

Biological and predatory characteristics of *Zicrona caerulea* (Hemiptera: Pentatomidae) on *Altica viridula* (Coleoptera: Chrysomelidae), under laboratory conditions

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Received: Dec., 07, 2024

11(2) 111–120

Accepted: Feb., 11, 2025

Abstract

Hyrceanian forests, which are 25 and 50 million years old, are among the most valuable forests in the world and are known as a natural reserve. These forests play a vital role in protecting biodiversity and maintaining the ecological health of the region. However, the pressures caused by the spread of pests are a serious threat to this forest ecosystem. Therefore, preserving and protecting them is very important. In recent years, the pest *Altica viridula* Weise, as one of the leaf-eating pests of the *Carpinus orientalis* mill., has become an outbreak in some areas of Mazandaran province. By feeding on the leaves, this pest causes the branches and foliage of trees to dry out, reduces forest density and even increases the risk of fire. Managing this pest requires solutions that are not only efficient but also environmentally friendly. The use of natural enemies is one of the effective approaches in biological pest control. In this regard, the species *Zicrona Caerulea* Linnaeus has been identified as a specialized predator of *A. viridula* in recent studies. Adults was collected from the forests of Marzanabad and transferred to the labs of Nowshahr Natural Resources Research Station. The biology and life stages of this predator on *A. viridula* larvae was investigated under laboratory conditions of a 13:11 L: D, 25 ± 2 °C, and $65 \pm 5\%$ RH. The studies were conducted in the laboratory based on a controlled descriptive statistical design. Also, randomization was implemented using 15 containers for replications to allow for a more reliable assessment of the results. The average pre-oviposition length was 7.1 ± 0.5 days, with an average of 20.15 ± 3.42 eggs laid. The embryonic period lasted 15.5 ± 0.7 days and the egg hatching rate was $93.1 \pm 2.18\%$. The average lifespan was 38.2 ± 1.78 days for males and 40.5 ± 2.35 days for females, respectively. Last nymph instar became an adult after 7 ± 0.3 days. An average generation took 44.5 ± 2.90 days to complete. First instar nymphs did not feed. The number of third instar larvae as food for first, 2nd, 3rd, 4th and 5th instar nymphs were 2, 4, 6, 9 and 16, respectively. The percentages of predation were 0, 85.71%, 88.09%, 92.59% and 91.96% for first to fifth instar, respectively. After the emergence of adults, 10 pairs were selected and the predation rate of each pair was monitored daily. The predation rate of male and female was 14.2 ± 0.96 and 14.7 ± 0.7 larvae, respectively. Totally, the percentage of larvae eaten increased from second instar nymphs to adult insects. The importance of this present study is due to the first time in Iran. This species has high potential for use as a biological control agent due to its characteristics such as short lifespan, high hunting power, and good reproduction and its protection, support and release are felt seriously. Previous research has shown the effectiveness of *Z. caerulea* in various regions, but this study aims to fill the gap in knowledge regarding its impact in Hyrcanian forests ecosystems. The primary objective is to evaluate the efficacy of *Z. caerulea* in managing *A. viridula* populations, contributing valuable insights for pest management strategies.

Keywords: Asopinae tribe, forest, leaf-eating beetle, predator, Mazandaran province.

Introduction

The Family Pentatomidae, also called stink bugs or turtle bugs, is the third most speciose family within the suborder Heteroptera and There are 4949 species scattered worldwide (Grazia *et al.*, 2015; Schuh *et al.*, 2020; Ali, 2021; Falcone & Villanueva, 2024). This family contains Asopinae and eight subfamilies (Henry, 2017; Rider *et al.*, 2018; Armando & Raul, 2024). Members of the subfamily Asopinae are set apart from the other pentatomid subfamilies by having robust labium adapted for predatory behavior (De Clercq, 2000, Rider *et al.*, 2018, Brugnera *et al.*, 2024).

