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Elaphoidella grandidieri (Harpacticoida: Copepoda): Demographic characteristics and possible use as live prey in aquaculture

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Abstract

In freshwater ecosystems, rotifers and cladocerans are ideal prey for fish larvae whereas copepods, due to their purported low growth rate and predatory tendency, are not. We recently isolated the parthenogenetic *Elaphoidella grandidieri* (Guerne et Richard, 1893) a benthic freshwater harpacticoid, from a fish farm in the State of Morelos, central Mexico and tested its potential as a live prey organism for larval vertebrates. Population growth and life table demography experiments were conducted, in 100 ml recipients with 50 ml of test medium on a diet of *Scenedesmus acutus* at a density of 1.0 X 10^6 cell ml^{-1}; the former on live algae alone while the latter on live algae as well as detritus. We also conducted experiments to document the prey preference for this copepod by the larval *Ameca splendens* (Pisces: Goodeidae) and *Ambystoma mexicanum* (Amphibia: Ambystomatidae), fed the rotifer *Plationus patulus*, the ostracod *Heterocypris incongruens*, and the cladocerans *Moina macrocopa* and *Daphnia pulex*. *Elaphoidella grandidieri* is relatively easy to maintain under laboratory conditions, reaching densities (copepodites and adults) of more than 10,000 l^{-1}. The generation time ranged between 30-45 days, depending on the diet. The net reproductive rate was as high as 60 nauplii female $^{-1}$ day $^{-1}$. Population growth rates ranged between 0.03 and 0.11 d^{-1}, live algae being the superior diet compared to detritus. Both predators showed no preference for *E. grandidieri*, but in the absence of alternate prey they consumed 80% of the harpacticoids offered. The data have been discussed in relation to the potential of *E. grandidieri* as live food for aquaculture.

Key words

Aquaculture, Copepods, Population density, Fish food, Mass culture

Introduction

Shortage of live food continues to be a major bottle neck in succesful aquaculture practices all over the world (Jana and Webster, 2003). Appropriate characteristics of live prey include a small body size, high maximum population densities and high population growth rates of the prey (Gerking, 1994). In freshwater systems, zooplankton less than 5 mm including rotifers such as *Brachionus calyciflorus* and cladocerans such as *Moina macrocopa* and various species of *Daphnia* are often used (Hoff and Snell, 1987). Several species of marine or brackish calanoid copepods (*Acartia tonsa* Dana, 1849, *Eurytemora affinis* (Poppe, 1880), *Calanus finmarchicus* (Gunnerus, 1770) and benthic harpacticoids (*Tigriopus japonicus* Mori, 1932; *Schizopera elatensis* Kahan and Bar-El, 1982) are used for mariculture (Lee *et al.*, 2005). In freshwaters, copepods are rarely used because many cyclopoids are voracious predators (Dussart and DeFaye, 1995; Fernando, 1998) and can feed even on fish larvae. The possibility of using non-predatory copepods as live prey for fish larvae has rarely been tested, despite the fact that many copepods are rich sources of HUFA's and PUFA's, regardless of the diet quality (Lee *et al.*, 2005). This is primarily because, being sexual and having several developmental stages they have lower growth rates (Dussart and Defaye, 1995). They usually reach lower population densities than rotifers or cladocerans which are mostly parthenogenetic forms (Williamson and Reid, 2001).

Harpacticoids are widely used in mariculture practices throughout the world (Zhang and Uhlig, 1993). They are highly tolerant to a wide range of environmental conditions and can feed successfully on a variety of diets (Stottrup and Nosker, 1997). Although their growth rates are lower than rotifers or cladocerans (Allan, 1976), they do reach and maintain high densities under less demanding culture conditions. These advantages greatly outweigh the disadvantage of not being able to store the eggs or nauplii of harpacticoids, implying the need of production on site (Lee et al., 2005).

