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LABORATORY OF ENTOMOLOGY**

**DETERMINATION OF FOOD CHAIN LENGTH
USING THE HYPERPARASITOID *GELIS AGILIS***

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Period: 2

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JANUARY 2011

ABSTRACT

Food chains reflect the interactions among species represented by links, and in turn, the number of links among these species reflects the trophic levels in a food chain. Moreover, the function of species of each trophic level affects other trophic levels above and below them. The interactions among species along with other factors such as the availability of food resources, host-prey size correlations, energy transfer efficiency, community organization, habitat stability and ecosystem size, affect the length of a food chain. Until now researchers have found that food chain length in endotherm-species ecosystems, usually reach no more that four levels. This study determines the food-chain length in an ectothermic-species food-chain, using the hyperparasitoid insect *Gelis agilis*, chosen for its general feeding preferences. Interestingly, I found out that *G. agilis* can parasitize and develop on con-specific body tissues, parasitizing host cocoons already parasitized by *G. agilis* individuals, thus lengthening the food chain up to the 5th and even to the 6th trophic level. The ability of organisms in converting nutrients plays a significant role in their development. This study showed that *G. agilis* is a highly efficient hyperparasitoid in nutrient conversion, an important factor for food chain length. Moreover, this study also demonstrated the importance of biology and abundance of species in a community, regarding the way these parameters affect food chain length. The correlation between body mass and number of individuals at each level is remarkably different between insect and mammal-based communities. Therefore, the factors limiting food chain length are different for different types of ecosystems.

Keywords: *Gelis agilis*, Food-chain length, hyperparasitoid, ecosystem, trophic levels.

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1. INTRODUCTION

In nature there are different species that occur in the same environment and that constitute a community. A community includes organisms that may occupy different trophic levels (Townsend 2003). The basal species of a chain are the primary producers, and have no other species as prey, while the top species of a chain are those that are not consumed by any other species (Schoener 1989). All communities start with the primary producers (plants) that in turn are eaten by herbivores, and herbivores in turn are eaten by carnivores and at the end of food chain are omnivores that eat both herbivores and carnivores (Cohen and Luczak 1992). Post (2002) claims that studying food chains is important in order to understand the structure of communities and the ways that ecosystems function. Food chains reflect trophic interactions among species represented by links showing which species consume other species. Therefore, the vertical number of these links represents the number of the levels in a food chain (Brown and Gillooly 2003).

Ecologists have posited several theories to determine food chain length. Elton (1927); (cited in Sterner et al. 1997) and have suggested that food chains in terrestrial ecosystems generally have no more than three to four levels. However, food chain length can go higher due to cycles. A cycle refers to the case when one species consumes individuals from the same species within a food chain (Cohen and Luczak 1992); (Ulanovicz and Kamp 1979, Cousins 1987 and Burns 1989 cited in Sterner et al. 1997). Trophic interactions are changing according to food chain length and as a consequence the community structure also changes. The reasons behind these changes are the cycling of nutrient resources and atmospheric C exchange affecting higher-level predators including those that humans consume (Post 2002). Other authors had different approaches determining food-chain length, for example, according to Kim (2006) food chain-length is determined and ultimately constrained by the energy or nutrient-transfers within it. Pimm and Kitching (1987) claim that the length of food chains is positively affected by the energy resource inputs. According to their research on rain-forest species including beetles, mites, mosquitoes and frogs, the number of predators as well as the number of links in a food web increased when the energy resources input increased. Brown and Gillooly (2003) suggested that the longer a food chain becomes, the more the body size increases, whereas the number of each species declines while the biomass quantity stays the same. For example in an aquatic ecosystem starting from phytoplankton, the chain goes to zooplankton and ends in large predatory fish. Indeed, the largest marine predator ever to exist was probably the Megalodon, a prehistoric shark that exceeded 20 m in length (Personal communication with Harvey J. A.)

