

Potential of *Sepedon ruficeps* Becker (Diptera: Sciomyzidae) larvae for biological control of intermediate snail hosts of schistosomiasis in Benin (West Africa)

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Abstract

Sciomyzids (Diptera) are malacophagous flies which have potential to be used as biological control agents of gastropod vectors of parasitic diseases. *Sepedon ruficeps* was previously identified as a potential biocontrol agent easy breeding and plastic enough to develop in different habitats. In this study, we investigated firstly the influence of prey (larval stage, species, size and parasitic status) and secondly the sex-ratio (number consumed, metamorphosis stage duration, length and width of pupa) on the predation activity of larvae along the complete larval development of *S. ruficeps*. The capacity of *S. ruficeps* was confirmed in the current study to be an effective predator of *Bulinus globosus* and, moreover, the role it can play in controlling *Biomphalaria pfeifferi* and *Bulinus globosus* was highlighted, a worldwide gastropod acting as intermediate host for many pathogen trematodes. *B. pfeifferi* was significantly more killed than *B. globosus* snail's species used in the trial and the parasitized specimens were more attacked than healthy ones. The prey consumption influences the sex-ratio, metamorphosis stage duration, length and width of pupa in a laboratory environment.

Keywords: Larval predation, Snails killed, parasitic diseases, Sciomyzidae Diptera, Sex-ratio, Benin.

1. Introduction

Evidence gathered by [1, 2] suggested that some species of Diptera Sciomyzidae as *Sepedon* spp., may be used as biocontrol agents. In fact, their larvae are malacophagous and many species are voracious predators of aquatic and terrestrial snails [3, 4, 5, 6, 7, 8]. The point of looking for a biological control of schistosomiasis is enhanced by the huge impact of this disease for human health and by the appearance of resistances to chemical control in the worm populations. There is a special concern for Praziquantel that could be put out of use [9, 10]. A high number of freshwater snails are implied in the cycle of pathogenic trematodes [11]. Consequently, the potential biocontrol agents have to be searched in the commonest, widespread and broad spectrum diet snail killing species. First of all, the capacity of predation by fly larvae needs to be assessed. [12], described factors that affect the feeding preference of praying mantis as a predator. According to other authors who studied other species, the metamorphosis stage duration can vary between males and females, with males often having shorter

durations, [13, 14, 15]. Sex ratios are a fundamental trait of sexually reproducing populations. Consequently, sex ratio is one of the most studied traits of natural populations, and the theory of sex allocation is one of the most successful in evolutionary ecology [16, 17, 18]. Most studies on Sciomyzids community structure rarely report sex ratios. Few studies were quantitatively assessed on Sciomyzidae sex ratio. [19] showed a sexual dimorphism in size of adults and puparia of *Tetanocera ferruginea* (Diptera Sciomyzidae).

Sepedon ruficeps Becker, 1923 is a common African species with a large distribution, whose larvae are known to kill almost systematically snails they meet without completely consuming their preys [20, 21, 22, 23]. The biology was described by [8]. Previously, *S. ruficeps* was identified as a potential biocontrol agent easy to rear and plastic enough to develop in different habitats [24].

As the efficiency of the larvae to feed upon various snails will determine their effectiveness as biocontrol agents, laboratory experiments were undertaken to determine the influence of (i) the metamorphosis stage of the fly larvae, (ii) the size of the snail preys, (iii) the identity of the snail species and (iv) the parasitic status of

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the snail preys on the predation exerted by *S. ruficeps* larvae. The current study proved that differential parameters of predation can modify the adult sex ratios in a laboratory condition.

Material and Method

Sepedon (P) ruficeps adults were caught at eight localities in the Southern Benin from August 2014 to July 2015 by sweeping above the low vegetation (Table 1) and were identified with [20] key. They were reared in plastic transparent boxes containing water, food. The eggs laid were placed in Petri dishes and allowed to obtain first-stage larvae. Then, neonate larvae were individually put in separate vial containing natural water and varying sizes of two aquatic snails. These species were *Biomphalaria pfeifferi* (Krauss, 1848), and *Bulinus globosus* (Morelet, 1866). *B. pfeifferi* was caught in Sonon, city located in the Northern Benin (35 km from Parakou city), and *B. globosus* from capture sites of *S. ruficeps*. All breeding were made at 32°C under the natural photoperiod (12/12).

