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Discovery of Larvae of *Haliplus kamiyai* Nakane, 1963 (Coleoptera, Haliplidae) and Implications for its Life Cycle

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Abstract We reported the morphology and feeding habits of the larva of the Japanese endangered crawling water beetle, *Haliplus kamiyai* Nakane, 1963 (Coleoptera, Haliplidae) and showed implications for its life cycle through field surveys and laboratory rearing. Total body length of larvae was approximately 3.4 mm in the first instar, 6.8 mm in the second instar, and 12.3 mm in the third instar. Body was elongate and pale yellowish to light brown. Dorsal and ventral surface were covered with small tubercles (microtracheal gills). Fore legs had thumb-like tibial lobes with two spines. In the field, *H. kamiyai* larvae were observed in summer and winter, and the adults were observed from summer to winter. These results indicate that *H. kamiyai* hibernates both in the larval and adult stages, and imply two possible life cycles: (1) the larval duration of this species is very long or (2) this species has a bivoltine or multivoltine life cycle. Under laboratory rearing, *H. kamiyai* larva grasped filamentous algae *Spirogyra* spp. (Zygnemataceae) with its fore legs using the thumb- like tibial lobes and ate it with falcate mandibles. The larva successfully reached the adult stage by feeding on *Spirogyra* spp. We concluded that shallow and permanent water bodies inhabited by abundant filamentous algae are crucial to maintain the population of *H. kamiyai*.

Introduction

Haliplus kamiyai (Coleoptera, Haliplidae) is a small water beetle (3.1 mm in body length) that inhabits shallow wetlands, such as paddy fields, ditches adjacent to paddy fields, and abandoned paddy fields (Vondel *et al*., 2006; Watanabe, 2017; Watanabe *et al*., 2019; Nakajima *et al*., 2020). This species is distributed locally in Honshu (from southern Tôhoku Region to Kantô Region), Japan (Nakajima *et al*., 2020) and enumerated in the Red List of Japan as "Endangered" (Ministry of the Environment of Japan, 2019). For successful recovery of this threatened insect species, it is crucial to fully understand its life cycle. However, little information is available on the life cycle and habitat requirements of this species, and its larvae have not previously been found (Satô & Yoshitomi, 2018; Nakajima *et al*., 2020). In the present study, we report some notes on the morphology and feeding habits of *H. kamiyai* larvae and showed implications for its life cycle through field surveys and laboratory rearing. In addition, we discuss the habitat requirements of this species for its conservation.

Material and Methods

To collect *H. kamiyai* larvae, we conducted field surveys in a ditch adjacent to a paddy field (Ditch) and an abandoned paddy field (Paddy A) at Ishioka-shi, Ibaraki Prefecture, Japan, and in an abandoned paddy field (Paddy B) at Ôtaki-machi, Chiba Prefecture, Japan (Fig. 1). The elevations of Ditch, Paddy A and Paddy B were 39, 43 and 176 m, respectively. The direct distance between Ditch

and Paddy A was approximately 30 m. Preliminary surveys confirmed the presence of adult *H. kamiyai* in all sites. During the survey period, Ditch had water depths of 6–16 cm, and was dominated by *Spirogyra* spp. (Zygnemataceae), *Chara braunii* (Characeae), *Monochoria vaginalis* (Pontederiaceae), *Murdannia keisak* (Commelinaceae), and *Persicaria thunbergia* (Polygonaceae). Paddy A had water depths of 1–10 cm, and we observed flourishing *Spirogyra* spp. and several water plants: *Leersia japonica* (Poaceae), *Oenanthe javanica* (Apiaceae), and *Typha latifolia* (Typhaceae). Paddy B had water depths of 1–7 cm, and contained *Spirogyra* spp. and several water plants: *Sagittaria trifolia* (Alismataceae), *Juncus effusus* (Juncaceae), and *O. javanica*).