Authors express different opinions in predicting food-chain length, but the question is, what are the real constraints on it? Many authors have tried to number and explain the factors that limit

food-chain-length. However, these factors may not always function alone; they can be determined by a combination of factors. Factors influencing food-chain length are: **(i) Availability of food resources:** resources should be adequate for development and survival of organisms in the next trophic level. However, Post (2002) claims that the availability of food resources that the ecosystem provides can be a constraint of the food-chain length, because, the more resources are available at lower levels, the less necessary is for the top predators to search for food at higher levels, thus keeping the food-chain short. **(ii) Host-prey size correlations:** in terrestrial and aquatic ecosystems the predator is usually larger than the prey (Kim 2006). For example, the Gray wolf in U.S. consumes relatively smaller mammals like the white-tailed deer and the snowshoe hare. In a reverse case, where the Gray wolves would have to feed on larger prey, (e.g. moose, caribou), they would risk their life during hunting (U.S. Fish and Wild life Service 2006). **(iii) Energy transfer efficiency:** each species of the trophic chain has to be efficient enough to transfer the available energy into their own body-tissues. Post (2002) suggests that if both energetic efficiency of organisms within a food chain and the amount of resource availability increases, then the food-chain length also increases. **(iv) Community organization:** the community organization depicts which types of species are involved in an ecosystem that determine the interactions among predators and prey. Throughout history, community organization has evolved allowing different species to enter the community or even go extinct from the community, thus affecting the predator-prey interactions (Post 2002). **(v) Habitat stability:** as the stability decreases, the length of the food chain increases, whereas, when the number of species remains the same there is stability in longer food chains (Sturner et al. 1997). And **(vi) Ecosystem size:** in natural aquatic and mammal-based communities, the size of an ecosystem affects the variability and availability of prey, offering to the predators more or even less choices influencing the number of levels within a trophic chain (Schoener 1989). For example, Roger and Segelken (2000) suggest that the small nutrient-rich and highly productive lakes have shorter food chains than the larger low-productivity, crystal-clear lakes.

However, not all communities follow the same rules. Communities that include insect populations may have longer food chains. According to Elton (1927); (cited in Cousins 1987) the food chain pyramid is expressed in terms of biomass where the weight of predators is lower than the weight of herbivores and the weight of herbivores is lower than the plant mass. Cousins (1987) suggests that vertebrate endotherms need large amounts of prey to survive and reproduce, thus for a top-level predator, many prey individuals are required during its life time. In contrast, for ectothermic animals such as insects, top-level predators/hyperparasitoids need only a few insects as prey for their survival and reproduction. Nutrient and energy conversion is different in insects and mammals and aquatic based ecosystems, thus the trophic chain length should also be

different and possibly longer in trophic food chains consisting of insects. For example, few plants can support the life of hundreds of herbivores potentially supporting large numbers of parasitoids that in turn support many hyperparasitoids. Thus, going higher in the trophic chain the size and the number of predators/hyperparasitoids decrease relatively slowly.

Plants offer only limited amounts of nitrogen and proteins for herbivores. These nutrients travel from plant tissues to herbivores to higher trophic levels, such as predators and hyperparasitoids. The higher up in the food chain, the more optimal concentrations of some nutrients become. Proteins and nitrogen are essential nutrients for parasitoid development and the C: N ratio sometimes differs according to the body size of the host, the phylogeny and the trophic status (Harvey et al. 2009a). Different insect species in a linear food chain have different nutritional needs for their development. Especially nitrogen is an important nutrient, which is usually increased in higher trophic levels. However, there should be a balance between the utilization of resources and their availability in the food web and this balance is highly affected by the biology of each species (Goldfray 1994).

For a better understanding of communities, it is essential first to study the biology of the species involved. For example, the body size of a parasitoid is often closely correlated with the size of the host in which it developed (Harvey 2008; Harvey et al. 2009b). Parasitoids have a remarkable ability to efficiently utilize the nutrient resources provided by their hosts. For example, *Lysibia nana* hyperparasitoid adults emerging from *Cotesia glomerata* cocoons have found to be up to 98% of the size of *C. glomerata* adults emerging from similar sized cocoons (Harvey 2008). Harvey et al. (2009a) argue that secondary parasitoids can make use of nutrient resources so efficiently that a trophic chain can go to higher levels than have ever been observed in nature.

