



Title	Plasticity in the timing of a major life-history transition and resulting changes in the age structure of populations of the salamander <i>Hynobius retardatus</i>
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1 Plasticity in the timing of a major life-history transition and resulting changes in the age
2 structure of populations of the salamander *Hynobius retardatus*

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7 Running title: Plasticity and age structure changes

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14 Variation in age and size at life-history transitions is a reflection of the diversifying
15 influence of biotic or abiotic environmental change. Examples abound, but it is not well
16 understood how such environmental change can influence a population's age structure. I
17 experimentally investigated the effects of water temperature and food type on age and
18 body size at metamorphosis in larvae of the salamander *Hynobius retardatus*. In
19 individuals grown at a cold temperature (15 °C) or given Chironomidae as prey, the
20 time to metamorphosis was significantly prolonged and body size at metamorphosis was
21 significantly enlarged compared with individuals grown at a warmer temperature
22 (20 °C) or fed larvae. I also examined whether larval density (a possible indicator of
23 cannibalism in natural habitats) generated variation in the age structure of natural
24 populations in Hokkaido, Japan, where the climate is subarctic. Natural ponds in
25 Hokkaido may contain larvae that have overwintered for 1 or 2 years, as well as larvae
26 of the current year, and I found that the number of age classes was related to larval
27 density. Although cool water temperatures prolong the larval period and induce later
28 metamorphosis, in natural ponds diet-based enhancement of development translated into
29 a shorter larval duration and earlier metamorphosis. Geographic variation in the
30 frequency of cannibalism resulted in population differences in metamorphic timing in *H.*
31 *retardatus* larvae. It is important to understand how environmental effects are ultimately
32 transduced through individual organisms into population-level phenomena, with the
33 population response arising as the summation of individual responses. Without a
34 thorough comprehension of the mechanisms through which population and individual
35 responses to environmental conditions are mediated, we cannot interpret the relationship
36 between population-level and individual-level phenomena.

37

38 **ADDITIONAL KEYWORDS:** amphibian - cannibalism - metamorphosis -

39 overwintered larvae - phenotypic plasticity

INTRODUCTION

40

41

42 Almost all life-history traits are phenotypically plastic (West-Eberhard, 2003). Variation
43 in age and size at life-history transitions is a reflection of the diversifying influence of
44 biotic or abiotic environmental change, and, because it is tightly linked to fitness, it is a
45 central topic in life-history evolution (Roff, 2002). The effects of variable
46 environmental factors on life-history parameters, from the viewpoint of plasticity in the
47 timing of life-history transitions, have been extensively studied in amphibians, which
48 have complex life cycles. The larvae of many species of amphibians cannot escape their
49 aquatic environment until metamorphosis, and thus plasticity in metamorphic timing
50 may be important in these species, especially those that develop in ephemeral ponds
51 (Travis, 1983; Denver *et al.*, 1998; Laurila & Kujasalo, 1999). Changes in biotic or
52 abiotic environmental factors such as larval density (Newman, 1998), presence of
53 predators (Laurila & Kujasalo, 1999; Lardner, 2000), type and quantity of available
54 food (Alford & Harris, 1988; Hensley, 1993), habitat desiccation (Travis, 1983; Denver
55 *et al.*, 1998; Laurila & Kujasalo, 1999), and water temperature (Stahlberg *et al.*, 2001,
56 Hickerson *et al.*, 2005) can affect rates of growth and development, and thus the
57 duration of the larval period and size at metamorphosis (Wilbur, 1980; Werner, 1986;
58 Rose, 2005). Despite an abundance of examples of environmental changes affecting age
59 and size at life-history transitions, how such environmental changes influence the age
60 structure of larval populations has rarely been investigated, and it not well understood.

61 The salamander *Hynobius retardatus*, which lives in Hokkaido, Japan, where the
62 climate is subarctic, has long been noted for its variable life history (Sasaki, 1924;
63 Iwasaki & Wakahara, 1999). This species spawns from early April to May in ponds, and

64 hatchlings appear from late May to June (Sato & Iwasawa, 1993). Most larvae in small,
65 ephemeral ponds metamorphose into terrestrial juveniles by late autumn (October) of
66 the same year. However, individuals in permanent ponds, ones that seldom dry up, may
67 retain larval features such as external gills and tail fins and overwinter once or twice in
68 their aquatic habitat, not metamorphosing until their second or third year (Iwasaki &
69 Wakahara, 1999). Thus, such a pond habitat may contain several year classes.

70 In amphibians, high water temperature is often associated with a rapid larval
71 development rate and therefore rapid timing of metamorphosis. In contrast, low water
72 temperatures lead to a slower development rate and delayed metamorphosis (Voss,
73 1993; Walsh *et al.*, 2008). Overwintering larvae obviously experience cooler
74 temperatures (Petranka, 1998; but not necessarily, see Freeman & Bruce, 2001), but a
75 previous field study of *H. retardatus* has shown that not only the water temperature but
76 also the stability of the water level in a pond significantly affects the timing of
77 metamorphosis (Iwasaki & Wakahara, 1999).

78 Theoretical and empirical studies have shown that cannibalism can affect the life
79 history of various taxa (Elgar & Crespi, 1992; Wildy *et al.*, 1998; De Block & Stoks,
80 2004; de Vries & Lakes-Harlan, 2007). In larval amphibian communities, cannibalism
81 can directly affect population density, size, and structure, and therefore may play an
82 important role in regulating populations (Crump, 1992; Maret & Collins, 1994). In
83 general, cannibalistic individuals develop faster, are larger, and have higher survivorship
84 and enhanced reproductive success than non-cannibalistic individuals, and these
85 beneficial effects of cannibalism on life history may be more pronounced when food
86 availability is low (Polis, 1981; Elgar & Crespi, 1992). In amphibians, however,
87 fast-developing larvae metamorphose at a smaller body size than do slowly developing

88 larvae from the same cohort (Wilbur & Collins, 1973). In particular, *H. retardatus*
89 larvae fed only conspecific larvae metamorphose much earlier and at a smaller size than
90 those fed only their typical prey (freshwater oligochaetes) (Michimae & Wakahara,
91 2002). The fast development associated with cannibalism may result in the
92 metamorphosis of larvae into terrestrial juveniles by late autumn, before ephemeral
93 ponds dry up. In amphibians, therefore, cannibalism may be an important mechanism by
94 which the larvae reach the necessary developmental stage and size before the pond in
95 which they were spawned dries up, thus reducing mortality due to desiccation (Lannoo
96 & Bachmann, 1984). Thus, there is a trade-off associated with cannibalism: even if it
97 increases the likelihood of survival during the larval stage, the associated accelerated
98 development can result in the larvae being smaller at metamorphosis, which can
99 negatively affect fitness-related traits expressed later in life (Altwegg & Reyer, 2003).