We found the harpacticoid *Elaphoidella grandidieri* in a shallow fish pond in the State of Morelos, Mexico (Gutiérrez-Aguirre et al., 2010). *Elaphoidella grandidieri* is a tropical, cosmopolitan species, originally found in Africa but also found in North America (Reid and Ishida, 1993), South America (Gaviria and Aranguren, 2007) and Asia (Kikuchi, 1985). Sarvala (1979) documented a few species of *Elaphoidella* as parthenogenetic and the possibility of *E. grandidieri* also being parthenogenetic, considering the fact that few males are found under natural conditions. Since we found that *Elaphoidella grandidieri* is indeed parthenogenetic, we analysed the possibilty of using this species as live prey in freshwater aquaculture by studying its life history traits and testing the preferences of fish and amphibian larvae.

In this study we present data on:

1. Population growth of *Elaphoidella grandidieri* on a monoalgal diet (*Scenedesmus acutus* at a concentration of 1.0 X 10^6 cells ml^{-1})
2. Life table demography of *Elaphoidella grandidieri* on live and detrital *Scenedesmus acutus* (at a concentration of 1.0 X 10^6 cells ml^{-1})
3. Ratio of females with and without eggs during the growth phase
4. Prey preference of larval fish (*Ameca splendens*) and amphibia (*Ambystoma mexicanum*) with an analyses of the relative advantages and disadvantages of *E. grandidieri* as prey with relation to rotifers and cladocerans

Materials and Methods

Elaphoidella grandidieri females were isolated from a sample collected in August, 2006 from a shallow (<1.0 m depth) ornamental fish pond in the State of Morelos, Mexico. Fifty liters of pond water was filtered thorugh a 50 mm sieve, diluted to two liters and transported to the laboratory. We cultured this species on moderately hard water (EPA medium), a diet of *Scenedesmus acutus* and at a temperature of 23 ± 2°C. The algal strain *Scenedesmus acutus* was obtained from the University of Texas and cultured on Bold medium (Borowitzka and Borowitzka, 1984). The algae were harvested after 8-10 days, allowed to sediment in a refrigerator, decanted and enumerated using a Neubaur haemocytometer. Cultures were changed twice a week once they were in one liter recipients. We found that it was rather easy to maintain this species even in aquaria because they did not need to be changed often as they browsed at the bottom and kept the vessel clean.

Twenty adult, non-egg bearing females were weighed individually following standard procedures. Small aluminium foil cups were weighed previously in a Cahn 33 electrobalance with a precision of 1 µg. The harpacticoid females were placed individually in each of them and then all were placed in an oven at 60°C for 24 hr (Downing and Rigler, 1984). The next day the foil cups were removed, placed in a dessicator to avoid the absorption of water and weighed. Corrections were made for changes in the foil weight.

Population growth studies: Population growth experiments were conducted at one food level of *S. acutus* (1.0X 10^6 cells ml^{-1}) at a temperature of 22 ± 1°C. The experiments were conducted in 100 ml recipients with 50 ml of the test medium with the desired algal concentration. We set up four replicates into which we introduced 25 nauplii that were less than 24 hr old. These were isolated from containers with gravid females that were set up the previous day. We counted and transferred the individuals daily to fresh medium with the appropriate algal concentration. The number of nauplii, copepodites, non-gravid and gravid females were enumerated. The experiments were continued over a two-month period, until the populations began to decline. Population growth rates were calculated using the formula $r = lnN_t - lnN_0 / t$ where N_0 is the initial population density, N_t is the population density at time *t* (in days) (Krebs, 1985).

For life table experiments we offered *S. acutus*, either fresh or in a detrital form which was prepared by keeping a pre-counted algal suspension in the dark at 30°C for 72 hr following Gulati et al. (2001). For each test jar with 50 ml of medium we began with a cohort of 20 nauplii. Thus, the experimental design consisted in a total of 8 test jars (2 food types X 4 replicates). Nauplii observed during the experiments were counted and removed and the experiments were terminated when every individual of each cohort died. Based on standard formulae (Krebs, 1985), we constructed age-specific survivorship, life expectancy, fecundity and stage age distribution curves. The results were analyzed using ANOVA and where needed Tukey's test was employed.

