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STUDIES ON THE ECOLOGY OF *CERCION SIEBOLDI* (SELYS) IN THE AOMORI PREFECTURE, NORTHERN JAPAN. I. LIFE HISTORY AND LARVAL REGULATION (ZYGOPTERA: COENAGRIONIDAE)

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The life history was studied during 1981-1985, using natural populations and reared specimens. The flying season lasts from late May to mid Sept., reproductive behavior was observed between mid-June and mid-Sept. The average egg stage duration was 10 days (at 21-33° C), or 20 days (at 11-32° C, in the laboratory). Two modes of larval development were discerned. Univoltine larvae developed slowly and hibernated usually between the F-3 and F-1 instars; the ultimate (i.e. the 10th, prolarva excl.) instar appeared in the next spring and the adults emerged between late May and mid July. Bivoltine larvae hatched about June 24, reached the final (9th) instar between late July and early Aug., and the adults emerged during Aug. and early Sept. The latter were smaller than those from the univoltine larvae. The antennal development is of the BBBA type of K. MIYAKAWA (*Odonatologica* 6 [1977]: 173-180).

INTRODUCTION

Until present, the life histories have become known of but a few Japanese Zygoptera (e.g. AINO, 1934; OKUMURA & ISHIMURA, 1941), although the ultimate instar larvae were adequately described and figured in many of them.

The present paper deals with the life history details in the endemic *Cercion Sieboldi* (Sel.), one of the common damselflies in Japan. The species is of a medium size (abd. 23-27 mm, hind wing 16-23 mm), and the male has a sky-blue body coloration, with black markings. It flies from late May to mid September. The emergence curve is bimodal, the season ranging from late May to mid July and during August (NARAOKA, 1976).

NARAOKA (1976) reported that the body size of the August individuals is smaller than that of those of the May-July class. It has been reported by INGRAM (1971) and INGRAM & JENNER (1976) that a part of the *Enallagma aspersum* population in North Carolina, originated from eggs deposited in early spring, also emerges in August of the same year. In the present paper, these matters are discussed and some structural features of various larval instars are described.

STUDY AREA AND METHODS

The study area is an unused irrigation canal and two ponds in Tsugaru District, Aomori Prefecture, northern Japan (latitude: 40°45'N, longitude: 140°20'E, altitude a.s.l.: 20 m). According to the Chronological Scientific Tables 1986 (MARUZEN, 1985), the monthly mean air temperature in this area is highest in August (22.3° C) and lowest in January (-2.7° C), with an annual mean at 9.1° C. The water temperature in the study area was not measured, but at 30-100 cm below the surface in some ponds of this area the temperature generally ranges from 2-3° C, in early February to 24-26° C, in late August.

The irrigation canal (where *C. sieboldi* was the only zygopteran breeder), was destroyed in 1983 and did not dry up throughout four seasons. Its vegetation was dominated by *Potamogeton crispus* and consisted further of *P. natans, Scirpus triqueter, S. juncoides, Equisetum limosum,* etc., all of which served *C. sieboldi* as oviposition substrate. Its waterside was also occupied by *Artemisia japonica, Polygonum cuspidatum, Rumex crispus japonicus, Trifolium repens,* etc., that were used as resting and roosting sites by the insects. The aquatic fauna of the irrigation channel included small fish.

C. sieboldii, C. calamorum, Ischnura asiatica, Lestes sponsa and *Copera annulata* commonly inhabit the man-made, Otameike pond (ca 288 ha), while in addition to these, the natural Hiratakinuma pond (ca 49 ha) also harbours *Coenagrion lanceolatum.*

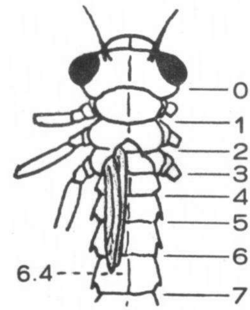

Fig. 1. Method applied for the measurement of the wing sheath length.