100 I hypothesized that higher density in natural ponds is likely to trigger cannibalism and
101 hasten metamorphosis, thus decreasing the number of age classes in a larval population
102 and altering its age structure. Moreover, this may occur even in ponds with relatively
103 cool water temperature, which tends to prolong the larval period and increase the
104 number of age classes, with the benefit of a larger body size at metamorphosis. On the
105 basis of this hypothesis I made the following predictions: (1) Relatively cool water
106 temperature prolongs the larval period in *H. retardatus*, but cannibalism reduces the
107 larval period. (2) In natural ponds characterized by cool temperatures, populations with
108 high larval density should have fewer age classes than those with low larval density.

109 I examined the first prediction by experimentally investigating the effect of water
110 temperature and cannibalism on the duration of the larval period in *H. retardatus*. To
111 my knowledge, this is the first study to jointly examine the relative influence of water

112 temperature and cannibalism. To test the second prediction, I conducted a field study of
113 larvae in seven natural ponds with either low or high larval density. In the field study I
114 addressed the following questions: (1) What is the age distribution of salamander larvae
115 in cool-water ponds in Hokkaido? (2) Is the number of age classes correlated with larval
116 density?

117

118 MATERIALS AND METHODS

119

120 REARING EXPERIMENT

121

122 I collected three fertilized egg clutches of *H. retardatus* in 2006 in the vicinity of
123 Sapporo, Japan, during the spawning season, transported them to the laboratory, and
124 placed all clutches in a large plastic tank (30 × 25 × 17.5 cm deep) filled with 5 L of
125 dechlorinated tap water. The tank was kept in the laboratory at 4 °C until use. The tank
126 was placed at room temperature (20–21 °C) to accelerate hatching before starting the
127 rearing experiment. All the clutches almost hatched on almost the same date (fewer than
128 3 days separated the earliest from the latest hatching), and then 60 of the newly hatched
129 larvae were separately reared for 1 week at room temperature (20–21 °C), each in a
130 small tank (8 × 8 × 8 cm) containing 0.3 L of dechlorinated tap water. The experimental
131 larvae were fed on days 3, 5, and 7 by being offered frozen Chironomidae from 20:00 to
132 22:00. They were always given enough food to eat within 2 h, and any food remaining
133 in their tanks was removed after the feeding period. The rearing water was also
134 exchanged on days 3, 5, and 7, after the feeding period.

135 Then, I randomly assigned a group of 15 1-week-old larvae to one of four
136 experimental conditions that were created by crossing two categories of water

137 temperature (15 °C or 20 °C) with two food type categories (Chironomidae or
138 conspecific larvae). These water temperature choices in the experiment were based on
139 the findings of our previous study (Sakata *et al.*, 2005). That study found that *H.*
140 *retardatus* larvae that were reared at 20 °C had the shortest larval period and those
141 reared at 16 °C had the longest larval period (data not shown in Sakata *et al.* 2005),
142 among larvae reared at four specific temperatures (16, 20, 23, and 28 °C). Larvae
143 continued to be reared separately in the small tanks in 0.3 L of dechlorinated tap water.
144 Each larva was placed in an electric incubator set at 15 °C or 20 °C and fed with one of
145 the two food type categories (one larva or frozen Chironomidae) from 20:00 to 22:00
146 every other day (no food remained at the end of the feeding period) until they completed
147 metamorphosis. All food types had about the same wet weight (all fed larvae were about
148 the same size, and frozen Chironomidae of about the same mass as one larva were fed).
149 The fed larvae were smaller than the experimental larvae because they were reared after
150 hatching in tanks (30 × 25 × 17.5 cm deep) maintained at 4 °C, which retarded their
151 growth. The wet weight of each food type was measured to the nearest 0.01 g with an
152 electronic balance. The rearing water was also changed every other day after the feeding
153 period. The time (days) from hatching to the completion of metamorphosis was
154 recorded for each larva. To compare the effects of the two treatments (food type and
155 water temperature) on body size at metamorphosis, I first anesthetized each new
156 metamorph by immersion in 0.01% MS222 (Sandoz). I measured the total length and
157 snout-vent length (SVL) of each metamorph to the nearest 0.05 mm with calipers and
158 weighed each metamorph to the nearest 0.01 g with an electronic balance.
159 Measurements of SVL were made from the tip of the snout to the anterior corner of the
160 cloaca. The metamorphs were afterward released into the ponds from which they had

161 been collected from as eggs.

162

163

FIELD SURVEY

164

165 I chose seven discrete *H. retardatus* larval habitats (permanent ponds) in Hokkaido,

166 Japan, each characterized by a different density of larvae, as described below (Fig. 1).

167 The seven permanent ponds were visited every month from May or June through

168 October in 2006 and 2007 to check whether spawning had begun and to monitor larval

169 growth and measure water temperature. During the winter (December to next April)

170 some sites were inaccessible because of the heavy snow fall. At each visit, water

171 temperature in the ponds was measured at 5 cm depth, and captured larval salamanders

172 were anesthetized by immersion in 0.01% MS222 (Sandoz). The SVL of each captured

173 larval salamander was then measured, and the developmental stage was determined

174 according to the normal table for *H. nigrescens* (Iwasawa & Yamashita, 1991). Iwasaki

175 & Wakahara (1999) previously observed two or three size categories in some permanent

176 ponds, but the categories may vary seasonally. Two studies used skeletochronology to

177 determine the age of salamander larvae of different sizes from the same pond (Iwasaki

178 unpublished data, Kanki & Wakahara, 2001). These studies identified larvae that had

179 overwintered either 1 or 2 years, as well as a strong positive relationship between age

180 and SVL (Iwasaki unpublished data, Kanki & Wakahara, 2001). I adopted the

181 methodology of assigning age classes based on SVL, although the possibility cannot be

182 excluded that the observed overlap in size of successive cohorts is due to differential

183 feeding histories or other factors.

184 The number of *H. retardatus* egg clutches in each pond and the number of eggs in each

185 collected clutch were counted to estimate the annual recruitment in each pond. Ten egg
186 clutches were collected from each pond in 2006 and ten in 2007. The annual recruitment
187 in each pond was estimated by multiplying the mean size of the collected clutches by
188 the estimated density of clutches in the pond. By visiting all seven ponds approximately
189 monthly from May or June through October, I was able to ascertain both the end of the
190 oviposition period of *H. retardatus* and also the total number of egg clutches of *H.*
191 *retardatus* in each pond. The annual recruitment was calculated for each pond each year,
192 and the mean annual recruitment (individuals/m² ± SD) in each pond was calculated by
193 dividing the sum of the annual recruitment by the number of years that clutches were
194 collected. Although total density in the ponds with two or three year classes cannot be
195 estimated by this method, larval density can be approximated because the number of 2-
196 or 3-year-old larvae was overwhelmingly smaller than the number of that year's
197 recruitment (personal observation). I therefore categorized each of the seven ponds into
198 two groups (low or high) according to the mean annual recruitment.