Prey consumption: The number of *Elaphoidella grandidieri* consumed by *Ameca splendens* (Pisces: Goodeidae) and *Ambystoma mexicanum* (Amphibia) larvae was tested. The predators were 4.5 ± 0.5 cm in length. We introduced the prey (non-gravid adult females) at a density of 0.95 ind.ml^{-1} into each recipient with 100 ml of EPA medium. We chose to use adults instead of naupliar stages since previous studies (Peña-Aguado *et al.*, 2009) indicate that these predators feed well on prey in the size range of cladocerans (>1 mm) rather than rotifers (< 0.25 mm). Four replicates were set up for each predator. *Ameca splendens* and *Ambystoma mexicanum* were starved for 30 minutes and then one individual of each was introduced into the test vessels where they were allowed to feed for 30 minutes in diffused fluorescent light.

At the end of the feeding period, we separated the predators and counted the remaining prey. The difference between the initial and final prey density was taken to be the prey consumed by the predators.

Prey preference: In order to test the prey preference of the fish and the amphibian larvae for *E. grandidieri*, we conducted an experiment where five different prey types were offered. The design of the experiment was the same as above, except that we introduced the rotifer *Plationus patulus* (25 ind), the cladocerans *Daphnia pulex* (10 ind.) and *Moina macrocopa* (25 ind), *E. grandidieri* (25 ind) and the ostracod *Heterocypris incongruens* (10 ind). The total density was also the same as above of 0.95 ind. ml^{-1}.

The preference index was claculated using Manly's alpha (α) (Krebs, 1999) where

$\alpha_i = r_i / n_i \, (1/\Sigma(r_j / n_j))$ where,

α_i = Manly's alpha for prey type *i*,

γ_b, γ_j = proportion of prey type *i* or *j* in diet (*i* and *j* = 1, 2, 3 ...,*m*)

n_i, n_j = proportion of prey type *i* or *j* in medium

m = number of possible prey types

If

$\alpha_i = 1/m$, non selective feeding

$\alpha_i > 1/m$, prey preferred in diet

$\alpha_i < 1/m$, prey species avoided

Results and Discussion

The duration of the naupliar and copepodite stages of *E. grandidieri* ranged between 10-12 days each. Gravid females bore egg sacs with 7-16 eggs, mostly with 10-12 eggs. The population densities of the adults (Fig. 1) began increasing only after 35 days of initiation of the experiment, peaking at densities as high as 6-8 ind. ml^{-1}. The proportion of egg bearing to non-egg bearing females was between 0.9-1.0, that is almost all females were egg bearing at low population densities (<2/ml) but at high densities, the number of egg bearing females began to decline (Fig. 2). The survivorship curves (Fig. 3) show a high (> 70%) mortality during the naupliar and copepodite stages, regardless of the food type. The reproductive phase lasted 65 days on the fresh alagl diet but only 15 days on the detrital diet (Fig. 3). All the survivorship and fecundity related variables (Fig. 4) are significantly lower on a diet of algal detritus (p<0.01, one-way ANOVA, Table 1). The prey preference experiments showed that both the predators, *Ambystoma mexicanum* and *Ameca splendens*, when offered with a choice of the rotifer *P. patulus*, the cladocerans *Moina macrocopa* and *Daphnia pulex* and the ostracod *Heterocypris incongruens*, preferred *Moina macrocopa* to *Elaphoidella grandidieri* (Fig. 5), regardless of the fact that both prey taxa were very similar in size. Nevertheless, when *E. grandieri* was the exclusive prey offered, both predators consumed it well (Fig. 6)

We observed that *E. grandidieri* could reproduce parthenogenetically, rare in copepods but suspected for this species (Sarvala, 1979). Parthenogenesis has been observed only in a few species of harpacticoids such as *Elaphoidella bidens*, *Epactophanes richardi*, and *Canthocamptus staphylinus* (Sarvala, op. cit.). The duration of the naupliar and copepodite stages of *E. grandidieri* was similar to that observed in other harpacticoids such as *Canthocamptus staphylinus* (Sarvala, 1979) and *Bryocamptus zschokkei* (O'Doherty, 1985). Although the generation time of *E.*

Table - 1: Statistical analyses of selected life history variables (mean adult lifespan (days), life expectancy at birth (days), gross reproductive rate (offspring $female^{-1}$), net reproductive rate (survival-weighted offspring $female^{-1}$), generation time (days) and the rate of population increase (*r*) per day) of *E. grandidieri* fed live and detrital *Scenedesmus acutus* (*Sa*)