Genus *Zicrona* (Amyot & Serville. 1843) is a member of the subfamily Asopinae, they are scattered in Africa, Myanmar, Syria, Equatorial Guinea, Pakistan, Australia, North America, Central America, India, Iran, China, Japan, Turkey, Greece Malawi and Madagascar (Altaf *et al.*, 2017; De Clercq, 2008). They are predator as biocontrol agents and identified on the lengthened rostrum (Joniora *et al.*, 2022; Claver & Jaiswal, 2013). It occurs on low vegetation in many habitats, including grassland and forest.

Zicrona caerulea (Linnaeus, 1758) is recognized worldwide for its beneficial biological and predatory characteristics. This predator plays an

important role in controlling pest populations across various ecosystems and agricultural settings. One of its key biological characteristics is its ability to adapt to diverse environments, allowing it to thrive in different regions around the world. As a predator, exhibits efficient predator behavior, making it an effective natural enemy against a wide range of insect pests. It preys on various pest species, helping to keep their populations in check and contributing to the overall balance of ecosystems. Additionally, is known for its reproductive capabilities and capable of consuming pests at an early stage of development. This reproductive strategy further enhances its effectiveness in controlling pest populations. Overall, the global presence of *Z. caerulea* and its valuable biological and predatory characteristics make it a valuable asset in pest management strategies worldwide, promoting sustainable and environmentally friendly pest control practices (Agasyeva *et al.*, 2016; Mangi *et al.*, 2023).

Agasyeva *et al.* (2023), The population dynamics of the insect *Perillus bioculatus* Fabr and the *Leptinotarsa decemlineata* Say were assessed from 2013 to 2015 and it was observed that in addition to this predator, *Z. caerulea* periodically feeds on the Colorado potato beetle and useful activity of *Z. caerulea* has been repeatedly observed. The results showed that the adapted population of *Z. caerulea* played a major role in reducing the Colorado potato beetle population.

Eggs are black and lays under the leaves. The color of the nymph's abdomen is red with black marks. Medium-sized with adult Length 5–8 mm. Blue–green metallic sheen and dark wing membrane. The blue coloring is unmistakable, not seen in any other bug samples. Head, pronotum are distinctly bluish and legs are all dark. Overwinters as an adult (Grey & Healey, 2016).

Carpinus orientalis (Miller) is known as the eastern *carpinus* and belongs to the plant family Betulaceae and is native to southeastern Europe and western Asia. It is distributed in the upper altitudes of Hyrcanian and Arsbaran forests (Yazdian & Sheikh Aleslami, 2010; Marvi Mohajer, 2019). A small to medium-sized tree that usually grows in hot, dry, sunny areas and on rocks. Among the important values of this type of tree, we can mention the restoring degraded lands and forest landscapes, reducing soil erosion and preventing climate change.

There are few studies about the biology, hosts and distribution of this important predator in the world. This bug collected from Romania and reared on *Melasoma populi*, also, research has shown that

is useful predator of leaf beetles in the genus *Altica*, larvae of various beetles and caterpillars of moths (Gima, 2015). The specimens of *Z. caerulea* in Britain and Mexico collected from *Altica* sp. and the life histories, host data, and distributions are fairly well known for a number of species found in the United States (Rider & Swanson, 2021). In Pakistan This species is predatory on different larvae which are pests on different crops and vegetables, so it is a very beneficial insect and has economic importance (Mangi *et al.*, 2023). One case of predation in Slovenia of *Zicrona caerulea* on *Ophraella communa* LeSage, 1986 larva was also observed (Sipek *et al.*, 2023). from Japan has introduced *Z. caerulea* as a predator (Souma *et al.*, 2023). No study has been done on biology and predation rate in Iran so far. Therefore, the necessity of research in this regard was felt.

The aim of this study is to preserve Oriental hornbeam as an ecological importance tree in the Hyrcanian forests, management and control the pest and also protection and expansion of this predator as biological control agent. So, it is a very beneficial insect and has economic importance. Due to the intense feeding of larvae and adults of *A. viridula* Weise, the leaves dry up and the risk of fire increases, especially at high altitudes and where the weather is hot and dry. For this purpose, identification of natural enemies as an approach compatible with the environment was used as a suitable method to manage this pest.