To investigate the influence of prey size on predation, snails were divided into three size classes according to the shell diameter: small (<3 mm), medium (3-7 mm) and large (>7 mm). The parasitic status of *B. pfeifferi* and *B. globosus* was determined by binocular observation after their exposure to intense light (40 W during 24h) inducing the emergence of *Schistosoma* larvae (cercaria).

In a first experiment, ten specimens of each of the first-, second- and third-stage fly larvae were individually placed in Petri dishes containing filtered water brought from the snail habitats. The number of snails killed and eaten by each larva was daily counted; the dead snails were removed and replaced by living specimens. Each larval moult by the presence of its exuvia was noted and conserved. The experiments were stopped during the pupa stage. Each experiment was replicated 30 times.

In a second experiment, ten third-stage larva were placed in presence of 25 large (size, >7mm) living *B. pfeifferi* and *B. globosus* with a similar parasitological status. Only *B. pfeifferi* and *B. globosus* were used because these species are known to be intermediate hosts of the schistosomiasis in Benin [23]. Each experiment was replicated 7 times.

In the third experiment, the influence of predation on the adult sex ratios in larval competition was studied. *B. globosus* of the large class in presence of (5; 10; 15 and 20) L3 were respectively separated in tubs 50 ml. The experiments were stopped with emergency of the imago. Two parameters of puparia (length and width) were measured. The measurements were obtained as distance between the two extremities of puparia. The number of snails consumed, pupal stage duration, size of each pupae and emerging sex were noted. Each experiment was repeated 5 times.

Statistical analysis

Data obtained were statistically analyzed. All analyses were performed using [25] software.

Two to three-way analysis of variance (ANOVA) was performed of several factors followed by the student's Least Significant Difference (LSD. test) allowed to estimate the variation of the number of snails consumed by a fly larva during each developing stage according to the size, the stage and the species of snail (library (Agricolae).

Logistic regression linear analysis was conducted to formulate a model by which the sex ratio of the larvae can be predicted by the number of snails consumed, the stage of larva, the size and the species of snails. Least squares sense was used to assess the correlation of sex ratio and to know the coefficient of determination and the regression line of sex ratio as a function of the different variables.

Table 1: Geographical positioning of catch locations of *Sepedon ruficeps*

Codes	Stations	environment	Geographic coordinates
Loc1	Cocotomey	Temporary	2°26'E ; 6°21'N
	Pahou	Permanent	2°12'E ; 6°37'N
Loc2	Djeffa	Temporary	2°36'E ; 6°23'N
	Accron	Permanent	2°42'E ; 6°27'N
Loc3	Za-zounmè	Temporary	2°13'E ; 7°13'N
	Djidja	Permanent	1°55'E ; 7°21'N
Loc4	Dah-Daho	Temporary	2°17'E ; 7°45'N
	Wlé-Wlé	Permanent	2°19'E ; 7°46'N

Results

Feeding behaviour of *S. ruficeps* during its larval stages

The number of snails killed by second or third fly stage larvae was significantly greater than the number of snails killed by the first stage larvae ($P<0.05$) (Figure 1). In comparison, second and third stages larvae consumed 2 and 5 times more than the first stage respectively. Third stage larvae attacked with success snails of all sizes.

The number of small and medium snails killed by flies during their larval development was greater than the number of large snails ($P<0.05$) (Figure 2). The number of *B. pfeifferi* killed by flies during their larval development was greater than the number of *B. globosus* ($P<0.05$). *B. pfeifferi* is much more consumed than *B. globosus*; the number of snails consumed increases according to the larvae developing stages and decreases according to larvae size.

The parasitic status had no influence on the predation by third stage larvae whereas parasitized *B. pfeifferi* were more attacked than non-infected snails (Figure 3). Moreover parasitized *B. pfeifferi* were more attacked than parasitized *B. globosus*.