Source of Variation	DF	SS	MS	F	P
Mean lifespan					
Food type	1	634.99	634.99	53.09	<0.001
Residual	4	47.84	11.96		
Life expectancy at birth					
Food type	1	640.15	640.15	52.97	<0.001
Residual	4	48.35	12.09		
Gross reproductive rate					
Food Type	1	86711.71	86711.71	80.20	<0.001
Residual		4	4324.53	1081.13	
Net reproductive rate					
Food Type	1	366.25	366.24	6.88	<0.001
Residual		4	212.89	53.223	
Generation time					
Food Type	1	484.32	484.32	121.54	<0.001
Residual	4	15.94	3.985		
Population growth rate					
Food Type	1	0.007	0.007	183.81	<0.001
Residual	4	0.0002	0.00004		

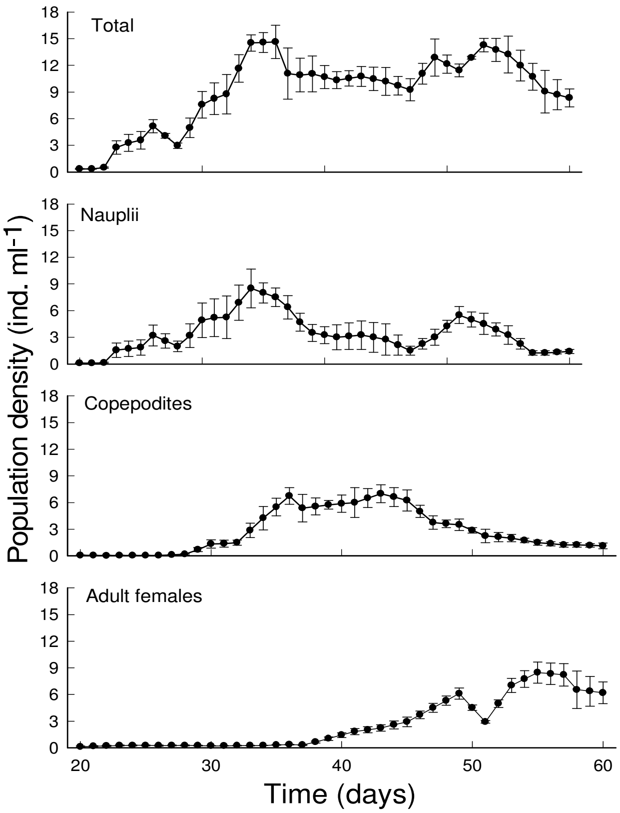

Fig. 1: Population growth of naupliar, copepodite and adult stages of *Elaphoidella grandidieri*. Shown are mean ± SD (n=4)

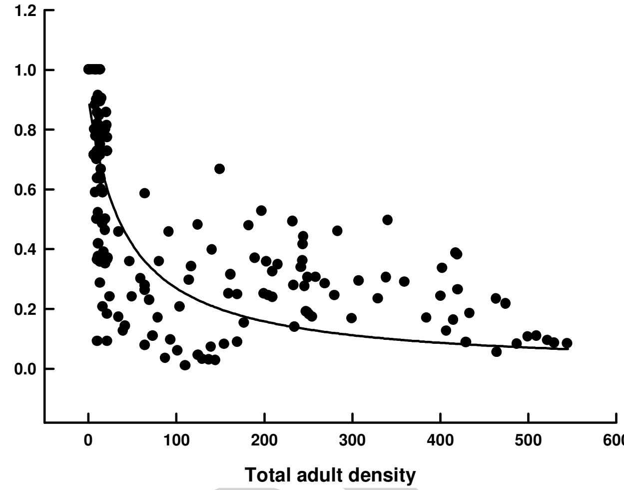

Fig. 2: Ratio of ovigerous to non-ovigerous females in relation to the total population density of *Elaphoidella grandidieri*

grandidieri is long (around 25 days) as compared to rotifers or cladocerans, it reaches high densities of around 15 ind. ml^{-1}, all stages considered. The cladoceran *Moina macrocopa* is also known to reach densities higher that 15 ind. ml^{-1} (Nandini and Sarma, 2003), yet it is not possible to maintain its high densites for a long period of time unlike *E. grandidieri*.