199

200

STATISTICAL ANALYSES

201

202 In the rearing experimental data, the effects of the two factors (food type and water
203 temperature), and the interaction between them on body size (total length, SVL, and
204 body weight) at metamorphosis were analyzed by multivariate analysis of variance
205 (MANOVA). After MANOVA, I assessed which variables were responsible for the
206 significant main effects by a univariate analysis of variance (two-way ANOVA) of each
207 response variable.

208

209

The Cox proportional hazards model was used to assess the effects of the two
treatments and their interaction on time to metamorphosis, which was measured from

210 the date of hatching to the date of completion of metamorphosis. I conducted a stepwise
211 model reduction and determined the final parsimonious model by comparing the
212 deviance (the difference in the $-2\log$ -likelihood values between two models) to evaluate
213 the fit of the models, which consisted of the different combinations of, and the
214 interaction between, the two independent variables. The effects of each independent
215 variable on the time to metamorphosis in the final model were adjusted for the other
216 independent variable by using the Cox proportional hazards model. Results were
217 calculated as the hazard ratio and 95% confidence interval (CI). Also, the distributions
218 of the time to metamorphosis between treatments were estimated by the Kaplan-Meier
219 method, and compared by using the log-rank test.

220 In the field study, three ponds, Tomaru, Teine1, and Teine3, contained two age classes
221 of larvae for 2006 and 2007, and the other four, Asari, Konuma, Jozankei, and Teine2,
222 contained three age classes for 2006 and 2007 (Fig. 1). To compare the SVL of the
223 oldest larvae just before metamorphosis (e.g., May) between the ponds with two age
224 classes and those with three age classes, I used one-level nested ANOVA for the factor
225 "age at metamorphosis" (two or three) and the subgroup "population" within age at
226 metamorphosis.

227 I then categorized each of the seven populations in a two-by-two factorial of the
228 factors larval density (low or high) and the number of age classes present (two or three).
229 The mean annual recruitment (individuals/m² \pm SD) was calculated for each pond: Asari
230 (142.7 \pm 10.1), Konuma (17.9 \pm 1.16), Jozankei (431.6 \pm 24.9), Teine2 (122.2 \pm 21.7),
231 Tomaru (2363.8 \pm 170.1), Teine1 (896.9 \pm 119.5), and Teine3 (928.7 \pm 61.1). Larval
232 density was low in Asari, Konuma, Jozankei, and Teine2, and high in Tomaru, Teine1,
233 and Teine3. To find the relationship between the number of age classes present and

234 larval density, I analyzed a table of frequency data cross-classified according to these
235 two categorical variables using Fisher's exact test.

236 I was not able to test for effects of water temperature differences between the natural
237 permanent ponds on the number of age classes present, because no permanent ponds
238 with a temperature exceeding 16 °C were found in my preliminary surveys. However,
239 the temperature of some temporary ponds exceeds 16 °C (e.g., 20°C in summer, Iwasaki
240 & Wakahara, 1999).

241

242 **RESULTS**

243

244 **REARING EXPERIMENT**

245

246 There were significant multivariate effects associated with both factors (food type and
247 water temperature), but not with the interaction between food type and water
248 temperature (Table 1). Subsequent ANOVAs detected that food type and water
249 temperature significantly affected body size at metamorphosis by all three measures:
250 total length, SVL, and body mass, but the interaction of the two factors had no effect on
251 any of these three variables (Table 1). Salamander larvae consuming Chironomidae or
252 reared at 15 °C had a larger body size at metamorphosis than those consuming larvae or
253 reared at 20 °C (Fig. 2).

254 The final model included food type and water temperature as explanatory variables but
255 no interactive effect was detected (Table 2). Therefore, only the additive effect of food
256 type and water temperature explained the time to metamorphosis. The estimated hazard
257 ratio of larvae to Chironomidae was 2.89 (95% CI, 2.03–4.27), and the estimated hazard
258 ratio of a water temperature of 15 °C to one of 20 °C was 0.18 (95% CI, 0.11–0.29).

259 Kaplan–Meier curves for time to metamorphosis are displayed in Fig. 3 for each
260 combination of water temperature (15 °C or 20 °C) and food type (Chironomidae or
261 larvae). The time to metamorphosis between larvae fed Chironomidae (median time, 70
262 days; 95% CI, 69–74 days) and those fed conspecific larvae (median time, 55 days;
263 95% CI, 52–60 days) was significantly different (log-rank test, $P < 0.0001$) at the water
264 temperature of 20 °C, and also at the water temperature of 15 °C: Chironomidae
265 (median time, 85 days; 95% CI, 79–87 days); conspecific larvae (median time, 74 days;
266 95% CI, 71–80 days) (log-rank test, $P = 0.0003$). Similarly, the time to metamorphosis
267 between water temperatures of 20 °C and 15 °C was significantly different in both
268 larvae fed Chironomidae (log-rank test, $P < 0.0001$) and those fed conspecifics
269 (log-rank test, $P < 0.0001$). Thus, the time to metamorphosis was prolonged by a
270 decrease in the water temperature from 20 °C to 15 °C, and also by feeding the larvae
271 Chironomidae instead of larvae (Fig. 3).

272

273

FIELD SURVEY

274

275 Figure 4 shows temporal changes in SVL (left axes: lines) of the collected *H. retardatus*
276 larvae in relation to the water temperature (right axes, bars) in the seven ponds (Fig. 1).
277 Larvae observed in May, the spawning season, at Tomaru, Teine1, and Teine3 were
278 those that had developed from egg clutches spawned the year before and overwintered
279 as larvae. These larvae were not observed in June (Teine1 and Teine3) or July (Tomaru),
280 probably because they had metamorphosed. Newly hatched larvae were first observed in
281 June at Tomaru and Teine3 and in July at Teine1. These larvae grew during the summer
282 and some probably overwintered as larvae in the aquatic habitat while others
283 metamorphosed by late autumn (October) of the year in which they were spawned. Only

284 full-grown larvae (stage 63) were observed at Tomaru, Teine1, and Teine3 in October
285 of both 2006 and 2007. Some of these may have hibernated during the winter in muddy
286 ground under the snow and not metamorphosed until the next year.