Some oviposition plants were collected in the habitat (1981-1985) and examined in the laboratory. The larvae were collected during 1983-1985, using a hand net of about 0.4 mm mesh. Within two days after examination almost all larvae were released at the original collection site. Some larvae were also reared in petri dishes and plastic containers, in the laboratory or in the garden. Throughout rearing food was not specially supplied, but the water and the aquatic plants were regularly replaced.

The head width (HW) was measured through a binocular microscope equipped with an ocular micrometer (1 UN = 0.048 mm), and the relative length of the wing sheath (RW) was estimated from the number of thoracic and abdominal segments covered by it (Fig. 1). Some other characters of each instar were also examined.

RESULTS

ADULT STAGE IN THE FIELD

In the study area, the adult season ranges from late May to mid September. The emergence occurs from late May to mid July and from early August to early September. Reproductive behavior was recorded between mid June and mid September.

DURATION OF EGG STAGE

The egg stage duration varied with the seasons (Tab. I). Larvae hatched in the laboratory within two weeks after oviposition on August 25 (21-33° C, photoperiod 12.8-13.7h). On the other hand, those collected on June 16 (16-31° C, 14.8-15.2h) and in early September (11-32° C, 11.9-12.8h) required 17 to 20 days until hatching. The eggs collected on August 25 and September 8 were batches from a single pair after copulation, but others were made up of batches of eggs originating from several pairs.

Table I

Egg hatching time in *C. sieboldi*

Date of oviposition	No. of larvae hatched	Days required for hatching		Temperature (° C)	
		mean	*range*	*mean*	*range*
June 16	203	21.5	13-34	22.4	17.0-31.5
Aug. 25	ca 300	10.0(?)	8(?)-13	25.4	21.0-33.0
Sept. 8	262	17.3	16-24	19.3	11.0-28.5
Sept. 15	several	—	20-?	—	—

RECOGNITION OF THE YOUNGER LARVAE

The indoor experiments commenced in mid September and were continued till midwinter in 1981 and 1983. Prolarvae were never recognized at hatching time, although some minute, freely swimming larvae were discovered (HW: 0.31-0.38 mm, total body length: 2 mm). Their antennae were three-segmented. Since larvae smaller than these were not seen, these are assumed to represent the first instar, excluding the prolarva. Some first instars were reared separately in petri dishes (diameter 70 mm), and the remainder, in mass, in a plastic container (height 12 cm, diameter 15 cm). The solitary larvae were checked daily. Most had died by the third instar. The second, third and fourth instars from mass culture

Table II

Development of young *C. sieboldi* larvae, reared from eggs. — The relative wing sheath size is defined in terms of the segments covered, counted from the prothorax (in brackets, where rudimentary)

Instar	n	Head width (mm)		Antennal segments	Relative wing sheath		No. of setae	
		mean±S.D.	*range*		*fore*	*hind*	*lateral*	*mental*
1	43	0.37±0.02	0.31-0.38	3	absent	absent	1 + 1	0 + 0
2	40	0.50±0.02	0.46-0.55	4	(1.5)	(2.6)	2 + 2	1 + 1
3	13	0.65±0.06	0.60-0.72	5	(1.7)	(2.7)	2 + 2	1 + 1
4	13	0.88±0.05	0.77-0.96	5	(1.8)	(2.8)	3 + 3	1+1 or 1+2
5	10	1.07±0.09	1.01-1.15	6	1.9	2.8	3 + 3	2 + 2
6	4	1.33±0.11	1.25-1.39	6	1.9	2.8	4 + 4	3 + 3

were also reared by the same solitary method. But all of the larvae died during the winter.

The larval instars, from the first to the sixth, were recognized by analysing the data obtained from the insects reared in the laboratory (Tab. II).