The highest population growth rate of *E. grandidieri* we observed was 0.11± 0.03 d^{-1}, lower than that recorded for

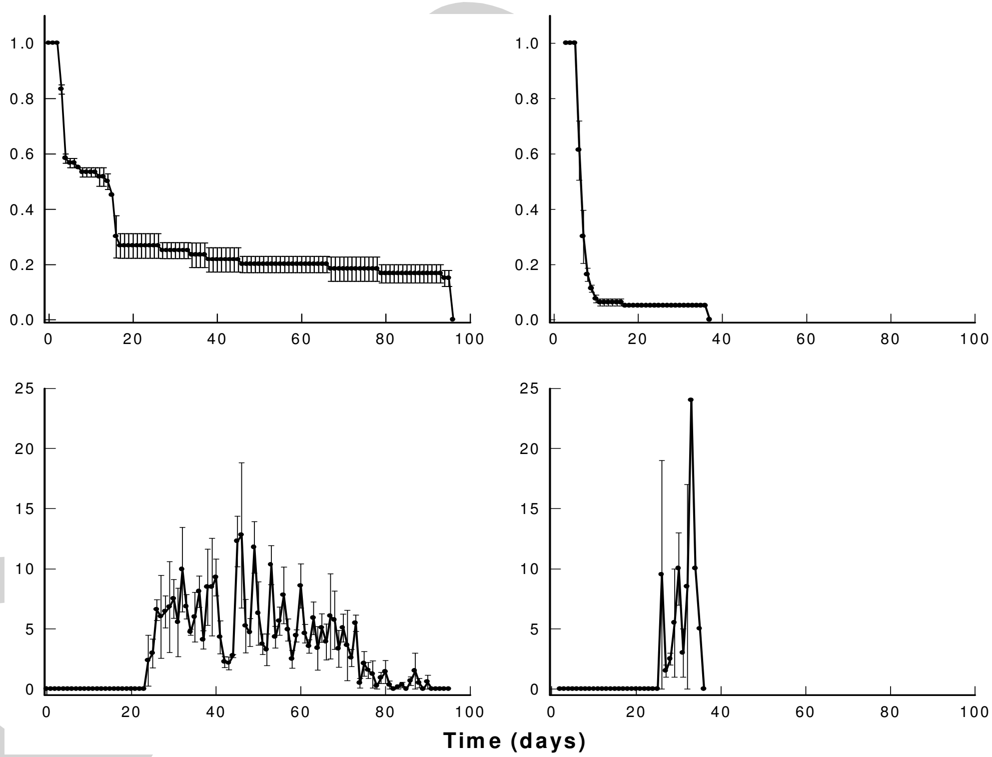

Fig. 3: Age-specific survivorship and fecundity curves of *E. grandidieri* reared on different diets. Values represent mean ± standard error based on 4 replicates (cohort)

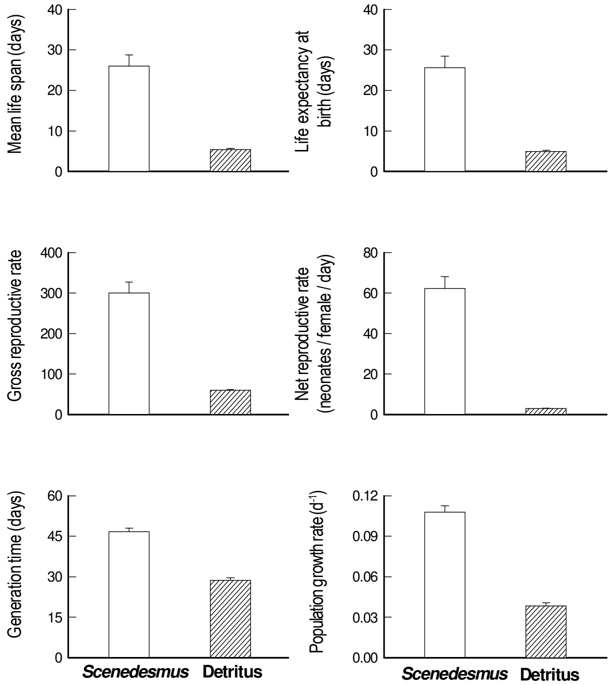

Fig. 4: Selected life history variables (AALS: average adult lifespan (days), ALE: adult life expectancy (days), GRR: gross reproductive rate (offspring female^{-1}), NRR: net reproductive rate (survival-weighted offspring female^{-1}), GT: generation time (days) and the rate of population increase (*r*) per day) of *Elaphoidella grandidieri*. Shown are mean ±SD (n=4)