287 Larvae that were observed in the spawning season at Asari, Jozankei, Konuma, and
288 Teine2 ponds belonged to two size categories, indicating the presence of larvae that had
289 overwintered 1 year as well as ones that had overwintered 2 years. The larvae that had
290 overwintered for 2 years (i.e., the larger larvae observed in the spawning season)
291 probably completed metamorphosis during the summer of their third year, as by July
292 they were no longer observed. The larvae that had overwintered for 1 year (i.e., the
293 medium-sized larvae observed in the spawning season) continued to grow in these
294 ponds during their second summer, and some may have completed metamorphosis by
295 the late autumn of their second summer, whereas others presumably again overwintered
296 as larvae in the aquatic habitat, to metamorphose during their third year. All 1
297 year-overwintered larvae observed at Asari, Jozankei, Konuma, and Teine2 ponds in
298 October of both 2006 and 2007 were full grown (stage 63). Small, newly hatched larvae
299 were first observed in June or July. These larvae grew during their first summer but
300 probably did not metamorphose by late autumn (none of these larvae at Asari, Jozankei,
301 Konuma, or Teine2 reached stage 63 in either 2006 and 2007). Instead, they probably
302 hibernated during the winter to become 1 year-overwintered larvae the following year.

303 In 2007, SVL in the ponds with three age classes (Asari, Konuma, Jozankei, and
304 Teine2) was significantly larger than that in those with two age classes (Tomaru, Teine1,
305 and Teine3), but in 2006, SVL did not significantly differ between these two groups,
306 indicating a very strong trend (Table 3). In natural environments as well as in the
307 experimental laboratory environment, a prolonged larval period led to a slightly larger

308 SVL just before metamorphosis (Figs 4, 5).

309 The number of age classes was significantly affected by larval density in natural ponds
310 (Fisher's exact test, $P = 0.0286$). Ponds characterized by high larval density contained 1
311 year-overwintered larvae along with the current year's larvae (i.e., two age classes), but
312 those characterized by low larval density had both 1-year- and 2-year-overwintered
313 larvae along with the current year's larvae (i.e. three age classes).

314

315 **DISCUSSION**

316

317 The experimental results showed that in individuals grown at the relatively cold
318 temperature of 15 °C the time to metamorphosis was significantly prolonged and their
319 body size (total length, SVL, and body mass) at metamorphosis was significantly
320 greater compared with individuals grown at the relatively warm temperature of 20 °C.
321 This impact of water temperature on metamorphic timing and body size at
322 metamorphosis is similar to the temperature effects seen in other amphibian larvae. For
323 example, larval anurans grown at cold temperatures take longer to develop but the
324 metamorphs are also larger than conspecifics grown at warmer temperatures (Smith-Gill
325 & Berven, 1979; Voss, 1993; Walsh *et al.*, 2008). After energy uptake, temperature can
326 be considered the most important proximal cause of variation in size and age at
327 metamorphosis in amphibians (Rose, 2005). In *H. retardatus* water temperature did not
328 itself directly affect the body size at metamorphosis; rather, the prolongation of the
329 larval period caused by the cooler water temperature caused the body size at
330 metamorphosis to be larger, as described below. Food type, which is independent of
331 water temperature, also affected the time to metamorphosis and body size at

332 metamorphosis. *Hynobius retardatus* larvae that consumed conspecifics had a shorter
333 larval period (Fig. 3), indicating that cannibalism can cause a fast development rate
334 (Michimae & Wakahara, 2002). This accelerated development led to smaller size at
335 metamorphosis (Fig. 2, Michimae & Wakahara, 2002), implying that metamorphic
336 timing may be accelerated by consumption of the thyroxine present in conspecific
337 larvae (Pfennig, 1992).

338 Larvae of *H. retardatus* living in cool, permanent habitats may prolong the larval
339 period into a second or third year by overwintering (Fig. 4), which ensures that they will
340 have attained a larger size at metamorphosis (Figs 4, 5; Table 3). Iwasaki and Wakahara
341 (1999) reported that the SVL of *H. retardatus* larvae just before completion of
342 metamorphosis differs significantly among three age groups; their results showed that
343 2-year-overwintered larvae are significantly larger than both 1-year-overwintered larvae
344 and those larvae that do not overwinter. Generally, low temperatures retard
345 differentiation more than growth, thereby increasing stage-specific size (Berven *et al.*,
346 1979; Voss, 1993; Walsh *et al.*, 2008). The longer larval periods of overwintering larvae
347 may benefit them by ensuring a larger body size at metamorphosis compared with their
348 nonoverwintering conspecifics (Berven *et al.*, 1979). In amphibians, a larger body size
349 is directly related to increased fecundity, and, in many cases, reproductive success
350 (Semlitsch *et al.*, 1988; Goater, 1994; Scott, 1994; Altwegg & Reyer, 2003). In addition,
351 larvae overwintering in cool permanent ponds may benefit by avoiding the additional
352 costs of terrestrial migration incurred by smaller adults. Thus, in Hokkaido, growth
353 conditions may be ideal for overwintering larvae.

354 The field survey results also suggest that time to metamorphosis in *H. retardatus*
355 larvae is influenced by larval density, that is, by cannibalism (Fig. 4). Metamorphosis

356 proceeds as soon as larvae reach a certain stage of development (i.e., stage 63) (Rose,
357 2005). Cannibalistic salamander larvae in ponds with high larval density might grow
358 faster and reach this stage earlier than non-cannibalistic larvae living in ponds with low
359 larval density (Fig. 4). This diet-based enhancement of development might translate into
360 a shorter larval duration and earlier metamorphosis, even though the cool water
361 temperatures of ponds in Hokkaido tend to prolong the larval period, leading to later
362 metamorphosis (Fig. 4). Thus, geographic variation in the frequency of cannibalism
363 may result in population differences in the metamorphic timing of *H. retardatus* larvae
364 (Fig. 4). Many *H. retardatus* larvae inhabiting permanent ponds in Hokkaido overwinter
365 as larvae in the aquatic habitat in which they were spawned instead of metamorphosing
366 during their first year, whereas most larvae inhabiting ephemeral ponds metamorphose
367 by August or September of their first year, even though the water temperature is not
368 different from that in the permanent ponds (Iwasaki & Wakahara, 1999). *Hynobius*
369 *retardatus* larvae spawned in temporary ponds must metamorphose by August or
370 September of the same year, like those of many other amphibians that breed in
371 temporary ponds and metamorphose before the ponds dry up (Travis, 1983, Newman,
372 1988b, Denver *et al.*, 1998, Laurila & Kujasalo, 1999). Cannibalism is thus an adaptive
373 behavior that, by accelerating larval development in drying ponds, reduces mortality
374 due to desiccation, even though accelerated development is associated with smaller size
375 at metamorphosis, which may negatively affect juvenile survival and the breeding
376 success of adults (Altwegg & Reyer, 2003). Indeed, accelerated larval development in
377 drying ponds is a classic example of adaptive plasticity (Travis, 1983; Lannoo &
378 Bachmann, 1984; Newman, 1988b). However, this cannibalism-induced shortening of
379 the larval period can be viewed as an unfavorable consequence for amphibian species in

380 permanent breeding habitats, where any extension of the larval period probably conveys
381 increased fitness (Semlitsch *et al.*, 1988; Goater, 1994; Scott, 1994; Altwegg & Reyer,
382 2003). Cannibalism is adaptive in that it reduces mortality due to desiccation by
383 accelerating larval development in drying ponds, but in permanent habitats the effects of
384 cannibalism on larval development might be maladaptive.