In Ditch, sampling was conducted weekly from May 8 to September 5, 2018, for a total 15 times. A D-frame net (30 cm width, 1 mm mesh) was pulled 20 times for 1 m along the shore of Ditch. Samples were transferred to a white plastic tray, and the numbers of *H. kamiyai* adults and unknown haliplid larvae were recorded. After recording, the adults were released to sampling sites and the larvae were taken to the laboratory in order to ensure they were *H. kamiyai*. During the survey period, we also observed two other halipild species: *Haliplus sharpi* WEHNCKE, 1880 and *Peltodytes intermedius* SHARP, 1873. Since larval morphology of these two species had previously been described (NAKANISHI, 2012; MITAMURA *et al*., 2017), we could distinguish unknown haliplid larvae from these two species. In Paddy B, we conducted a total of six surveys: June 9 and 16, July 16, August 20, November 30, and December 4, 2018. Mud and the base of aquatic plants (approximately 1.0 L) were collected using an aquatic net (12 cm length, 15 cm width, 0.5 mm mesh); the presence or absence of *H. kamiyai* adults and the number of unknown haliplid larvae were recorded. In Paddy B, other haliplid species were not collected during the study period. To confirm whether the larvae emerge during winter in Ibaraki Prefecture as observed in Chiba Prefecture (see below), an additional survey was conducted in Paddy A. Mud and the roots of aquatic plants (approximately 1.5 L) were collected from Paddy A on January 30, 2020 and taken to the laboratory. In the laboratory, we transferred the sample onto a plastic screen placed on a white plastic tray with tap water, and we recorded the number of larvae in the plastic tray. This method allows the efficient collection of small aquatic insects from debris (MILLER & BERGSTEN, 2016).

In Ditch, we collected two individuals of the unknown haliplid larvae on July 14, 2018 (Fig. 1). Based on the external morphology, the larvae appeared to be conspecific. We reared one of them in order to confirm whether the larva was *H. kamiyai*, while the other was fixed in 99.5% ethanol for morphological measurements. The larva was reared in a plastic cup (129 mm diameter, 65 mm height) with 200 mL dechlorinated tap water and kept at 25 ℃ in a 14 h light/10 h dark cycle. Since haliplid larvae are known to feed on filamentous algae or characeans (SEEGER, 1971 a; VONDEL, 1997), the larva was fed *Spirogyra* spp. and *Chara braunii* collected from Ditch. A wet filter paper was attached to the inside of the plastic cup to provide a terrestrial surface before pupation. If the larva climbed on the filter paper, we ascertained that the larva was approaching pupation and we transferred the larva to another plastic cup (78 mm diameter, 37 mm height) with wet peat moss. Some larvae collected in Paddy A and Paddy B were fixed in 99.5% ethanol for morphological measurements. Measurements of larvae were conducted by using a Nikon Multi Zoom AZ100M stereo microscope (Nikon, Tokyo) and microscope imaging software, NIS- Elements 4.2.0 (Nikon). We measured several larval body parts following VONDEL (2011 & 2012).

Results

In Ditch, a total of 60 adults and two larvae were collected. Adults were observed from May to September and peaked in August (Fig. 2), while larvae were collected only in July (Fig. 2). In Paddy

Fig. 1. Habitats of *Haliplus kamiyai*. —— a, Ditch; b, Paddy A; c, Paddy B.

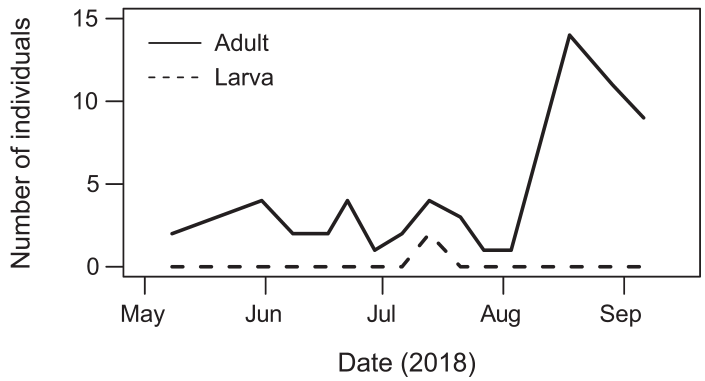

Fig. 2. Seasonal changes in the abundance of *Haliplus kamiyai* adults & larvae in an agricultural ditch.