Acanthocyclops americanus (0.3-0.6 d^{-1} Enríquez-García, 2009) but higher than that of *Eucyclops serrulatus* (Nandini and Sarma, 2007). Several studies on marine and freshwater harpacticoids indicate that they have low growth rates. Just to mention a couple, O'Doherty (1985) showed that the freshwater *Bryocamptus schokkei* has apopulation growth rate of 0.03-0.05 d^{-1}, while Tawfiq *et al.* (1997) showed that the marine *Tisbe furcata* had growth rates of 0.07-0.13 d^{-1}. Nevertheless, ease of maintenance is an important factor while selecting a species as live food for aquaculture (Lee *et al.*, 2005).

The proportion of egg bearing females declined with an increase in population density, probably due to the intense competition for the limited food resources as has been observed in several other zooplankton taxa (Lampert and Sommer, 2007). During our experiments we found that less than 10% of the algae left after 24 hr. It is advisable to regularly harvest the adults from the *E. grandidieri* population in order to keep the population density of the adults below the 2 ml^{-1} limit and achieve higher growth rates. It has been shown that crowding lowers female productivity, egg viability, and naupliar production in copepods (Williamson and Reid, 2001). The peak population density we obtained was more than 10,000 l^{-1} and could have been higher had we separated the adults from the naupliar and copepodite stages in order to avoid competition.

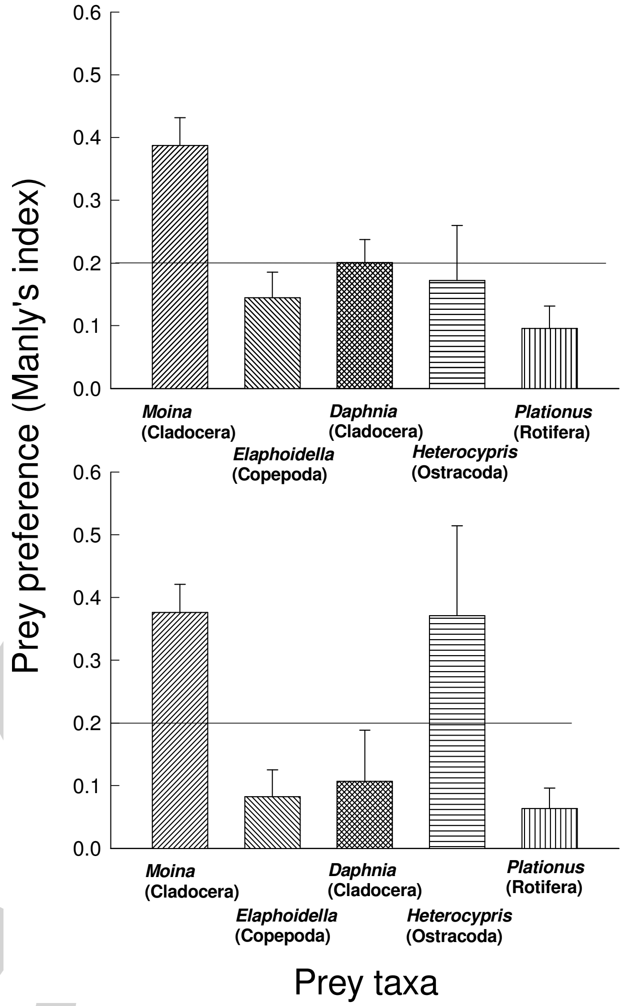

Fig. 5: Prey preference of *Ameca splendens* larvae and *Ambystoma mexicanum* tadpoles offered *M. macrocopa, E. grandidieri, D. pulex, P. patulus* and *H. incongruens.* Shown are mean ±SD (n=4)