HIBERNATING LARVAE UNDER NATURAL CONDITIONS

To obtain information on larval development after hibernation, the larvae were periodically collected from early May to early July, 1982 in the irrigation channel, particularly among the *Potamogeton crispus* vegetation. These were brown in color and had a HW of 1.5-3.8 mm. They were divided into four size classes on the basis of the HW and RW (Fig. 2, Tab. III). Group "IV" consists of the largest members and they have remarkably long wing sheaths, which go beyond the third abdominal segment, with apparent venation, and their antennae are seven-segmented. They are thus distinguished from the smaller members of the three other classes. Because these characters are similar to those of the exuviae found in the field, it is obvious that group "IV" is the final instar, while the rest belong to F-1 to F-3 instars.

Final instars occupied 5% of the total collection of larvae in early May. Then, the rate increased rapidly to 66% in early June and to 90% in mid-June, but it decreased in early July (Fig. 2A).

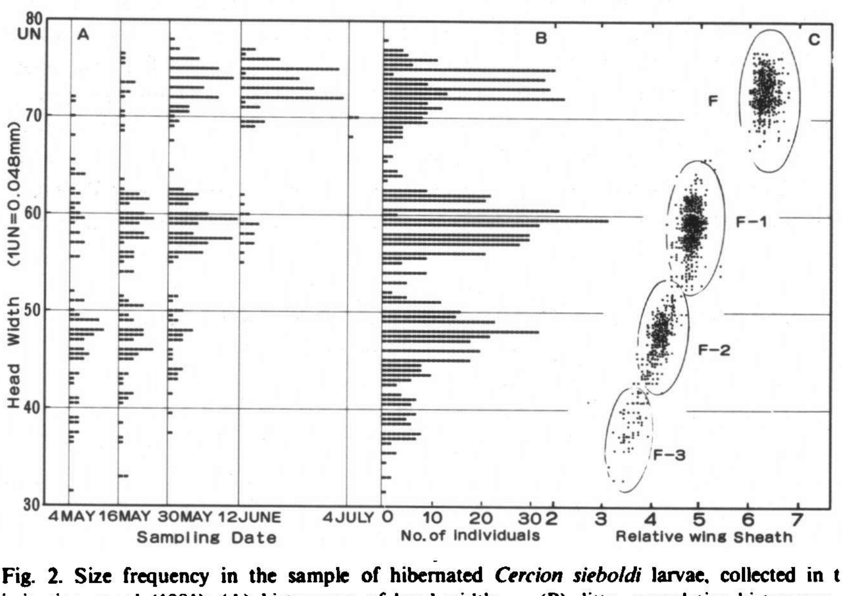

Fig. 2. Size frequency in the sample of hibernated *Cercion sieboldi* larvae, collected in the irrigation canal (1981): (A) histograms of head width; — (B) ditto, cumulative histogram; — (C) relationship between hind wing sheath length and head width (F: final instar).

Table III

Some characters of *C. sieboldi* late instar larvae, hibernated under natural conditions — The relative wing sheath size is defined in terms of the segments covered, counted from the prothorax

Instar	n	Head width (mm)		Antennal segments	Relative wing sheath		No. of setae	
		mean ± S.D.	*range*		*fore*	*hind*	*lateral*	*mental*
F-3	61	1.85±0.11	1.51-1.99	6	2.7	3.5	4+4, 5+5	3+2, 3+3, 3+4
F-2	217	2.28±0.11	2.04-2.50	6	3.4	4.1	4+5, 5+5	3+3, 3+4
F-1	312	2.82±0.11	2.54-3.17	6	4.5	4.8	5+5	4+4
F	254	3.49±0.10	3.24-3.74	7	6.2	6.3	5+5, 5+6, 6+6	3+4, 4+4, 4+5

DEVELOPMENT OF PROGENY

As sexual behavior was observed in mid June, it was thought that the progeny would hatch in July. On July 18, 1982, the irrigation channel was carefully searched, and a large number of younger larvae were obtained from the vegetation of *Scripus triqueter*. These small larvae were white, almost transparent, like the specimens reared in 1981 and 1983. Brownish large larvae were not collected at this time. Judging from the HW obtained with the larvae reared in 1981 and 1983, almost all of these collected are referable to the fifth to seventh instars. In addition, the collection included two specimens of the second instar and one each of the third and the fourth instars. This may indicate the escape of most of the minute larvae of the first to fourth instars from the collection.