This study explores the potential food chain length using the generalist hyperparasitoid *Gelis agilis* (Hymenoptera; Ichneumonidae) (4th trophic level). *Gelis agilis* is a wingless solitary asexually reproducing hyperparasitoid of e.g. *Cotesia* species. It has also been reported that *G. agilis* can attack and parasitize moth pupae and spider egg sacs (Jervis and Kidd 1986; Rivero and West 2005). *Cotesia rubecula* (Hymenoptera: Braconidae) (3rd trophic level), is a primary larval parasitoid of the herbivore *Pieris rapae* (Lepidoptera: Pieridae) (2nd trophic level) which feeds on brassicaceous plants including Brussels sprouts cabbage (*Brassica oleracea*) (1st trophic level). Poelman et al. (2009) investigated the insect community associated with white cabbage plants and found that the community consisted of at least four trophic levels.

2. HYPOTHESIS

We hypothesize that *Gelis agilis* being an extreme generalist can develop on con-specific body tissues, allowing it to parasitize and develop in *Cotesia rubecula* cocoons already parasitized by *G. agilis* individuals.

Research questions:

Under this hypothesis the following questions can be posed:

Question 1: Is *G. agilis* able to produce offspring developing on conspecific tissues? If so, are the resulting adults able to reproduce?

Question 2: If *G. agilis* is able to cannibalize and produce viable offspring, how many trophic levels could this system facilitate?

3. MATERIALS AND METHODS

3.1. Plants

Brussels sprouts plants were obtained from Unifarm of Wageningen UR and were used for *Pieris rapae* caterpillar development. In total 15 plants were consumed by the caterpillars throughout the experiments.

3.2. Insects

Cotesia rubecula were reared on *P. rapae* caterpillars. They feed primarily on haemolymph and egress from the host when they have completed their larval development. Completely formed cocoons of *C. rubecula* were collected and placed in cages in a greenhouse compartment, where adult wasps emerged. *Cotesia glomerata* cocoons were obtained from the institute of ecology NIOO in Heteren, and had been reared in the same way as the *C. rubecula* wasps. *C. rubecula* cocoons were used for parasitism because they are relatively larger than the *C. glomerata* cocoons. However, for the reproduction experiment the size of the host cocoon was of low importance, therefore, we used *C. glomerata* cocoons.

Pieris rapae caterpillars were fed on Brussels sprouts plants and were maintained in a climate room ($22 \pm 1^\circ\text{C}$, 50-70% r.h., L16:D8) in Wageningen UR. For parasitism, Brussels sprouts leaves with L1 instar caterpillars feeding on them were placed in the rearing cages of *C. rubecula* wasps for about 1h and 30 min. depending on the number of wasps in the cage. After parasitism, *P. rapae* caterpillars were placed in cages in a greenhouse compartment ($22 \pm 2^\circ\text{C}$, 50-70% r.h., L16:D8) and fed on Brussels sprouts plants until the emergence of *C. rubecula* larvae and cocoon formation. *Cotesia rubecula* cocoons were collected from the cages and transferred to

a Petri dish (10 cm diameter) and placed in a climate cabinet at 10°C to arrest further development. Prior to experiments they were kept at room temperature for several hours.

Gelis agilis hyperparasitoid wasps were originally collected from *C. glomerata* cocoons from *B. oleracea* plants growing in a garden plot near the institute of ecology NIOO in Heteren. Adult wasps were collected and placed in a cage, kept in a climate cabinet at 10°C and fed on honey and water. Adults of these hyperparasitoids feed on carbohydrates containing food such as floral nectar. This food resource is low in proteins and mainly used for maintenance in parasitoids. *Gelis agilis* females host-feed in order to obtain extra protein resources for egg production (Jervis and Kidd 1986; Rivero and West 2005). We allowed the wasps to host-feed by placing in the cage a Petri dish with fresh *Cotesia* cocoons for two days at room temperature several times during their life.

3.3. Experimental protocol

To investigate whether *G. agilis* can successfully develop on con-specific tissues, the following experiment was conducted.