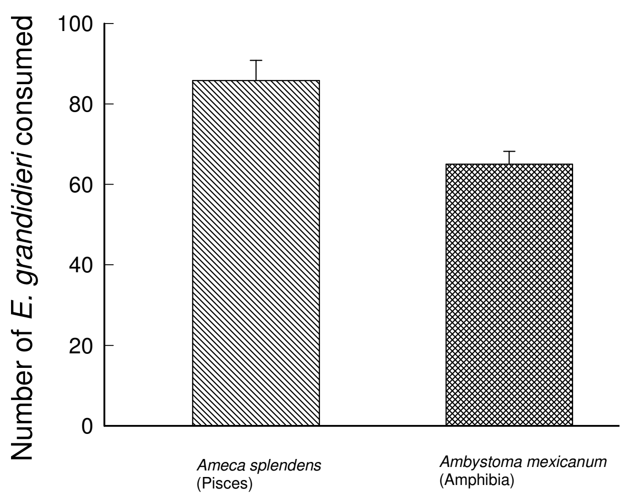

Fig. 6: Prey consumption by *Ameca splendens* larvae and *Ambystoma mexicanum* tadpoles offered *E. grandidieri* alone. Shown are mean ± SD (n=4)

Separating adults from the developing stages can be achieved by using special culture vessels with screens of 100µm pore size.

The survivorship and fecundity of *E. grandidieri* was similar to that observed in other copepods on a diet of fresh *S. acutus* (Williamson and Reid, 2001) but it was significantly lower on a detrital diet. Although harpaticoids feed on detritus in nature it has been shown that detrital diets are not nutritionally adequate (Roman, 1984). There is a more than 3 fold decrease in survivorship on detritus; this is in accordance with the nutritional deficiency hypothesis which states that fecundity and growth is lower on nutritionally inadequate diets (Pond *et al*., 1996). *Elaphoidella grandidieri* has a high fecundity of more than 300 neonates per female, which is considerably higher than that reported for the cyclopoid *Eucyclops serrulatus* (Nandini and Sarma, 2007) but close to the range of 400-700 reported for *Calanus helgolandicus* (Roman, 1984) or the predatory cyclopoid *Acanthocyclops americanus* (Enríquez-García *et al*., 2010). Although the generation time of around 30 days is quite high, the high gross reproductive rate offsets this disadvantage, resulting in an overall growth rate of a 0.1 d^{-1}, which is in the range reported for several copepods (Allan, 1976). It remains to be tested whether the survivorship and fecundity could be higher on other green algae, since it has been shown that *Scenedesmus* is often not an adequate diet for several copepod taxa (Hart and Santer, 2006).

In order to confirm the viability of *Elaphoidella grandidieri* for aquaculture we tested whether it would indeed be consumed by larval stages of vertebrate predators. Both, the larval fish *Ameca splendens*, and the axolotl, *Ambystoma mexicanum*, preferred *Moina macrocopa* to *Elaphoidella*. It has been well documented that *Moina macrocopa* is an attractive species for aquatic vertebrate predators because of its size, visibility, ease of capture, high protein and low ash content (Zaret, 1980; Kibria *et al*., 1995; Peña Aguado *et al*., 2009) and is the prey of choice. Nevertheless, when *E. grandieri* was the exclusive prey offered, both predators consumed it well which indicates that this species could be effectively used as live food for fish larvae.

The three most important factors to be considered before selecting a species as a live prey for aquaculture purposes are its population growth rate, maximal population density and the biomass of each individual. The biomass of each adult of *E. grandidieri* is 2.8 µg, about four times less than an individual of *M. macrocopa* (Nandini and Sarma, 2006). However, the fact that it can reach and maintain very high densities compensates for the individual weight of each copepod. Thus, although *M. macrocopa* appears to be more useful as live prey, it is not as easy to culture as *E. grandidieri* is. In order to realize the full potential of *E. grandidieri*, further studies are needed on the growth of fish larvae fed this harpacticoid, its nutritional value and standardization of techniques for maximal growth rates in large scale cultures.

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