Materials and Methods

Rearing of *Altica viridula* and establishing a colony in the laboratory

The studies were conducted during the years 2022 and 2023. overwintering adults emerged in nature from late April. The initial population of adult beetles was collected from the main source of infested *Carpinus orientalis* trees in forests of Chalus city, Mazandaran provinces and transferred to the laboratory and after laying eggs larvae reared on fresh leaves of the *C. orientalis*. The test conditions were the normal conditions of the room included 14:10 L: D, 25±2 °C, 65±5% RH. Each rearing container containing a complete pair of male and female insects. To prevent fungal growth, the dishes were cleaned every three days and fresh leaves were provided to larvae and adults (fig 1). Third instar Larvae from the same cohort were used to feed the predator.



Fig. 1. *Altica viridula*: A & B– Overwintering adults mating in nature and in the laboratory; C– Cluster of eggs during hatching in the laboratory; D– First instar larvae feeding of epidermal surface of *Carpinus orientalis* leaves; E– Molting of the first instar larva and transformation into the second instar larva; F & G– Third instar larvae feeding (original)

Determination of predation rates of different instars of nymphs and adults of *Zicrona caerulea* and rearing by feeding on third-instar larvae of the *Altica viridula*

The overwintering adults were collected at the late April 2024 and transferred to the laboratory. The biology and life stages of this predator feeding on *A. viridula* larvae was investigated under laboratory conditions of a 13:11 L: D, 25 ± 2 °C, and $65 \pm 5\%$ RH. The studies were conducted in the laboratory based on a controlled descriptive statistical design. It also implemented randomization using 15 containers for replication to allow for more reliable assessment of the results. After mating and egg-laying, eggs transferred to sterile containers. One-day-old first instar nymphs were transferred individually to experimental units (9 cm diameter Petri dishes) using a soft brush and were fed individually with third-instar larvae of *A. viridula*. The Petri dishes were inspected daily and live larvae were counted. According to the certain number of larvae available, the feeding rate of nymphs and adult insects was determined every 24 hours. Nymphs and adults were transferred to new

dishes every 24 hours. To determine the predation rate of female individuals, the predation rate of 15 male was examined separately from the female. To calculate the predation rate of female individuals, the predation rate of males was subtracted from the average predation rate of each pair, thus separating the predation rates of males and females. After the emergence of adults, the male and female beetles were fed with larvae of the 3rd stage beetles and their predation rate was recorded until death (fig. 2).

The dried samples were mounted for or external morphological analyses with a micros Austria stereomicroscope and the other samples preserved in 75% ethanol. All measurements of the specimens performed in Digimizer software (6.4.0). Rearing predators' technique were performed Mangi *et al.* (2023) method. Voucher specimens were deposited at the Nowshahr Natural Resources Research Station (Botanical Garden), Research Institute of Forests and Rangelands, Agricultural Research Education and Extension Organization (AREEO), Mazandaran, Iran.



Fig. 2. Rearing of *Zicrona caerulea* in the laboratory: A– adults mating; B– Cluster of eggs during hatching; C, D, E– two, three, four and five instar nymphs feeding on the larvae of *Altica viridula*; (original)

Data analysis

Studies were conducted based on a controlled descriptive statistical design. Mean and standard deviation of biological parameters and predation rate was done using Excel 2010 software.

Results

The results indicate significant trends in the effectiveness of *Z. caerulea* as a biocontrol agent and suggest that *Z. caerulea* could play a crucial role in pest management.

Biology and life stages of *Zicrona caerulea*

The average pre-oviposition length was 7.1 ± 0.5 days, with an average of 20.15 ± 3.42 eggs laid. The embryonic period lasted 15.5 ± 0.7 days and the egg hatching rate was $93.1 \pm 2.18\%$. The average lifespan was 38.2 ± 1.78 days for males and 40.5 ± 2.35 days for females, respectively. Last nymph instar became an adult after 7 ± 0.3 days. This predator has five nymph instars and the average lifespan of first, second, third, four, and five instar nymphs is 5 ± 0.5 , 7 ± 0.7 , 7 ± 0.6 , 6 ± 0.4 , and 7 ± 0.3 days, respectively. An average generation took 44.5 ± 2.90 days to complete (Table 1).