The larvae were reared in mass in a large plastic container (30x40x20 cm height) buried in the garden. The container was filled up with water and aquatic plants from their habitat, and the larvae were examined periodically. The mode of larval development was divided into quick and slow growth types (Tab. IV, Fig. 3). The larvae belonging to the quick type developed to the final (ninth) instar after eight moults, between late July and early August, and 23 (14♂, 9♀) out of 26 final instar individuals emerged during August 9-29 (mean: Aug. 18). They belonged to the second generation or the bivoltine type and were in the sixth

Table IV

Development of the larval stages of *C. sieboldi*. — The relative wing sheath size is defined in terms of the segments covered, counted from the prothorax

	Univoltine						Bivoltine					
Instar	n	Head width (mm)		Antennal segments	Relative wing sheath		n	Head width (mm)		Antennal segments	Relative wing sheath	
		mean ± S.D.	*range*		*fore*	*hind*		*mean ± S.D.*	*range*		*fore*	*hind*
1	15	0.36±0.03	0.33-0.38	3	—	—	—	—	—	—	—	—
2	43	0.50±0.02	0.48-0.53	4	—	—	4	0.50±0.02	0.48-0.53	4	—	—
3	52	0.71±0.06	0.60-0.79	5	—	—	—	—	—	—	—	—
4	29	0.89±0.05	0.77-0.96	5	1.9	2.8	5	0.93±0.02	0.91-0.96	5	—	—
5	39	1.08±0.09	0.96-1.25	6	1.8	2.9	3	1.19±0.04	1.15-1.22	6	2.0	3.0
6	58	1.49±0.11	1.20-1.68	6	2.3	3.2	27	1.42±0.06	1.25-1.51	6	2.9	3.4
7	66	1.85±0.10	1.68-2.06	6	2.9	3.8	34	1.85±0.09	1.63-1.97	6	3.2	4.1
8	33	2.35±0.10	2.16-2.45	6	4.2	4.6	34	2.39±0.07	2.26-2.59	6	4.6	5.0
9	99	2.77±0.11	2.59-2.88	6	5.0	5.4	45	2.98±0.08	2.78-3.17	7	6.4	6.5

and seventh instars at the time of collection. The mean rearing duration of the emerged larvae was 30 days (22-42 days), and the final instars were smaller than those of the first generation or the univoltine type. Body size of bivoltine adults was similar to that of the August-September specimens observed in the field.

Although development of the larvae of the slow type reached only the fifth to seventh instar during August, they developed mostly to the seventh to ninth instar by winter. And five bivoltine finals of the ninth instar were discovered in late November. Numerous small larvae below the fifth instar at the time of collection