From the collected *C. rubecula* cocoons, 300 cocoons were left at room temperature for a few hours and then numbered and weighed individually on a Metter-Toledo MTS microbalance (accuracy 1µg) before they were offered to *G. agilis* for parasitism. After parasitism they were placed in numbered glass vials and kept in a climate cabinet (10°C; L16:D8).

For parasitism, the cocoons were individually presented to *G. agilis* wasps. Taking into account that *G. agilis* store no more than 3 mature eggs in their ovaries at given time; they were allowed to sting only one cocoon each (Harvey 2008). The whole procedure of parasitism was completed in about 6 hours.

All parasitized cocoons were kept in a climate cabinet at 25°C (L16:D8). Fifty (50) cocoons out of the 300 parasitized cocoons were separated and were left for adult wasps to emerge, while the rest of the 250 cocoons were used for the next parasitism one week later. Ten of the newly emerged *G. agilis* were used for the reproduction test.

The same *G. agilis* individuals parasitized the remaining 250 cocoons again one week later. The procedure of parasitism was repeated in consecutive weeks, and each time 50 cocoons were separated from the sample after parasitism to be checked for emergence and reproduction ability.

Newly emerged wasps were immediately narcotized using CO₂ and then weighed on the microbalance. Every week, of the newly emerged wasps, 10 were used for the reproduction test by offering each of them 15 fresh *C. glomerata* cocoons for 2 days. Prior to the reproduction test, the wasps were allowed to host-feed for one day on 5 fresh *C. glomerata* cocoons and provided

with honey and moist cotton wool.

In order to test the quality of the host cocoons, 50 non-parasitized *C. rubecula* cocoons were placed in a climate cabinet at 25°C, L16:D8. The number of successfully emerging *C. rubecula* adults was recorded.

4. RESULTS

Parasitism and performance of *Gelis agilis* wasps.

Although the mortality ratio of *C. rubecula* cocoons was high, 78%, I proceeded with the experiments following the protocol. After each parasitism, 50 cocoons were separated and allowed to emerge; however, none of them produced new *G. agilis* wasps after the 1st parasitism. Therefore, reproduction ability could not be determined.