A, six adults, two first instar larvae, and nine third instar larvae were collected in January. In Paddy B, adults were observed from June to December except for July, and larvae were captured only in November (16 individuals) and December (43 individuals).

Larval measurements are shown in Table 1. Body was elongate and pale yellowish to light brown (Fig. 3a). Dorsal and ventral surfaces were covered with small tubercles (microtracheal gills). Head was rounded and dark brown. Abdomen was 10- segmented. 10th abdominal segment was long with two appendices. Fore legs had thumb-like tibial lobes with two spines (Fig. 3b).

During laboratory rearing, the larva grasped *Spirogyra* spp. with its fore legs using the thumb-like tibial lobes (Fig. 3b) and ate it with falcate mandibles (for the movie: Watanabe, 2020) (Fig. 3c). Conversely, we did not observe it feeding on *C. braunii*. The larva landed on the filter paper on August 23, and the larva was transferred to the plastic cup containing wet peat moss for pupation. The larva dove into the peat moss on August 23, but the larva emerged on the peat moss after two days. Thus, we transferred the larva back into the plastic cup with water and kept rearing. On September 3, the larva landed on the filter paper and was transferred back to the plastic cup with wet peat moss. To confirm whether the larva was pupating, we exposed the peat moss and found a pupa on September 23 (Fig. 3d). The pupa sufficiently eclosed and the adult emerged on October 4. We identified the adult as *H. kamiyai*, referring to Vondel *et al*. (2006).

Discussion

In the present study, we observed *H. kamiyai* larvae in summer (July) (Fig. 2) and winter (January) in Ibaraki Prefecture and in winter (November and December) in Chiba Prefecture, while

Table 1. Measurements (mean ± SD) of *Haliplus kamiyai* larvae (length in mm).

	1st instar (n = 2)	2nd instar (n = 19)	3rd instar (n = 3)
Total length	3.38 ± 0.19	6.76 ± 0.92	12.29 ± 1.99
Length from mandible to apex of abdominal segment 9	2.80 ± 0.14	5.35 ± 1.21	9.42 ± 1.71
Length of abdominal segment 10	0.58 ± 0.01	1.49 ± 0.39	2.87 ± 0.55
Length of head including mandibles	0.21 ± 0.02	0.34 ± 0.05	0.54 ± 0.09
Width of head including eyes	0.35 ± 0.02	0.42 ± 0.03	0.79 ± 0.02
Antenna			
Length of segment 2	0.02 ± 0.00	0.03 ± 0.01	0.06 ± 0.00
Length of segment 3	0.08 ± 0.02	0.10 ± 0.01	0.17 ± 0.01
Ratio of segment 3 among segment 2	3.50 ± 0.71	3.21 ± 0.46	3.41 ± 0.02
Mandible			
Length from apex to hind lobe	0.12 ± 0.01	0.15 ± 0.03	0.25 ± 0.04
Spines on outer margin	2	2	2
Pronotum			
Length	0.31 ± 0.05	0.37 ± 0.05	0.70 ± 0.21
Width	0.52 ± 0.15	0.77 ± 0.04	1.41 ± 0.06
Mesonotum			
Length	0.23 ± 0.07	0.37 ± 0.06	0.69 ± 0.11
Width	0.47 ± 0.11	0.74 ± 0.05	1.35 ± 0.06
Metanotum			
Length	0.20 ± 0.001	0.39 ± 0.06	0.70 ± 0.13
Width	0.44 ± 0.1	0.74 ± 0.05	1.38 ± 0.05

