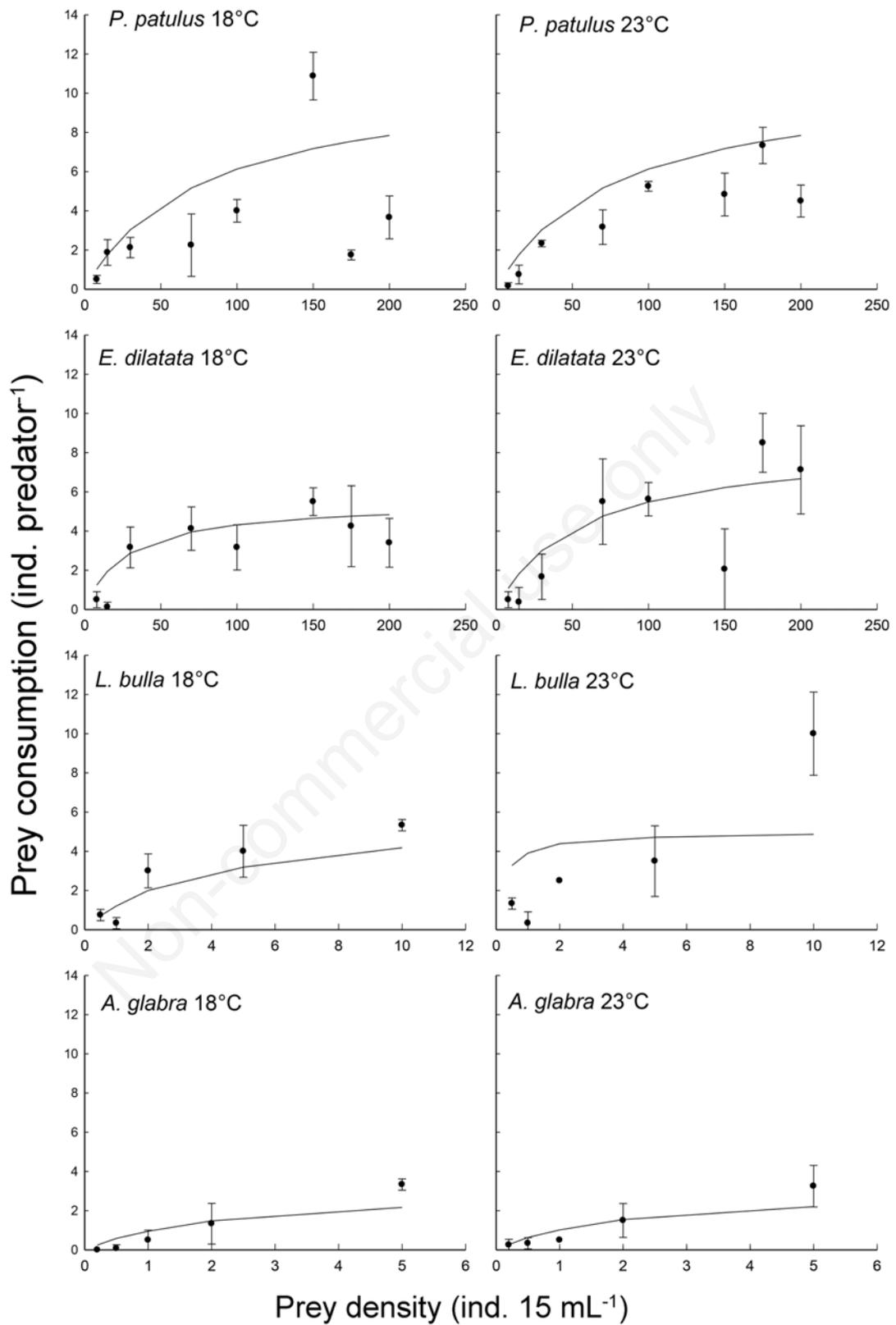


Fig. 4. Functional response of *S. leucops* offered four preys at two temperatures: a) *P. patulus* at 18°C; b) *P. patulus* at 23°C; c) *E. dilatata* at 18°C; d) *E. dilatata* at 23°C; e) *L. bulla* at 18°C; f) *L. bulla* at 23°C; g) *A. glabra* at 18°C; h) *A. glabra* at 23°C. The mean \pm SE values of the actual prey consumed based on four replicate observations, and the transformation of the curve using the Michaelis-Menten equation are shown.