385 The aim of the laboratory experiment was to determine the association between key
386 life-history characteristics of salamander larvae (body size and larval period) and
387 environmental conditions (water temperature and diet). The extended temporal scope of
388 the field observation allowed a description of the variation in population age structure
389 under a range of environmental conditions (larval density) (Fig. 4). Populations of
390 different density categories had very different population age structures and were
391 composed of individuals with strikingly different life history characteristics (Table 3,
392 Fig. 4). The population age structures of *H. retardatus* larvae may depend primarily on
393 individual phenotypic plasticity in response to environmental variability. It is important
394 to understand how environmental effects are ultimately transduced through individual
395 organisms into population-level phenomena, with the population response arising as the
396 summation of individual responses. Without a thorough comprehension of the
397 mechanisms through which population and individual responses to environmental
398 conditions are mediated, we cannot interpret the relationship between population-level
399 and individual-level phenomena.

400

401

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403

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502 growth in larval long-toed salamanders (*Ambystoma macrodactylum*). *Journal of*
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504 Figure legends

505

506 **Figure 1.** Map of sampling sites and ponds used in the field study (open circles) in

507 Hokkaido, Japan

508

509 **Figure 2.** Effects of water temperature and food type on body size at metamorphosis.

510 Total length (a), SVL (b), and body mass (c) at metamorphosis of larvae under four

511 experimental conditions created by crossing two categories of water temperature (15 °C

512 or 20 °C) with two food types (Chironomidae or larvae). In each case the mean and SD

513 are shown. Total length, SVL, and body mass at metamorphosis were significantly

514 different between larvae reared at 15 °C or 20 °C and fed with Chironomidae or larvae.

515 Total length (water temperature, $P < 0.0001$; food type, $P < 0.0001$), SVL (water

516 temperature, $P < 0.0001$; food type, $P < 0.0001$) and body mass (water temperature, $P <$

517 0.0001 ; food type, $P < 0.0001$)

518

519 **Figure 3.** Kaplan–Meier estimates of time to metamorphosis for each of combination of

520 water temperature (15 °C or 20 °C) and food type (Chironomidae or larvae).

521

522 **Figure 4.** Longitudinal growth data (snout-vent length, SVL) in larval *Hynobius*

523 *retardatus* (left axes, symbols \pm SD, lines) and water temperature (right axes, bars) in

524 seven ponds surveyed in 2006 and 2007. Asari, Jozankei, Konuma, and Teine2, in

525 which larval density was low, contained three age classes of larvae (larvae of the current

526 year and 1-year- and 2-year-overwintered larvae), whereas Tomaru, Teine1, and Teine3,

527 where larval density was high, contained two age classes of larvae (the current year's

528 larvae and 1-year-overwintered larvae). The numbers above each symbol show the

529 sample size (n)

530

531 **Figure 5.** Numbers of larvae (larvae of the current year and 1-year- and

532 2-year-overwintered larvae) in each pond in relation to snout-vent length (SVL) during

533 2006 (left) and 2007 (right).

Table 1. Results of MANOVA for effects of food type and water temperature on body size (total length, SVL and body mass). ANOVA results for each response variable are also shown.

MANOVA				
Factor	Wilks' lambda	d.f.	<i>F</i>	<i>P</i>
Food type	0.228	3, 54	60.825	<0.0001
Water temperature	0.377	3, 54	29.714	<0.0001
Food type x Water temperature	0.944	3, 54	1.064	0.3721
ANOVAs				
Variables	MS	d.f.	<i>F</i>	<i>P</i>
Total length				
Food type	3.587	1, 56	51.763	<0.0001
Water temperature	5.998	1, 56	86.555	<0.0001
Food type x Water temperature	0.067	1, 56	0.972	0.3285
SVL				
Food type	0.905	1, 56	25.577	<0.0001
Water temperature	2.293	1, 56	64.790	<0.0001
Food type x Water temperature	0.076	1, 56	2.136	0.1494
Body mass				
Food type	1.162	1, 56	67.029	<0.0001
Water temperature	2.485	1, 56	143.325	<0.0001
Food type x Water temperature	0.051	1, 56	2.944	0.0917

Table 2. Models used in the Cox proportional hazards analysis, consisting of various combinations of two independent variables (T, water temperature; F, food type) and their interaction. Constant + T + F was selected as the final model.

Variables in model	-2Log-Likelihood	<i>df</i>	Variable evaluated	Deviance (<i>df</i>)	<i>p</i>
Constant + T + F + T*F	279.0396	3			
Constant + T + F	281.2178	2	T*F	2.1782 (2)	0.14
Constant + T	340.0858	1	F	58.8680 (1)	<0.0001
Constant + F	362.5096	1	T	81.2918 (1)	<0.0001

535

Table 3. Nested ANOVA results for the effect of age at metamorphosis (two or three) and population (Asari, Konuma, Tomaru, Jozankei, Teine1, Teine2, Teine3) within age at metamorphosis on SVL.

2006	MS	<i>df</i>	<i>F</i>	<i>P</i>
Age at metamorphosis	24.666	1	5.123	0.07
Population within Age	4.815	5	1.191	0.34
Error	4.043	23		
2007	MS	<i>df</i>	<i>F</i>	<i>P</i>
Age at metamorphosis	44.947	1	10.264	0.02
Population within Age	4.379	5	1.151	0.36
Error	3.803	21		