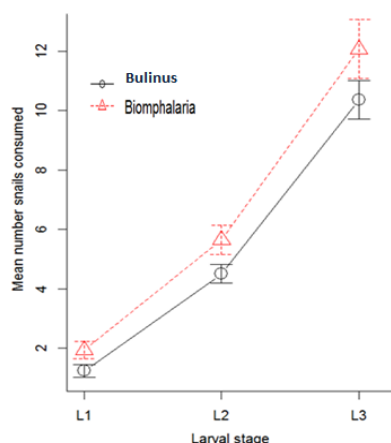


Fig. 1 Number of snails killed during the three larval stages

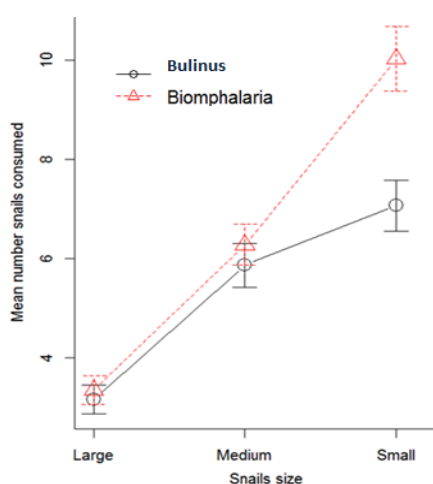


Fig. 2 Number of snails killed according to the size and species of snails

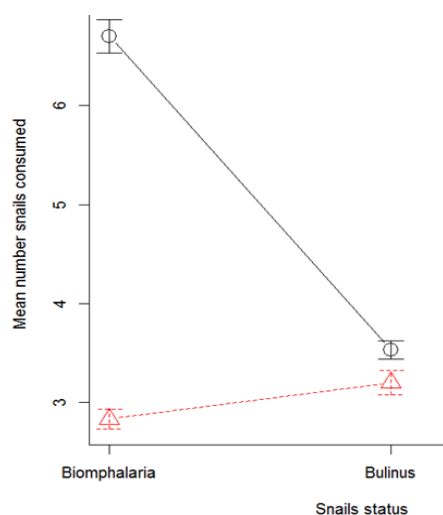


Fig. 3 Number of killed snails according to their parasitic status for *B. pfeifferi* and *B. globosus*

The output of the R software of the ANOVA of the number of molluscs consumed as a function of the larval stage, size of the larva and the species is as follows:

Aov (formula = Number consumed ~ Stage * Species * Size, data = pr).

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Df Sum Sq Mean Sq F value Pr(>F)
Stage      2  8574   4287 5931.78 < 2e-16 ***
Species    1   190    190  262.38 < 2e-16 ***
Size       2  2516   1258  1740.39 < 2e-16 ***
Stage: Sp   2    23    12   15.99 1.8 < 2e-07 ***
Stage: Size  4   656    164  226.75 < 2e-16 ***
Sp: Size    2   213    106  147.13 < 2e-16 ***
Stage: Sp: Size 4    63    16   21.81 < 2e-16 ***
Residuals 522  377     1

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 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

This result shows that the number of snails consumed varies according to species, larval stage and size. All variables show significant effect. This variation is observed in the following table 2.

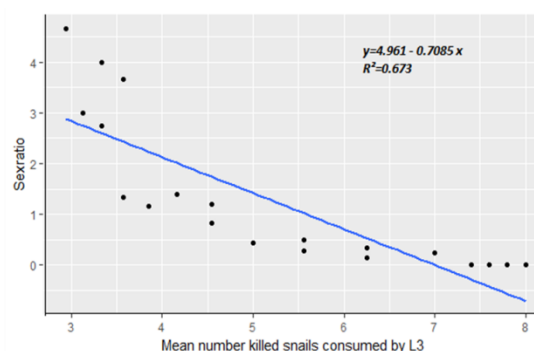
Table 2: Modalities of Anova model fitted for the number of killed snails

	Modalities	Means	IC-95%
Species	<i>B.globosus</i>	5,37a	5,26 - 5,47
	<i>B.pfeifferi</i>	6,55b	6,45 - 6,55
Larval stage	L1	1,58a	1,45 - 1,71
	L2	5,07b	4,94 - 5,20
	L3	11,22c	11,02 - 11,34
Size	Small	8,54a	8,41 - 8,67
	Medium	6,07b	5,94 - 6,20
	Large	3,26c	3,13 - 3,39