adults were observed from summer to winter at both sites. These results indicate that *H. kamiyai* hibernates both in the larval and adult stages, and imply two possible life cycles: (1) the larval duration of this species is very long or (2) this species has a bivoltine or multivoltine life cycle. The developmental period of the third instar larvae of Haliplidae is variable among species (SEEGER, 1971 a). Larvae of some species hibernate and newly eclosed adults emerge in the spring of the following year (VONDEL, 1997). Larval hibernation in the genus *Haliplus* was also observed in a pond by HICKMAN (1931), while VONDEL (1997) reported that some larvae hibernated twice before adult emergence under laboratory rearing. Therefore, *H. kamiyai* larvae might also require more than one year to reach the adult stage. To better understand the life cycle of *H. kamiyai*, it is necessary to investigate the phenology of adults and larvae throughout the year and elucidate the duration of each immature stage.

Feeding habits of haliplid larvae are divided into two groups based on morphological characters of the fore legs and mandibles: one group feeds primarily on filamentous algae and the other feeds primarily on characeans (SEEGER, 1971 a, b; VONDEL, 1997; VONDEL & DETTNER, 2005). The morphological characters of fore legs are differed even within the genus *Haliplus* (VONDEL & DETTNER, 2005). We report that *H. kamiyai* larvae have the thumb-like tibial lobes on the fore legs, a known adaptation for feeding on filamentous algae (SEEGER, 1971 a) (Fig. 3b). Under laboratory rearing, *H. kamiyai* larva successfully reached the adult stage by feeding on *Spirogyra* spp. (Fig. 3c), but we did not observe it feeding on *C. braunii*. These results indicate that *H. kamiyai* should be categorized into the former group that feeds primarily on filamentous algae. However, *Haliplus confinis* Stephens, 1828, which mainly feeds on characeans, feed on *Cladophora* algae when characeans are not available, although their survival rate is much lower when feeding on *Cladophora*