Table 1. Biological parameters of *Zicrona caerulea* reared on 3th instar larvae of *Altica viridula* under laboratory conditions at 13:11 L: D, 25±2 °C, 65±5% RH.

Development stages	Min– Max	Mean (±SD)
Preoviposition period (day)	6–8	7.1±0.5
Hatched eggs (percent)	90–95	93.1±2.18
Embryonic period	14–17	15.5±0.7
Nymphs period	24–26	24.6±1.07
Female longevity (day)	38–43	40.5±2.35
Male longevity (day)	36–40	38.2±1.78

The overwintering form is an adult and they appear a little earlier or at the same time as *A. viridula* adults and lay their eggs under the leaves in nature. The first instar nymphs did not feed. The second

and some instar nymphs start their predatory activities at the same time as the larvae of the pest until the first week of July (Fig. 3).

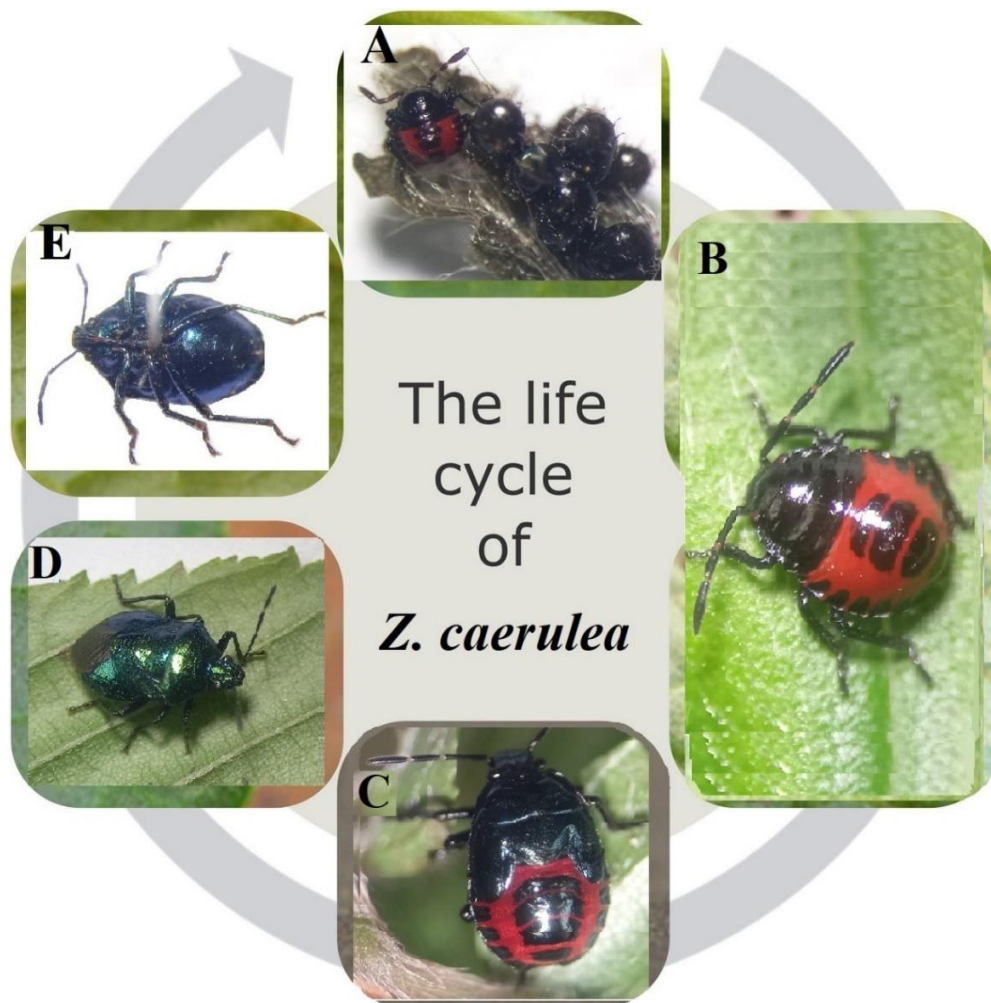


Fig. 3. *Zicrona caerulea*: A– Eggs and first instar nymph; B– three instar nymph; C– five instar nymph; D– Adult (dorsal view); E– Adult (ventral view) (Original).