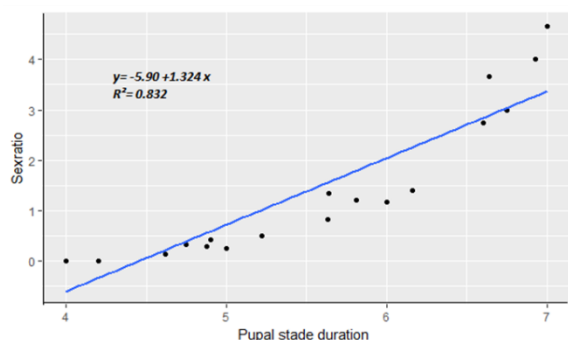
In columns the averages bearing the different letters differ significantly.

Factors affecting the Sciomyzids sex ratio

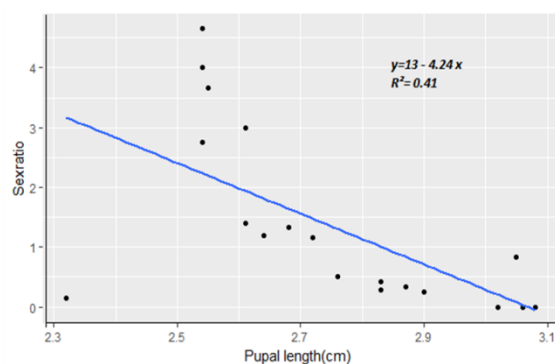
In all species examined on larval competition, male are smaller in size than females. The average amount of time by a female is short than in the male.



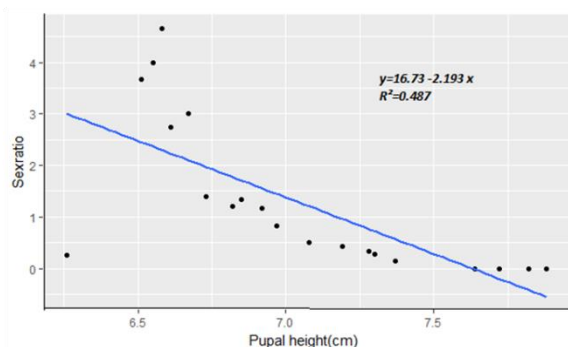
(1)



(2)



(3)



(4)

Fig. 4 Regressions between sex ratio and mean number of snails consumed, pupal stage duration, length and height

The linear least squares regression models of sex ratio as a function of the number of snails consumed (1), pupal duration (2), pupal length (3) and pupal height (4) are all significant ($p < 0.05$).

The model (1) explains 67.3% of the variability of the sex ratio ($R^2 = 0.673$) and the adjustment line shows that the sex ratio decreases with the average number of snails consumed. This makes it possible to say that more females are obtained when the number of snails consumed is high.

A significant difference was noted between males and females over the pupation period.

Thus, 83.3% of the sex ratio is explained by the regression model (2) with ($R^2 = 0.832$) and the adjustment line shows that the sex ratio increases according to the pupal duration. It follows from this model that when the pupal duration is long more males are obtained.

The Models (3) and (4) account for 41% and 48.7% of the variability of sex ratio, respectively. This means that 0.41% and 0.487% of the sex ratio is explained by this model. A significant difference is noted between pupal length and height ($P < 0.05$). These two models show that the sex ratio decreases according to the length pupae and the size. More females are obtained when the pupae length and height are small. An interaction between the three parameters studied was observed, implying that the number of snails consumed, the duration of pupation, increased the size of the pupae in females and these parameters were reduced in males.

Discussion

Feeding behaviour of *S. ruficeps* during the larval stages

In the present study, attempts were made to record the biology, the feeding behaviour and the predatory efficiency of *S. ruficeps*.