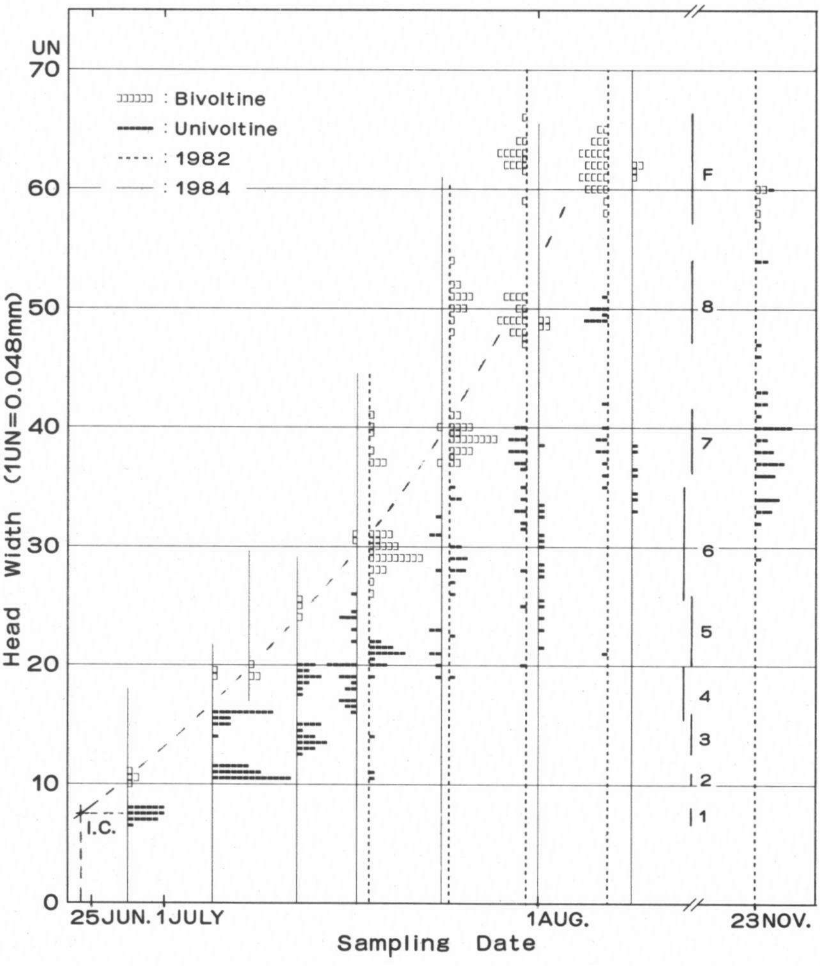

Fig. 3. Size frequency of the head width in the samples of the reared *Cercion sieboldi* larvae. (Numbers indicate the instars, — F: final instar, — I.C.: intersecting point, close to June 24).

belong to the univoltine type. They hibernated, stopping further development, and all died during winter. This might be attributed to freezing of water in the container, due probably to its small volume.

As the development of the progeny begins before mid July, some aquatic plants used for oviposition by *C. sieboldi* were collected on June 24, 1984 in the pond and kept in the laboratory. On June 28 some minute larvae were noticed: they were fifteen specimens of the first instar and four of the second. But, judging from the dates shown in Table I, these were rapidly grown, exceptional specimens, and should be regarded as immigrant larvae, adhering to the aquatic plants, or they might have hatched from eggs laid before collection of these aquatic plants. These larvae were reared under the same conditions as those of 1982, and four adults emerged (from the ninth instar) during August 11-17 (mean: Aug. 15). These were also small, like those reared in 1982. Developmental progression is shown in Figure 3. From this it appears that the bivoltine generation that emerged had reached the second instar in late June. The larval duration from the second instar was 44-50 days (mean: 48).

In the two experiments carried out in 1982 and 1984, the eighth and ninth instars of the bivoltine larvae were larger than the univoltine ones, especially in the ninth instar (Tab. IV). These mature larvae were brown in color, like the spring specimens obtained in 1982.

SEASONAL REGULATION OF LARVAE OF TWO *CERCION* SPECIES

The irrigation channel was destroyed in the spring of 1983. Subsequently, the larvae were collected from two nearby ponds. Larvae of six zygopteran species were obtained by scooping with a net from the shore, but those of *C. sieboldi* and *C. calamorum* could not be distinguished morphologically. The seasonal development of the two species together was investigated, because both the flying season and body size of their adults are very similar (mean HW of adults collected from May to July: *sieboldi* ♂: 4.1 mm [n=26], ♀: 4.1 mm [n=25], — *calamorum* ♂: 3.8 mm [n=40], ♀: 3.9 mm [n=16]). The adults of *sieboldi* were more numerous than those of *calamorum* (summation of 50 censuses on Aug. 12, 1984 taken along the 150 m shore line of Ôtameike; *sieboldi:* 2643 ♂, 157 ♀, *Calamorum:* 145 ♂, 14 ♀).