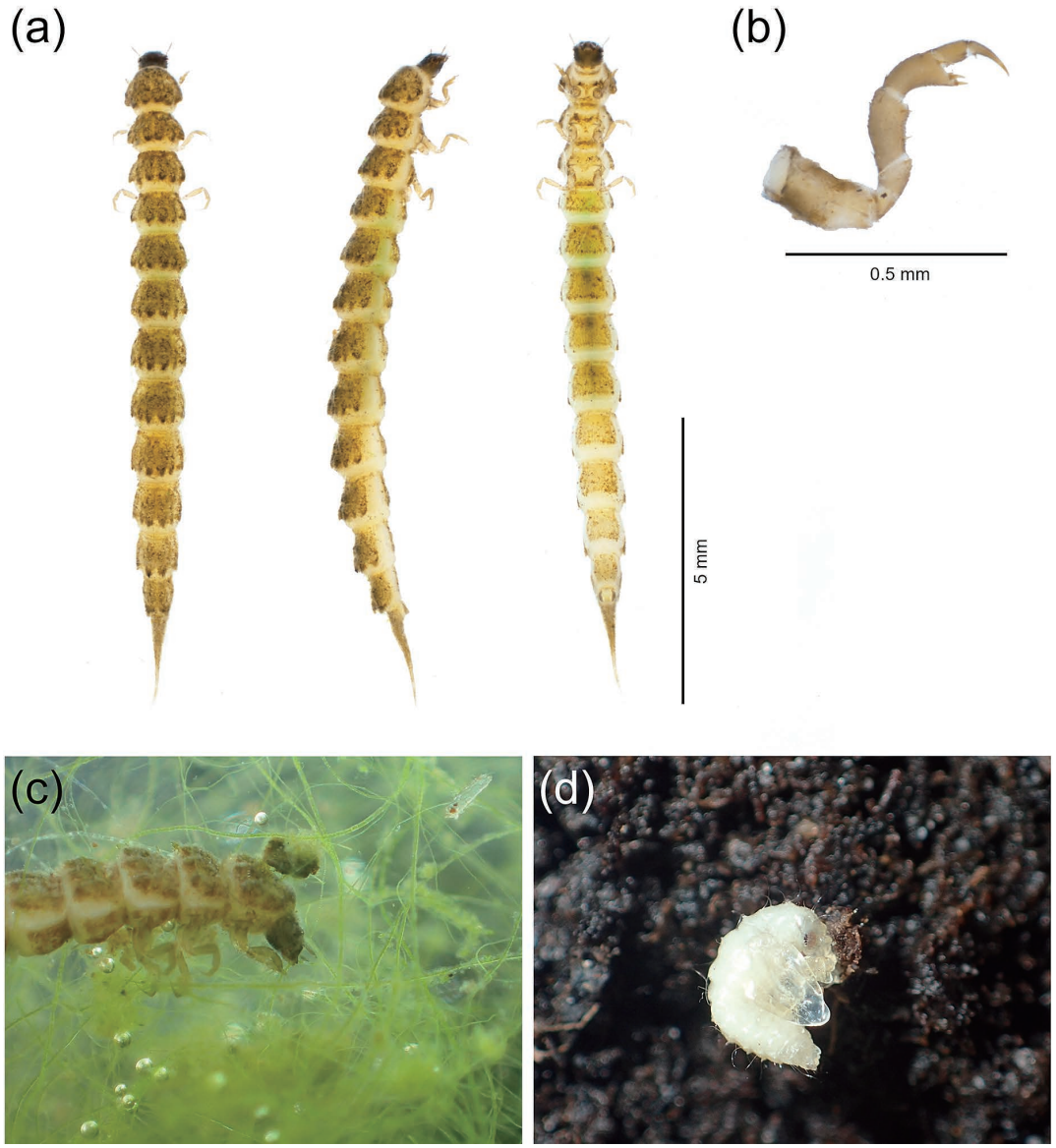

Fig. 3. Immature stages of *Haliplus kamiyai*. —— a, Ventral, lateral & dorsal views of the third instar larva; b, fore-leg of the third instar larva; c, feeding on the algae *Spirogyra* spp. by the third instar larva; d, pupa.

than on characeans (SEEGER, 1971 a). Therefore, to plan the most efficient rearing method of *H. kamiyai*, additional experiments are needed to confirm the growth performance of larvae when fed both filamentous algae and characeans. In conclusion, we suggest that shallow and permanent water bodies inhabited by abundant filamentous algae are needed to maintain the population of *H. kamiyai*.

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要　　約

渡辺黎也・山﨑　駿：絶滅危惧種カミヤコガシラミズムシ（鞘翅目コガシラミズムシ科）の幼虫の発見及び生活環への示唆．――――カミヤコガシラミズムシ *Haliplus kamiyai* NAKANE, 1963 は，環境省レッドリストにおいて絶滅危惧 IB 類に選定されている希少種であるが，幼虫形態を始めとした生態的知見は未解明であった．本研究では，本種幼虫の形態及び摂食様式を明らかにするとともに，野外調査により得られた本種の生活環に関する知見を報告した．幼虫の体長は 1 齢幼虫で約 3.4 mm，2 齢幼虫で 約 6.8 mm，3 齢幼虫で約 12.3 mm であった．体形は細長く，体色は淡黄色から淡褐色であり，体表面は背側，腹側ともに小突起（微小気管鰓）で覆われていた．野外調査の結果，本種の幼虫は夏と冬に，成虫は夏から冬にかけて観察されことから，本種は幼虫，成虫ともに越冬することが明らかになった．また，本種は (1) 幼虫期が非常に長い，あるいは (2) 年 2 化または年多化の生活環をもつことが示唆された．室内飼育下において，本種の幼虫は親指状の頸節葉をもつ前脚を用いてアオミドロ属藻類をつかみ，大顎まで運び摂食していた．本種の 3 齢幼虫はアオミドロ属藻類のみを摂食し，成虫まで成長した．以上により，本種の個体群を保全するためには，繊維状藻類が豊富に生育する，水深の浅い恒久的な水域を維持することが重要であると考えられる．

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