The predation by *S. ruficeps* was dependent on its larval stage as well as on the characteristics of its preys. The number of killed snails significantly increased with the age of the fly larvae and the third stage larvae killed five times as more snails than did the first stage. It was already shown that older larvae are more effective in the destruction of snails [26]. Third stage larvae especially consume more preys [27]. This increasing appetite may be due to increasing of nutritional needs [27, 28]. Although some larvae did attacked and killed snails continually without eating. This strict killer behavior was already point to some *Sepedon* larvae by [3]. *S. ruficeps* proved to be a generalist predator and attacked all two prey species. Only first and second stage larvae were not able to attack large prey size. *B. pfeifferi* were more attacked than *B. globosus*. This gradient may be due to differences in the capacities of snails either to flee or to defend themselves with the secretion of mucus [29]. The lower capacity of *S. ruficeps* to kill *B. pfeifferi* may be related to differences in the biogeographic origin of species. *B. pfeifferi* and *B. globosus* are African species [30, 31]. The parasitic status was shown to have an influence upon the predation of *B. pfeifferi*. That may be explained by the slower movements of parasitized snails. Alternatively, choice by the larvae can be caused by chemical emissions by the parasitized snails whose metabolism is altered. Haematophagous Diptera is known to be able to locate their hosts by cues and to detect various organic compounds [32, 33]. Although the results of laboratory experiments need to be confirmed by field experiments. The predatory efficiency of *S. ruficeps* was documented with other snail species [8, 22, 28, 34, 35]. Along its complete development, a fly larva consumes up to forty snails belonging to Lymnaeidae, Physidae or Planorbidae. Current study confirmed the capacity of *Sepedon* to be an effective predator of *Bulinus spp.* [36] and, moreover, the role this species can play in controlling *B. pfeifferi* which belongs to the five

Planorbidae species is highlighted. *Bulinus spp.* can be considered as worldwide pests because of their role in the transmission of parasites [37].

Factors affecting the Sciomyzids sex ratio

The effect of predation on sex ratio of Sciomyzidae (*S. ruficeps*) was investigated. An experiment carried out in the laboratory shows that the sex ratio of *S. ruficeps* in larval competition tends in favor of females.

Many parameters could induce the sex ratio variation. Usually, the male is smaller than the female. According to many authors, the determination of sexes is based on few parameters such as average weight [38], color of the puparium [39], examination after a non-toxic chemical treatment [40], external morphological characters [41]. In this study, the theory is based on the consumption that increases the predation risk and influenced the sex of adult sciomyzids.

The determination of sex of Sciomyzidae is an important factor in studies of the basic biology of these flies. Few studies have been mainly focused on the sex ratio or sexual dimorphism of Sciomyzidae [19]. Differential predation can modify the adult sex ratios in a laboratory environment. Adult sciomyzids sex ratio is as a function especially of the number of snails consumed, pupae duration, pupae length and pupae height. For a fixed amount of prey, the duration of development, the sexes of the adults obtained are correlated with the intensity of the larval competition. There is a clear correlation between the size of the pupae and the sex of the imago obtained. Similar results were observed by [19] who reported that three parameters (length, width and weight) were highly different between the sexes. Variations in sex ratio are observed mainly when there is larval competition in the sciomyzids by the growth of the number of females. Thus, according to [42], the number of males in *Anopheles stephensi* (Diptera: Culicidae) decreases, whereas the number of females decreases in hymenoptera [43, 44]. Food and larval density are therefore determining factors. In mosquitoes, for example, the main limiting factor is food and not larval density [45]. As other authors have shown, a significant interaction between density and sex was present in *Ae. Aegypti* [46].

With the model considered in this study, it is possible to have male and female of Sciomyzidae simply with the larval food. This model clearly indicates that these parameters were an important factor when predicting sex.

Conclusion

The specific high mortality inflicted to parasitized *B. pfeifferi* was specially promising. The high survival rate obtained for third stage larvae during the experiment could justify using this late stage for massive release in the wild. The capacity of *S. ruficeps* to feed on various

snail species could be an advantage for biocontrol programs: it makes easier the breeding mass; it should also help the potential control agent [47]. These observations suggest that these sex ratio variation parameters of Sciomyzids could potentially be used to select adult or pupae sex to biocontrol programs.

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