Collection of the larvae was made twelve times, except during the icy seasons, from 1983 to 1985. In early spring and early winter, the collecting was difficult, but it was easy between May and October. On the basis of HW and RW the larval populations were divided into ten size classes, from the first to the final (tenth) instars (Fig. 4). However, the larvae collected in late summer and early autumn (Aug. 26, Sept. 1 and 15), could not be divided strictly into size groups, since their body size increased gradually.

In early spring (April 29), most of the larvae obtained were seventh to ninth

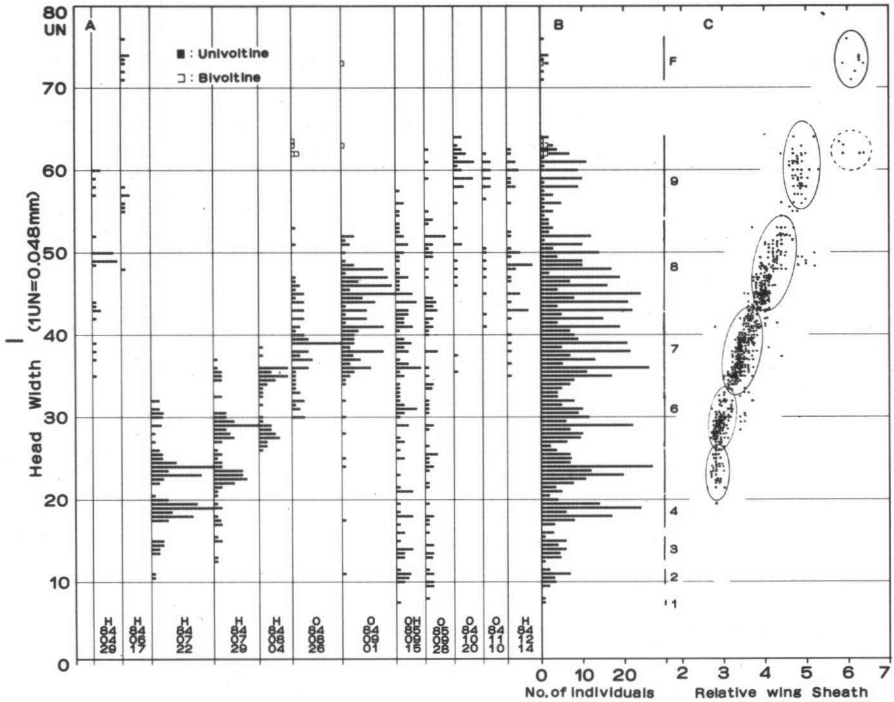

Fig. 4. Size frequency in the samples of *Cercion sieboldi/calamorum* larvae from the Otameike (O) and Hiratakinuma (H) ponds: (A) histograms of head width per sample; — (B) ditto, cumulative histogram; — (C) relationship between hind wing sheath length and head width. — (Numbers indicate the instars, — F: final instar, — Dotted line circle: final instar of the bivoltine population).

instars. Final stages were found only three times (June 17, Aug. 22 and Sept. 1), and all of them emerged by September 6. One final larva of September 1 was remarkably larger than others of the bivoltine type, but the emerged adult was equal in body size to univoltine adults.

ANTENNAL DEVELOPMENT

The antenna of a newly hatched larva is composed of three segments, namely, from base to apex, the scape, the pedicel and the flagellum. From the basal part of the flagellum, a new segment is formed in the second, third and fifth instars. On the other hand, in the final larvae of the tenth (univoltine) or the ninth (bivoltine) instar, the apical part of the flagellum is divided into two segments. Therefore, the antenna in the final instar is composed of seven segments: the scape, the pedicel and the five-segmented flagellum (Tabs II-IV).