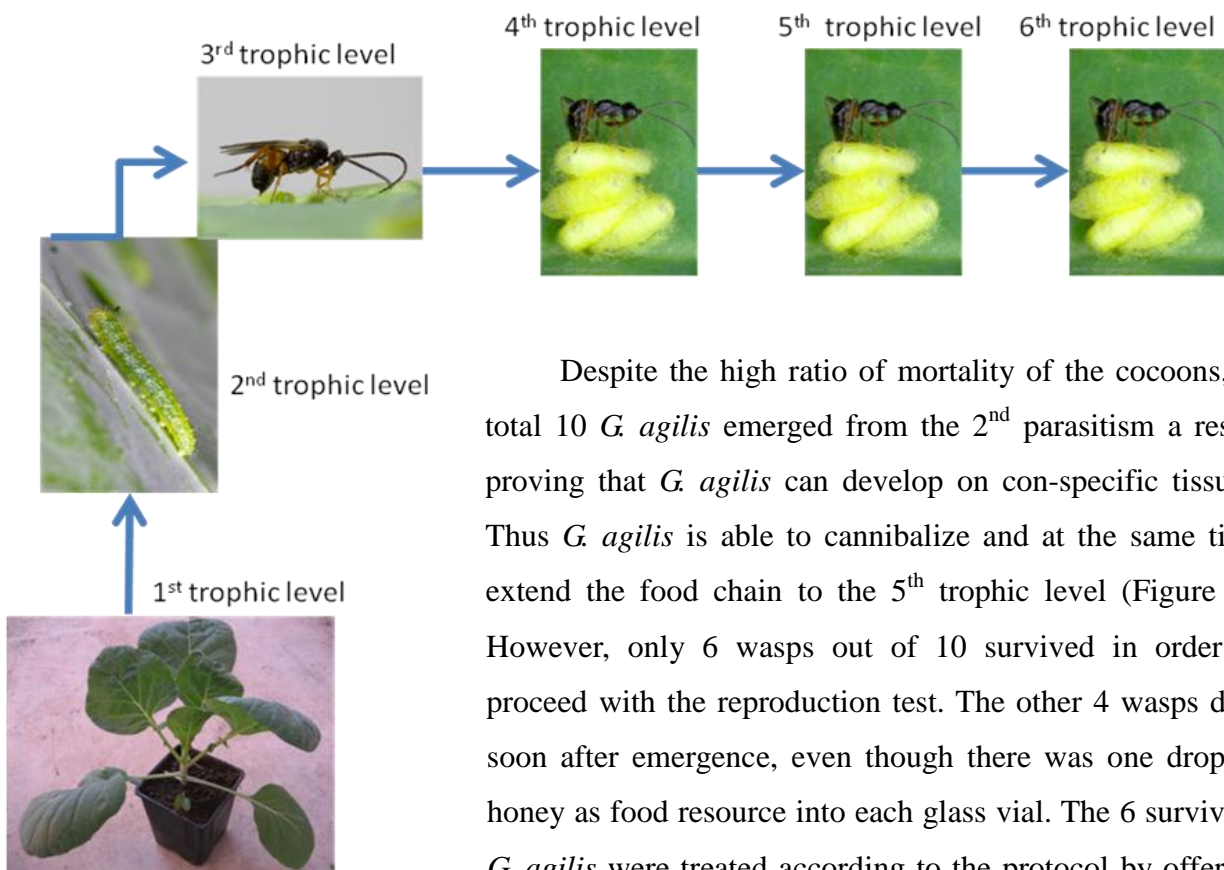


Figure 1 Trophic levels within the study system: Brussels sprouts, *Pieris rapae*, *Cotesia rubecula* and *G. agilis* (Pictures: Tibor Bucovinszky and Hans Smid).

Despite the high ratio of mortality of the cocoons, in total 10 *G. agilis* emerged from the 2nd parasitism a result proving that *G. agilis* can develop on con-specific tissues. Thus *G. agilis* is able to cannibalize and at the same time extend the food chain to the 5th trophic level (Figure 1). However, only 6 wasps out of 10 survived in order to proceed with the reproduction test. The other 4 wasps died soon after emergence, even though there was one drop of honey as food resource into each glass vial. The 6 surviving *G. agilis* were treated according to the protocol by offering 10 *C. glomerata* cocoons to each of them for parasitism. However, none of the wasps were observed stinging any of those cocoons.

Surprisingly, 2 *G. agilis* wasps emerged after the 3rd parasitism, proving the ability of this species to cannibalize and at the same time extend the food chain to the 6th trophic level (Figure 1). However, the only one wasp that survived and treated according to the protocol for the reproduction test, did not sting any of the cocoons offered.

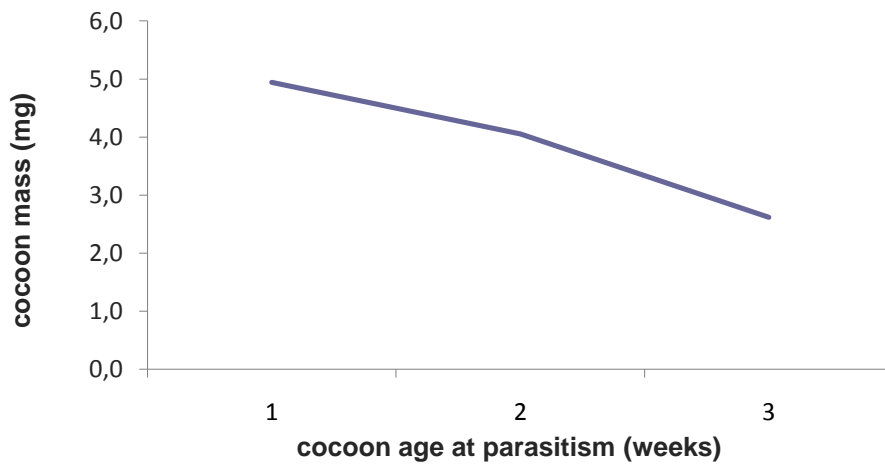


Figure 2 The decline in cocoon mass (in mg) before each parasitism.