536

Figure 1

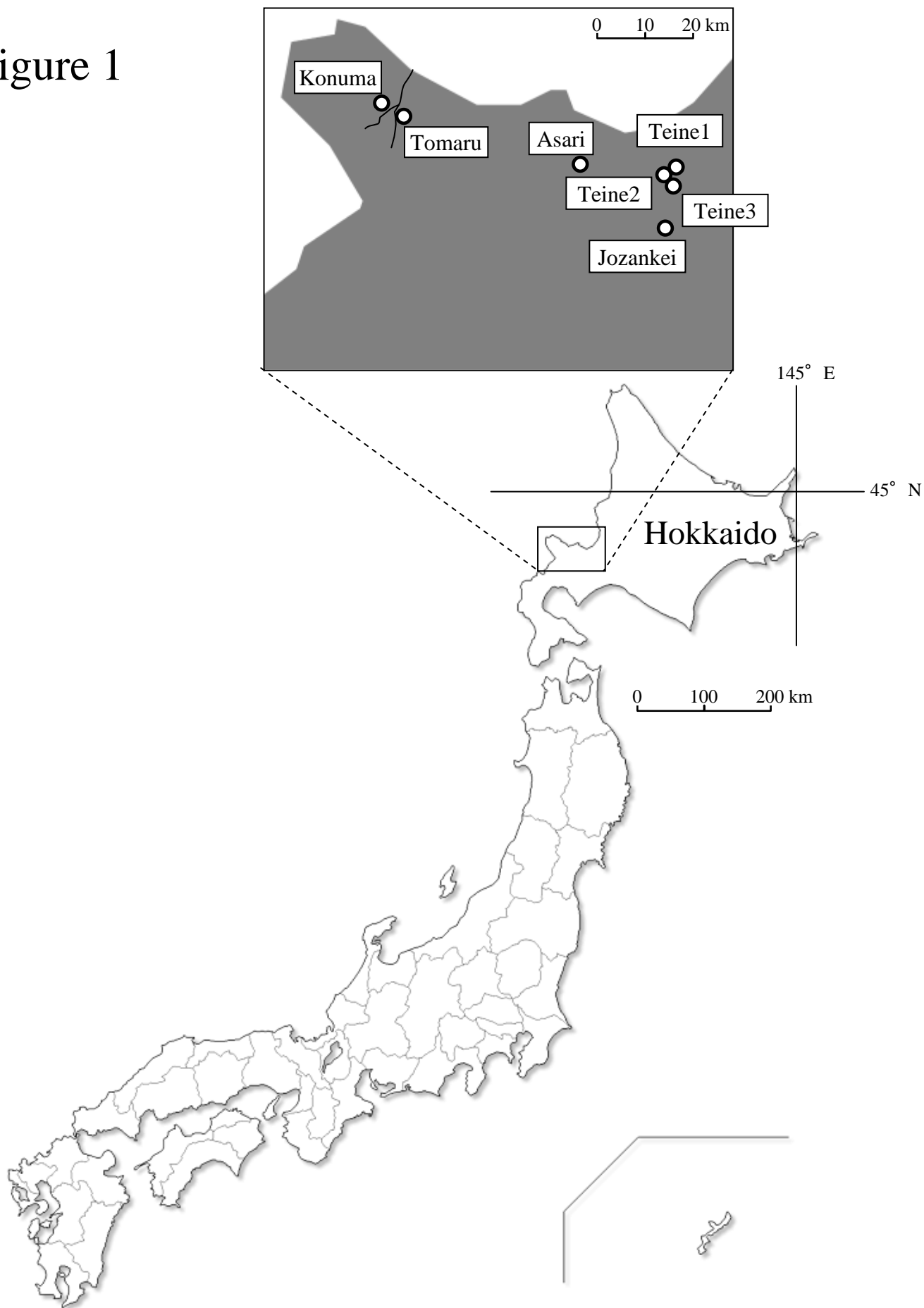


Figure 2

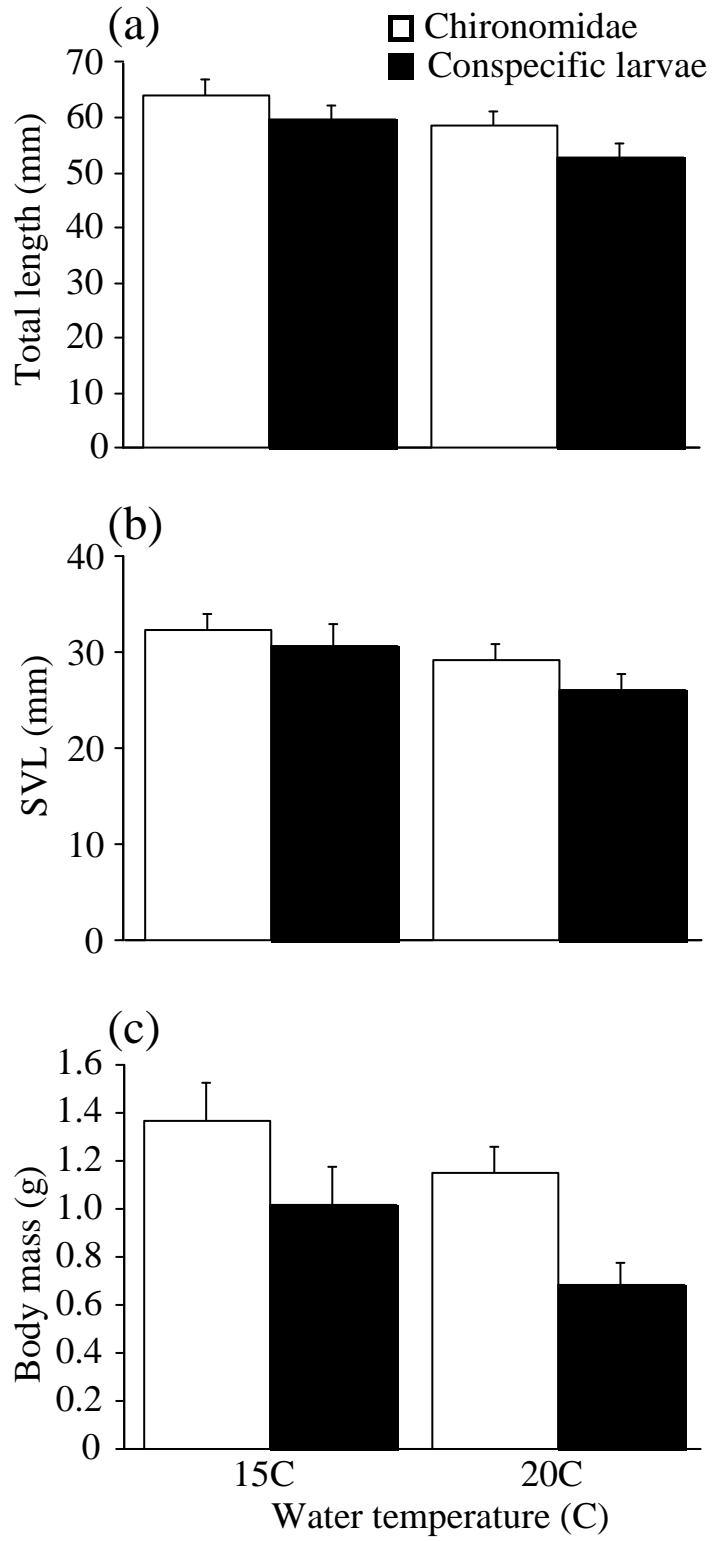