Predatory percentage of *Z. caerulea*

The first instar nymphs did not feed and had an aggregate behavior. For the second instar nymphs, four number of third instar larvae were given to the nymphs daily as food. Every 24 hours the information about the eaten larvae and the

developmental stage of the nymphs was recorded. With increasing instars to complete the molting period, the number of larvae as prey increased so that 6, 9 and 16 third instar larvae were placed for the 3rd, 4th and 5th instar nymphs, respectively. The percentage of predation was for second instar nymphs to adults is shown in Table 2. Adults, not

only had a somewhat longer feeding period compared to nature, but 16 third instar larvae were provided to them daily. In total, the percentage of

larvae eaten increased from second instar nymphs to adult insects (Table 2).

Table 2. Mean number of 3th instar larvae of *A. viridula* fed upon by different stages of *Z. caerulea* in laboratory conditions at 13:11 L: D, 25±2 °C, 65±5% RH.

Developmental stages (days)	Feeding period (days)	Total number of larvae presented	Total number of larvae eaten	Total feeding of nymphs	Number of larvae eaten daily	percentage of predation (%)
First nymphal instar	5	10	0		0	0
2 nd nymphal instar	7	28	24		3.42 ± 0.7	85.71%
3 rd nymphal instar	7	42	37		5.28±0.7	88.09%
4 th nymphal instar	6	54	50	214	8.33±0.8	92.59%
5 th nymphal instar	7	112	103		14.7±1.11	91.96%
Adults: male	38	608	568		14.2±0.96	93.42
Adults: female	42	672	630		14.7±0.7	93.75

Discussion

This research has demonstrated biology and potential value of *Z. caerulea* for the management of *A. viridula*, but it is clear that small proportion of this species was studied an enormous potential remains to be investigated. Understanding the life of the pest is very important in designing its management strategy. Since *Altica* species are separated based on the host plant (Jenkins *et al.*, 2009, Pettis *et al.*, 2004). In this regard, the management options of this pest are limited. Therefore, a combination of different types of control such as mechanical, biological and chemical control should be used. Species of subfamily Asopinae are obligate predators of other insects. Two of these specialize on chrysomelid beetles; *Rhacognathus punctatus* Linnaeus feeding mainly on the larvae of *Lochmaea suturalis* Thomson and *Z. caerulea* predated the larvae of *Altica* species, while *Troilus luridus* Fabricius is more of a generalist and has been recorded feeding on a range of invertebrates (Bantock, 2016). A study conducted in Italy vineyards, identified *Z. caerulea* as a beneficial natural enemy of *Altica ampelophaga* Guerin and the bug have one generation per year (Fauvel, 1999). Likewise, Chen (1982) reported *Z. caerulea* to be an important natural enemy of larvae and adults of *Altica* spp. in China. In Europe and Asia, *Z. caerulea* is particularly noted as a predator of Chrysomelidae and mentioned that a single adult can kill 12 larvae per day of a flea beetle *Altica ampelophaga* (De Clercq, 2000). In this research, results showed that a single adult can kill 14–15 larvae of *Altica viridula* per day which difference in the present study compared to the De–Clercq (2000) study can be attributed to the differences in environmental temperature, experimental precision and predator host. According to calculation of Balcells (1951), however, *Z. caerulea* can reduce populations of *Altica* under optimal conditions by only one–

seventh, because of its low reproductive potential. Zeki & Toros (1990) and Augustin & Leveux (1993) stated that populations of poplar beetles (*Chrysomela* spp.) in Turkey and France, respectively, are heavily attacked by *Z. caerulea*.