Paramecium allows predators satisfy their metabolic demands but the carbon input is insufficient to permit adequate reproduction in cladocerans, copepods and rotifers (De Biase *et al.*, 1990; Mohr and Adrian, 2002). The quality of the different diets was reflected in the turbellarian population growth even when the diet consisted only of algae or detritus; this suggests that *S. leucops* acquires different nutrients, the availability of which, in turn, affects its demographic parameters.

The energy designated for reproduction depends on the amount of food available and consumed (Sarma and Rao, 1991) which, in turn, depends on the vulnerability of the prey. Vulnerability is affected by characteristics such as size, presence of spines, swimming behavior, *etc.* (Iyer and Rao, 1996). These characteristics affect directly the prey selection by the predator; but also affect the functional response; which helps us understand how a predator plays a role in structuring prey populations. In the food preference experiment, we observed that in both temperatures *S. leucops* prefer *E. dilatata* compared to other prey; probably because *E. dilatata* is an organism with benthic habits. Among the rotifers offered, *E. dilatata* is the fastest achieving speeds up to 0.98 mm s^{-1} (Rico-Martínez and Snell, 1997), but despite this, probably the hungry turbellarian is able to swim faster to capture this spineless prey easily. *P. patulus* was not positively selected at any temperature even though its speed (0.69 mm s^{-1}) is lower than that of *E. dilatata* as it has numerous anterior (10) and posterior spines (4), features not present in *E. dilatata* and *L. bulla*. Although it was surprising to note that the flatworm did not prefer and neither did it grow well on the seemingly defenseless *Lecane bulla* similar trends are not uncommon in other invertebrate predators (Iyer and Rao, 1996). On the other hand our results indicate that it is not easy to extrapolate findings from preference studies to possible growth patterns. Although *M. triserialis* was the only cladoceran positively selected, the growth of the turbellarian on this species was low as compared to *M. macrocopa* and *A. glabra* perhaps because the feeding time was only two hours and it was observed that *S. leucops* tends to prey on larger organisms (such as cladocerans).

We observed that the number of prey items consumed increased with increasing prey density. The asymptote was achieved with prey such as *P. patulus* and *E. dilatata*, but not in the case of *A. glabra*. In the case of *A. glabra* consumption was very low, probably due to its size, and ornamentation that can modify handling time, capture and ingestion and decreasing consumption rates. For *Mesostoma lingua* a maximum intake of between 4 and 5 *Daphnia* per day, on a density of 10 daphniids per day (Dumont and Schorreels, 1990; Schwartz and Herbert, 1982); and in the case of *S. leucops* it was observed that the maximum density of cladocerans (*Alona* 70 mL^{-1}) *per capita* consumption was 3.25 *A. glabra* in 2 h. The effectiveness

of predator depends on many factors which have not yet been analyzed, such as the ability to form groups to hunt which reduces *per capita* consumption or slow hunting speeds (Kratina *et al.*, 2009). We also observed a general increase in prey consumption with increasing temperature, most probably to meet the increased metabolic demands (Lampert and Sommer, 2007).

CONCLUSIONS

Our study showed that *S. leucops* could have an important role regulating the population density of their prey, influenced by the selectivity of the predator and vulnerability of the prey (Lampert and Sommer, 2007). Our observations suggest that turbellarians may play an important role in transferring energy to higher trophic levels especially under conditions in which rotifers and cladocerans face competitive stress for limiting food or space habitats and predation as a result of fish or amphibian breeding.

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