Figure 3

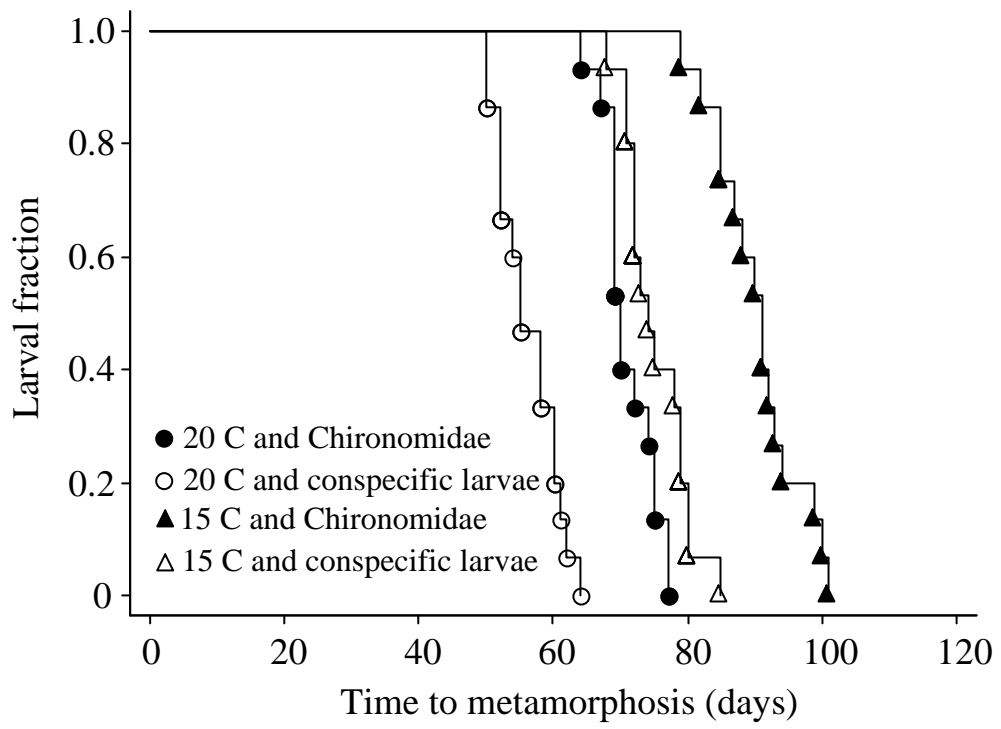
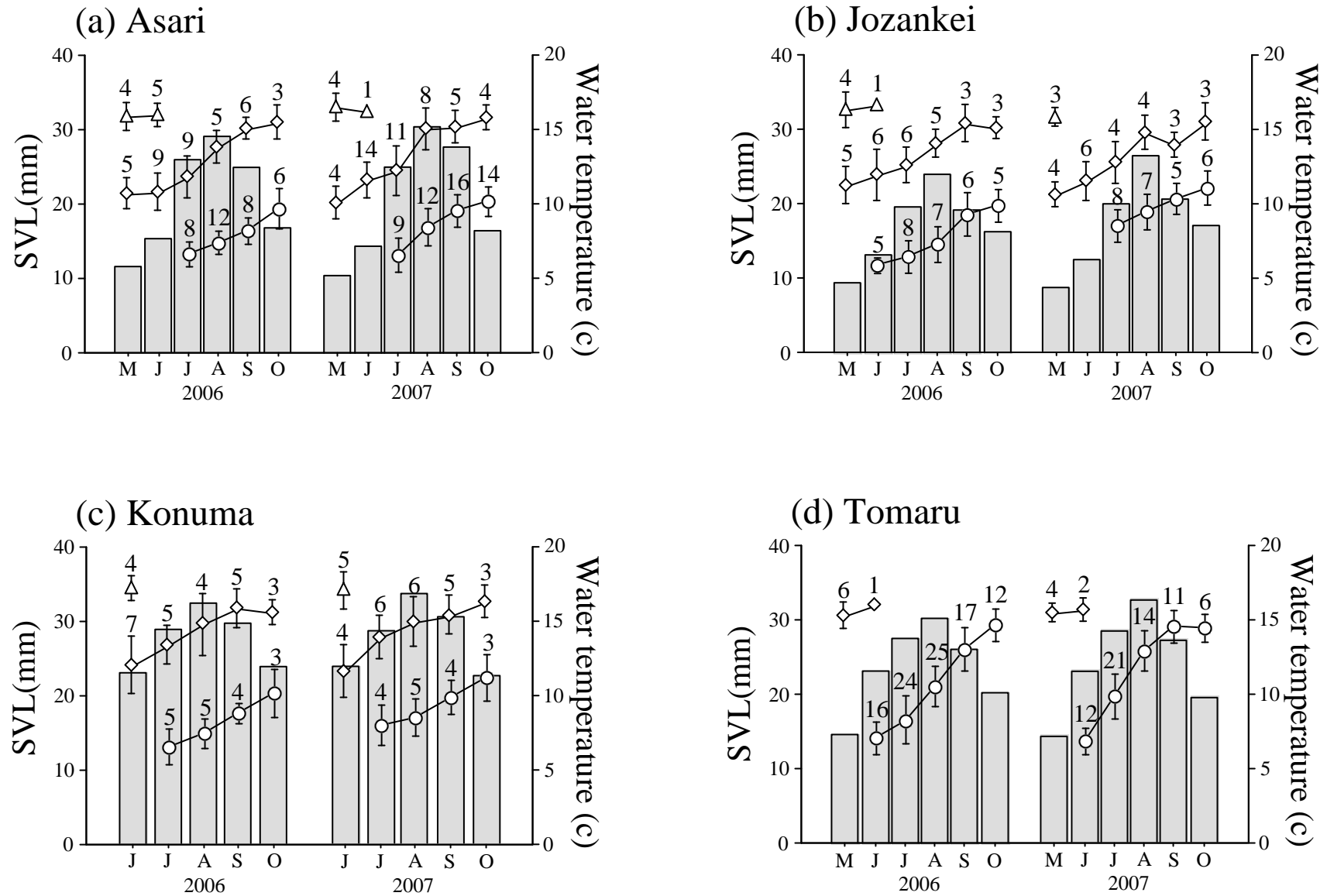