Each cocoon was weighed before each parasitism to determine the mass flow during time. The measurements show that there was a decline in cocoon mass (Figure 2). Before the 1st parasitism the average cocoon weight was around 5 mg, around 4 mg before the 2nd and around 2,6 mg before the 3rd parasitism.

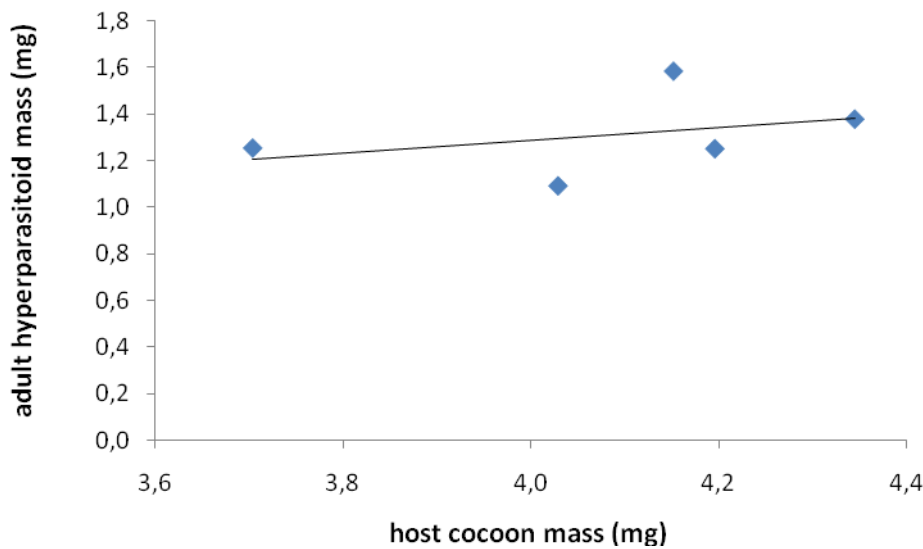


Figure 3 Relationship between cocoon mass (in mg) of five individual *Cotesia rubecula* cocoons and emerging adult *G. agilis* wasps, after the 2nd parasitism.

The weight of only five adult *G. agilis* that emerged after the second parasitism (Figure 3) was shown to be close to its normal weight (Harvey et al. Unpublished). According to Harvey et al. (unpublished) the weight of adult *G. agilis* emerging from *C. glomerata* cocoons is 1.4 mg on average. The lower weight of some *G. agilis* adults was maybe

due to the reduction in cocoon weight from the first until the third parasitism (Figure 2). However, the graph shows no correlation between the host cocoon mass and the adult hyperparasitoid mass. The adult *G. agilis* that emerged from the 3rd parasitism weighed 1.187 mg and emerged from a cocoon weighing 3.770 mg before the 3rd parasitism occurred.

5. DISCUSSION

In this study I determine potential food chain length in a food chain consisting of insects and discuss the results in relation to factors (indicated in bold font) that can influence food-chain length.

Gelis agilis is a wingless insect with an ant-like behavior, thus exploring a relatively small area while foraging. In addition, this hyperparasitoid is a generalist species that is able to develop on a variety of hosts, like spider sacks, and moth pupae along with many *Cotesia* species (Harvey 2009). This study showed that the secondary parasitoid *G. agilis* is able to develop on conspecific tissues, thus lengthening the food chain to the 5th trophic level. Moreover, two individuals emerged from double parasitized cocoons extending the food chain to the 6th trophic level. Although only a small number of wasps emerged, (twelve; 12), *G. agilis* offspring exhibits cannibalistic behavior, showing that it is highly efficient in utilizing different nutrient resources, making it an extreme generalist. However, the few experimental data generated in this study provide only limited evidence on how efficient *G. agilis* is in terms of nutrient conversion. Harvey et al. (2009) argue that important nutrients increase in quality at higher food chain levels. Regardless of the **availability of food resources** at higher levels, the ability of organisms to convert all nutrients play a significant role in their development. This study showed that *G. agilis* development is not constrained by the **energy transfer efficiency** factor. Moreover, considering the facts that *G. agilis* can develop on a range of host species and also on itself, this wasp is therefore only constrained by the **availability of food resourced** regarding the distribution and abundance of host resources in the wasp's habitat and its ability to find them.