DISCUSSION

In the study area the oviposition of *C. sieboldi* was observed from mid June to mid September. Fertilized eggs completed the embryonic development within two weeks in late summer, 13-34 days in early summer, and 16-24 days in early autumn, in the laboratory. Therefore, this species is thought to belong to the "direct development type" (CORBET, 1962). The seasonal variation in egg stage duration seems to depend on variations in water temperature. A similar situation was recorded in some other Odonata (JOHNSON, 1964; MIYAKAWA, 1969; BEESLEY, 1972; SAWCHYN, 1972; PILON, 1982; WARINGER, 1982). BEESLEY (1972) suggested that the photo response might affect embryonic development. But, in the present observation, the influence of day-length was unknown.

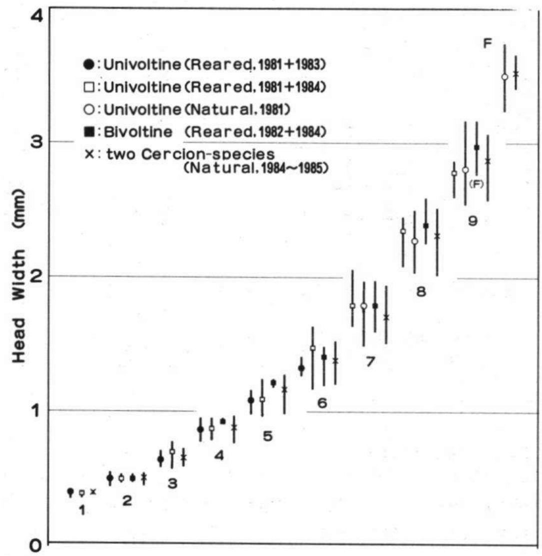

Fig. 5. Average head width and its range (in mm) in *Cercion sieboldi* and in *C. sieboldi/calamorum* samples. — (Numbers indicate the instars, — F: final instar).

In the three studies, viz. spring observations on hibernated larvae, rearing of progeny in summer, and seasonal regulation of two *Cercion* species, the size classes of larvae correspond well with the HW (Fig. 5). Namely, the seventh, eighth and ninth instars of the reared progeny are F-3, F-2 and F-1 instars of the spring population, respectively (Tabs III, IV, Fig. 5), and the ultimate larva of the univoltine generation is of the tenth instar. This is also ascertained through the observations on both *Cercion* species. Therefore, it is obvious that the life history of the hibernating generation is composed of ten instars excluding the prolarva. The ten-instar life history is common in Zygoptera. The duration of zygopteran larval life ranges from 9 to 15 instars, excluding the prolarva, and that of *Epiophlebia* and of some anisopterans also falls within this range (KINOSHITA & OBI, 1931; AINO, 1934; OKUMURA & ISHIMURA, 1941; BEESLEY, 1972; KUMAR, 1972a, 1972b; MIYAKAWA, 1977; PELLERIN & PILON, 1977; UBUKATA, 1980; TABARU, 1984).

From rearing of a new year class in two summer seasons it became known that several portions of the progeny emerge during August in the same year (Fig. 3). Multivoltinism is known in the tropics: in India, for example, *Ischnura delicata, Pseudagrion rubriceps, Ceriagrion coromandelianum, Crocothemis servilia* and *Orthetrum sabina* are bivoltine (KUMAR, 1972b). In southern France, *I. elegans* and *Sympetrum fonscolombei* are also bivoltine species (Aguesse, 1955, 1959 *in* INGRAM & JENNER, 1976). Furthermore, *P. salisburyense* completes three generations within a year in southern Africa (Chutter, 1961 in INGRAM & JENNER, 1976). Also in temperate latitudes, bivoltinism of *Enallagma aspersum* in North Carolina has been recorded (INGRAM, 1971; INGRAM & JENNER, 1976).