In this research, *Z. caerulea* was collected and reported as specific biological control agent for the first time from Iran. The association of *Z. caerulea* and *A. viridula* as predator and host is new.

So far, few studies have been on the biology and efficiency of this predator. For studies of biological control in the laboratory, the nymphs fed on the *A. viridula* larvae until early July. From the middle of July, new adults observed and they feed on the remaining larvae. This predator hibernates as an adult insect and has one generation in the climatic conditions of Mazandaran province. So, we reared *Z. caerulea* for the first time on *A. viridula* in Iran. Based on the investigations, the simultaneous activity of the predator and the pest, it is the best option for biological control.

In addition, various studies showed that the lifespan of Asopinae adult female *Andrallus spinidens* Fabricius was longer than that of males (Javadi *et al.*, 2006). In this research, it was found that the lifespan of female insects is slightly longer than male insects.

A study showed that the first instar in Asopinae subfamily do not attack prey and only need moisture, mainly plant juices, to develop. Nymphs from the second instar on require animal food to complete development (De Clercq, 2000). According to the results of this study and De–Clercq (2000) study, it was determined that feeding occurs from the second instar nymph, which is consistent with each other.

The potential of Asopinae species as biological control agents has been demonstrated, both in agricultural systems and forests. However, only

10% of species have been used as biological control agents (De Clercq, 2000). All this information is of extreme importance for the correct identification and understanding of the biology and distribution of *Zicrona* species (Roca–Cusachs *et al.*, 2020). Also, a discussion on the potential usefulness and viability of blue shield bug as biological control agents in Iran was presented. Therefore, further studies are required to elucidate the basic biology and studies to measure the role of predatory in regulating populations of economically important insect pests. Emphasis should be placed on the field evaluation of their effectiveness and climatic adaptation. Alternatively, predatory stinkbugs may have potential for use in augmentative biocontrol in such varied agroecosystems as field crops, greenhouse crops and forests.

In our opinion, a good knowledge of the feeding habits and nutritional needs of a natural prey

species is necessary for the design of biological control strategies.

A final decision on the selection of this predator for biological control of this pest can only be made when all suitable options are re-evaluated under the same conditions. The results of the present study showed that *Z. caerulea* is in a favorable condition in terms of its ability and population growth rate and can be used as a selected option for biological control of *A. viridula*. However, this predator, like other natural enemies, needs support.

Acknowledgments

The authors are grateful to the research institute of forest and rangeland for supporting the project of monitoring pests and diseases of forests and rangelands of Hyrcanian zone.

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زیست‌شناسی و شکارگری سن *Zicrona caerulea* (Hem.: Pentatomidae) روی سوسک برگ‌خوار لور *Altica viridula* (Col.: Chrysomelidae) در شرایط آزمایشگاهی

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تاریخ پذیرش: ۱۴۰۳/۱۱/۲۳

۱۲۰-۱۱۱ (۲)