The limiting factor **host-prey size-correlations** affect the development of hyperparasitoids and therefore the length of food chains. Parasitoid adult body size often depends upon the host size; i.e., the bigger the host, the more nutrients there will be for the parasitoid and thus it will develop a larger body size. A weight loss was observed in cocoons over time from the 1st until the 3rd parasitism covering a 3-week period, possibly due to water loss in older pupae. However, bearing in mind that each week a *G. agilis* larva was consuming the host larvae inside the cocoon and that *G. agilis* can practically consume all tissues of the host pupa, the loss in adult mass of the hyperparasitoid is expected to be lower but still very similar to the host pupa when developing into an adult (Harvey et al. 2008). Due to small sample sizes, loss in adult size over consecutive parasitisms, was impossible to be determined.

Insects are much more efficient in utilizing nutrients compared with endotherms such as mammals. The correlation between body mass and number of individuals at each level is remarkably different between insect and mammal-based communities.

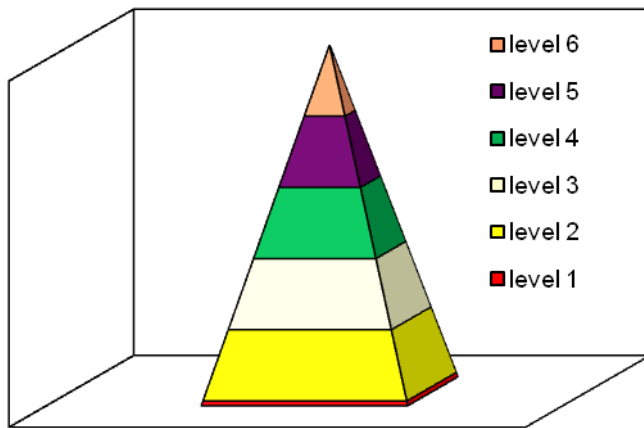


Figure 4 Insect-based community with 6 levels; Level 1: plants as the basal species (20); level 2: the herbivore *Pieris rapae* (300); level 3: the primary parasitoid *Cotesia rubecula* (300); levels 4, 5&6: the secondary parasitoid *Gelis agilis* (300)

In the current study, higher trophic level insect species utilize efficiently the available resources lengthening the food chain up to the 6th trophic level. The basal species, -plants-, are smaller in terms of numbers, efficiently supporting the mass of higher trophic level species (Figure 4).

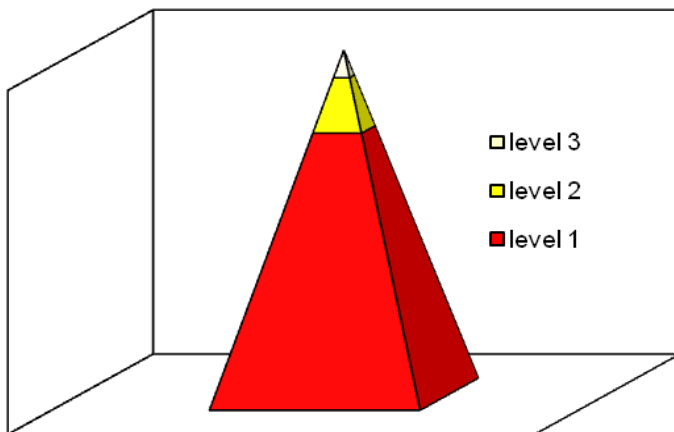


Figure 5 Mammal-based communities with 3 levels: Level 1: plants as the basal species (500); level 2: a herbivore e.g. rabbit (100); level 3: a carnivore e.g. wolf (50).

In mammal-based communities the basal species, -plants-, are considerably larger in mass, supporting lower masses of higher trophic-level species, resulting the short food chains (Figure 5).