The bivoltine *C. sieboldi* completes its larval stage through nine instars and its adult is smaller than that of the univoltine population from ten instars, but it is equal in size to the individuals of the August-September population in the field. Now, from the smaller body size and the emergence period during August, it is clear that the bivoltine adults are the population usually called "Summer type" by the Japanese odonatologists. This may be the case with some other zygopteran species also (cf. NARAOKA, 1976).

Judging from the rearing period, the larval duration of bivoltine *C.sieboldi* probably ranges from 45 to 65 days (mean: 54 days) (Fig. 3) and is shorter than that (70 days) estimated for the second generation of *E. aspersum* (INGRAM & JENNER, 1976). This larval duration of about two months is thought to be the result of the adaptation to accomplish one generation by an accelerated development in a short period of summer, under a high effective temperature.

As shown in Figure 3, the intersecting point of the curve of development and the size of the first instar is about June 24. The larvae hatched before this date had grown to the second instar by about June 28 and followed the course for the bivoltine type. On the other hand, the larvae hatched after June 24 were all univoltine (Fig. 3). In the study area, therefore, the "critical day" of hatching to determine the voltinism is thought to be about June 24. INGRAM (1971) has reported that the new year class, developed to the F-3 instar until August 4, emerge during August and the critical day of development is nearly July 28. In *C. sieboldi*, however, the new year class, developed to the eighth (F-2) instar until late July, emerge during August (Fig. 3).

Under natural conditions final instar larvae were not recorded between late autumn and early spring. They appear for the first time in early May of the next year and their number rapidly increases until mid June. Then the number gradually decreases and they are scarcely seen by mid July. In August and early September final-instar larvae appear again, and they emerge in the same year. Therefore, hibernated ultimate instars were not discovered. Consequently, this species belongs to the "summer species" of CORBET (1962). The life history of *C. sieboldi* in the study area is summarized in Figure 6.

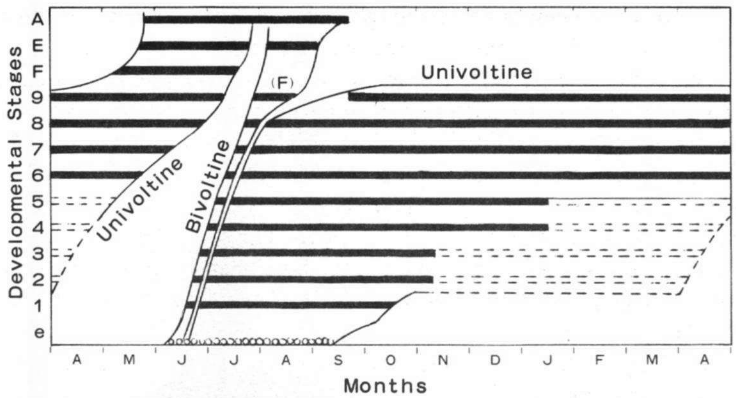

Fig. 6. *Cercion sieboldi*, Tsugaru District: life history as conjectured from periodical field samples and rearing. — (e = egg stage, — 1-9; larval instars, — F: final instar, — E: emergence period, — A: adult stage, — dotted lines: not evidenced).

It was shown by collecting in the natural pond that the larvae were few in number there in early spring as well as in late autumn. This may mean that their hibernating places differ from their summer habitats, as noticed by MIYAKAWA (1969) in *Pseudothemis zonata*. A large-sized final instar collected on Sept. 1 may be a tenth instar, due to extra moults (INGRAM, 1971; INGRAM & JENNER, 1976).

The mode of antennal development of this species apparently belongs to the "BBBA" type of MIYAKAWA (1977), which is commonly seen in damselflies.

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