تاریخ دریافت: ۱۴۰۳/۰۹/۱۷

چکیده

جنگل‌های هیرکانی، با قدمتی بین ۲۵ تا ۵۰ میلیون سال، از با ارزش‌ترین جنگل‌های جهان به‌شمار می‌آیند و به‌عنوان یک زیستگاه طبیعی شناخته می‌شوند. این جنگل‌ها نقش اساسی در حفاظت از تنوع زیستی و حفظ تعادل اکولوژیک منطقه دارند. فشارهای ناشی از گسترش آفات، تهدیدی جدی برای این زیست بوم ارزشمند به‌حساب می‌آید. لذا حفظ و حراست از آن خیلی مهم می‌باشد. در سال‌های اخیر، آفت *Altica viridula* Weise به‌عنوان یکی از آفات برگ‌خوار *Carpinus orientalis* mill. در برخی از مناطق استان مازندران محسوب می‌شود. این آفت با تغذیه از برگ‌ها موجب خشک شدن شاخ و برگ درختان، کاهش تراکم جنگل و افزایش آتش‌سوزی می‌شود. مدیریت آفت مذکور نیازمند راهکارهایی است که علاوه بر کارایی و تاثیر روی آفت با محیط‌زیست نیز سازگار باشد. استفاده از دشمنان طبیعی یکی از رویکردهای موثر در مهار زیستی آفات به‌شمار می‌آید. گونه *Zicrona caerulea* Linnaeus به‌عنوان یک شکارگر اختصاصی *A. viridula* در مطالعات اخیر شناسایی شده است. شکارگر یاد شده متعلق به خانواده Pantatomidae است. سن‌های بالغ از جنگل‌های ایت-دلیر شهرستان مرزن‌آباد جمع‌آوری و به آزمایشگاه ایستگاه تحقیقات منابع طبیعی نوشهر منتقل شدند. زیست‌شناسی و مراحل زندگی شکارگر مذکور در آزمایشگاه با تغذیه از لاروهای سوسک برگ‌خوار لور در شرایط ۱۳ ساعت روشنایی و ۱۱ ساعت تاریکی، دمای 25 ± 2 درجه سلسیوس و رطوبت نسبی 65 ± 5 درصد انجام شد. مطالعات در آزمایشگاه بر پایه طرح آماری توصیفی کنترل شده انجام شد. همچنین تصادفی‌سازی را با استفاده از ۱۵ ظرف برای تکرار اجرا کردیم که امکان ارزیابی مطمئن‌تری از نتایج فراهم شود. متوسط طول دوره قبل از تخم‌گذاری $7/1 \pm 0/5$ روز، میانگین تعداد تخم گذاشته شده $20/15 \pm 3/42$ عدد، دوره جنینی $15/5 \pm 0/7$ روز، میانگین میزان تفریح تخم $93/1 \pm 2/18$ درصد و میانگین طول عمر سن‌های نر و ماده به ترتیب $38/2 \pm 1/78$ و $40/5 \pm 2/35$ روز بود. این شکارگر دارای پنج سن پورگی است. پوره سن پنجم پس از $7 \pm 0/3$ روز بالغ شد. تکمیل یک نسل به طور متوسط $44/5 \pm 2/90$ روز طول کشید. در بررسی میزان شکارگری مشاهده شد که پوره‌های سن اول تغذیه نکردند. تعداد لاروهای سن سوم به‌عنوان غذا برای پوره‌های سن ۱، ۲، ۳، ۴ و ۵ به ترتیب ۲، ۴، ۶، ۹ و ۱۶ عدد بود. درصد شکارگری سن یک تا پنج به ترتیب صفر، $85/71$ ، $88/09$ ، $92/59$ و $91/96$ درصد بود. بعد از ظهور سن‌های بالغ، سن‌های نر و ماده جفت شدند، سپس ۱۰ جفت انتخاب و میزان شکارگری هر جفت روزانه مورد بررسی قرار گرفت. نرخ شکارگری سن‌های نر و ماده به ترتیب $14/2$ و $14/7$ لارو بود. در مجموع، درصد لاروهای خورده شده از پوره سن دوم تا حشرات بالغ افزایش یافت. این گونه به‌دلیل ویژگی‌هایی نظیر طول عمر کوتاه، قدرت شکارگری بالا و تولیدمثل مناسب، پتانسیل بالایی برای استفاده به‌عنوان عامل مهار زیستی دارد و حفاظت، حمایت و رهاسازی آن به طور جدی ضروری است. تحقیقات قبلی اثربخشی *Z. caerulea* را در مناطق مختلف نشان داده است، اما هدف این مطالعه پر کردن

شکاف دانش در مورد تأثیر آن در اکوسیستم جنگل های هیرکانی است. هدف اصلی، ارزیابی کارایی *Z. caerulea* در مدیریت جمعیت های *A. viridula* است که بینش های ارزشمندی را برای استراتژی مدیریت آفت ارائه می دهد.

واژه های کلیدی: استان مازندران، آفت برگخوار، جنگل، شکارگر، قبیله Asopinae