According to Post (2002) species involved in ecosystems and the interactions among them, determine food chain length. In the current study system, exploring the food-chain length, the community was structured in a way that there was only one species available as a host and therefore hyperparasitoids were forced to parasitize the only available host species for their own survival. Here we observe that the physiology of this species was not a constraint for reaching the 6th trophic level. By contrast, in nature, the **community organization**, as a factor limiting food-chain, is different compared to optimal laboratory conditions. The diversity of species and the

population densities of species are higher, offering much greater resource choices to top-level species. In nature, due to the higher number of species and the different interactions among them, other hyperparasitoids may compete with *G. agilis* for hosts. Moreover, in nature, large carnivores may also eat many smaller carnivores or herbivores. In a large ecosystem with a high abundance of each species, the large carnivore should choose the smaller prey (the herbivore), which is easier to find, thus keeping the food chain short. The same analogy occurs also in insect-based communities, where i.e. *G. agilis*, consumes unusual hosts due to lack of the first choice hosts, resulting in a longer food chain. However, insect biology is different from mammal biology making the extension of insect-food chains possible.

Ecosystem size and habitat stability, are factors influencing the food chain length. **Ecosystem size** depends on factors such as the size of the area, the biodiversity of species and on other unexplored factors, indirectly affecting **habitat stability**. According to Sterner et al. (1997) when the number of species in a habitat remains the same, the length of the food chain remains also the same. In nature, there is variability in the size of ecosystems, and probably also in habitat stability. However, these factors have not been studied here.

It was expected that there would possibly be reluctance in *G. agilis* to parasitize the same cocoon in consecutive weeks, because *G. agilis* might mark cocoons during oviposition by leaving pheromones on them. I cannot exclude the existence of marking pheromones because of the observed reluctance of some wasps to sting. Alternatively, reluctance to parasitize cocoons can be explained by the poor health of the host. Moreover, the selection of *G. agilis* individuals used for parasitism was randomly made, leading to the possibility that the same individual could sting the same cocoon (containing her own progeny) twice. Nevertheless, *G. agilis* developed on conspecific tissues, behavior not much studied before in parasitoids. However, viability and reproductive ability of these offspring could not be determined in this study.

It is important to mention that at the time when the experiment was conducted, there was a significant problem with the WUR entomological rearing, causing high mortality in the *Cotesia* species and other insects in the department. This rearing problem could explain the low emergence rate of *C. rubecula* and *G. agilis* wasps during this experiment and also the fact that there were no chances for reproduction tests. The quality and health of each species in a trophic level determines the performance of species in the next trophic levels in terms of quality and abundance. For example, Bukovinszky et al (2008) found out that the quality of plants; 1st trophic level, influence the other trophic levels, either directly (body size of aphids) or indirectly (density of hyperparasitoids).

6. CONCLUSIONS

Gelis agilis is an extreme generalist and highly efficient in nutrient conversion, being able to develop in con-specific body tissues, thus lengthening the food chain up to the 5th, but also to the 6th trophic level.

The biology, the number, and abundance of each species in a community affects the inter-links in food chains.

Communities consisting of ectotherms, like insects, are different from communities consisting of endotherms, like mammals, with respect to their biology and physiology. Consequently, the constraints determining food chain length are different for these two types of communities.

7. FUTURE PERSPECTIVES

Due to severe problems regarding the health of insects in these experiments, it is recommended that this study is repeated with healthy insects in order to develop a better understanding of the interactions among insect species in this community.

Gelis agilis can indeed develop on con-specific tissues, which is a behavior not much studied before. However, the viability of these offspring is unknown. Performance tests, fecundity and longevity measurements should be done.

A deeper approach of the factors determining food-chain length might further develop the theoretical basis determining food-chain length.

ACKNOWLEDGMENTS

First of all, I would like to thank my supervisors Rieta and Jeff for the confidence they provide and the inspiring discussions we made. Thanks also to Ecological institute NIOO and WUR Entomological rearing group for providing insects for my experiments. Thanks to Hans and Tibor from “Bugs in the Picture” for the nice pictures. Thanks to my friends who made my stay in Wageningen unforgettable experience. Last but not least, I would like to thank all colleges of Ento-group for the relaxing atmosphere during breaks.

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