Figure 4



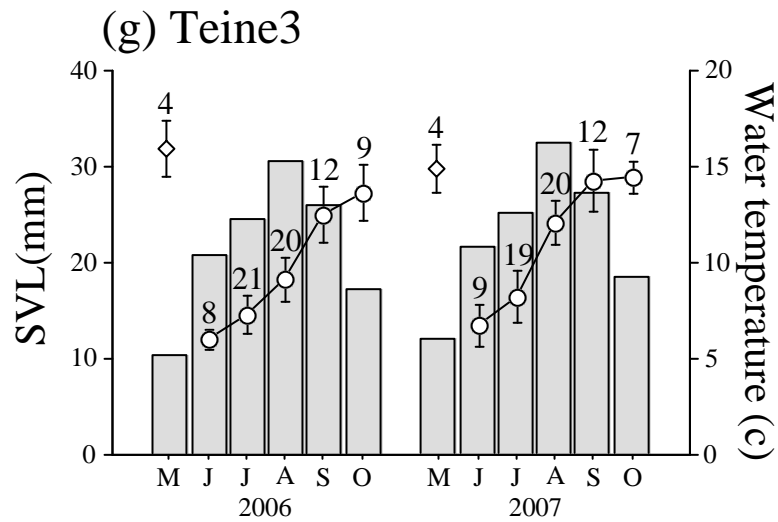
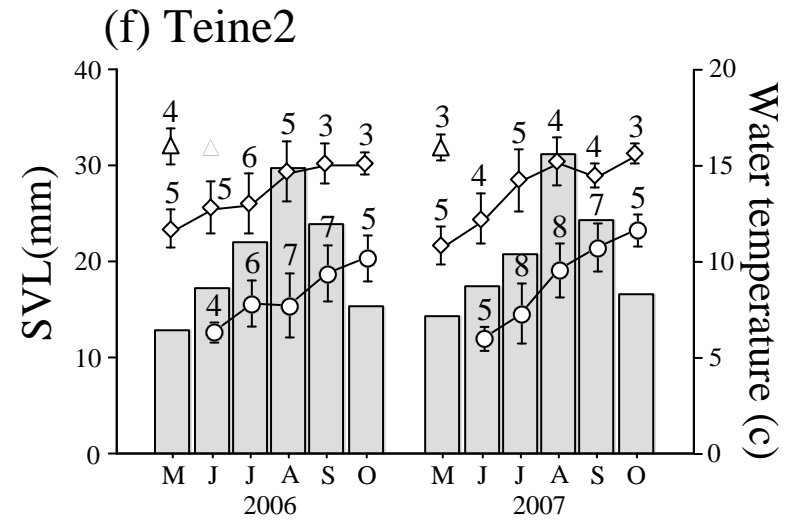
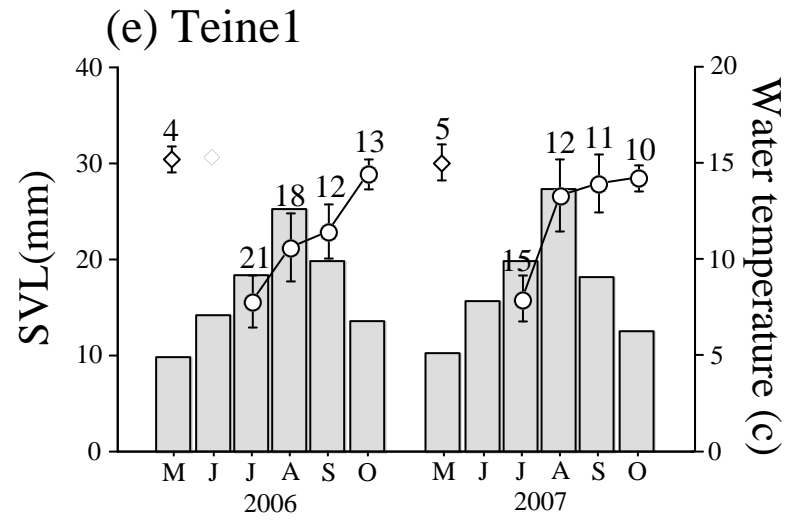
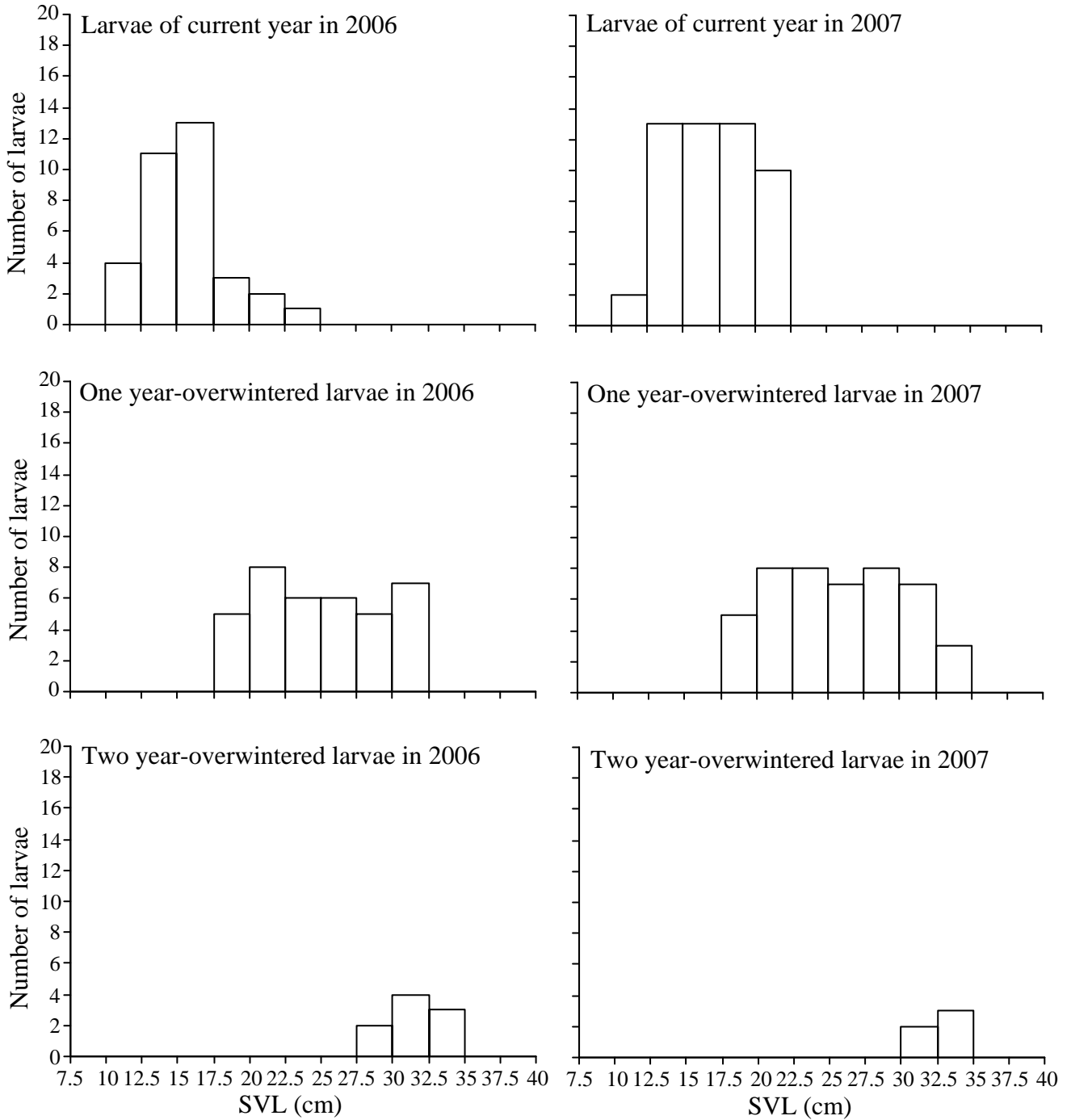
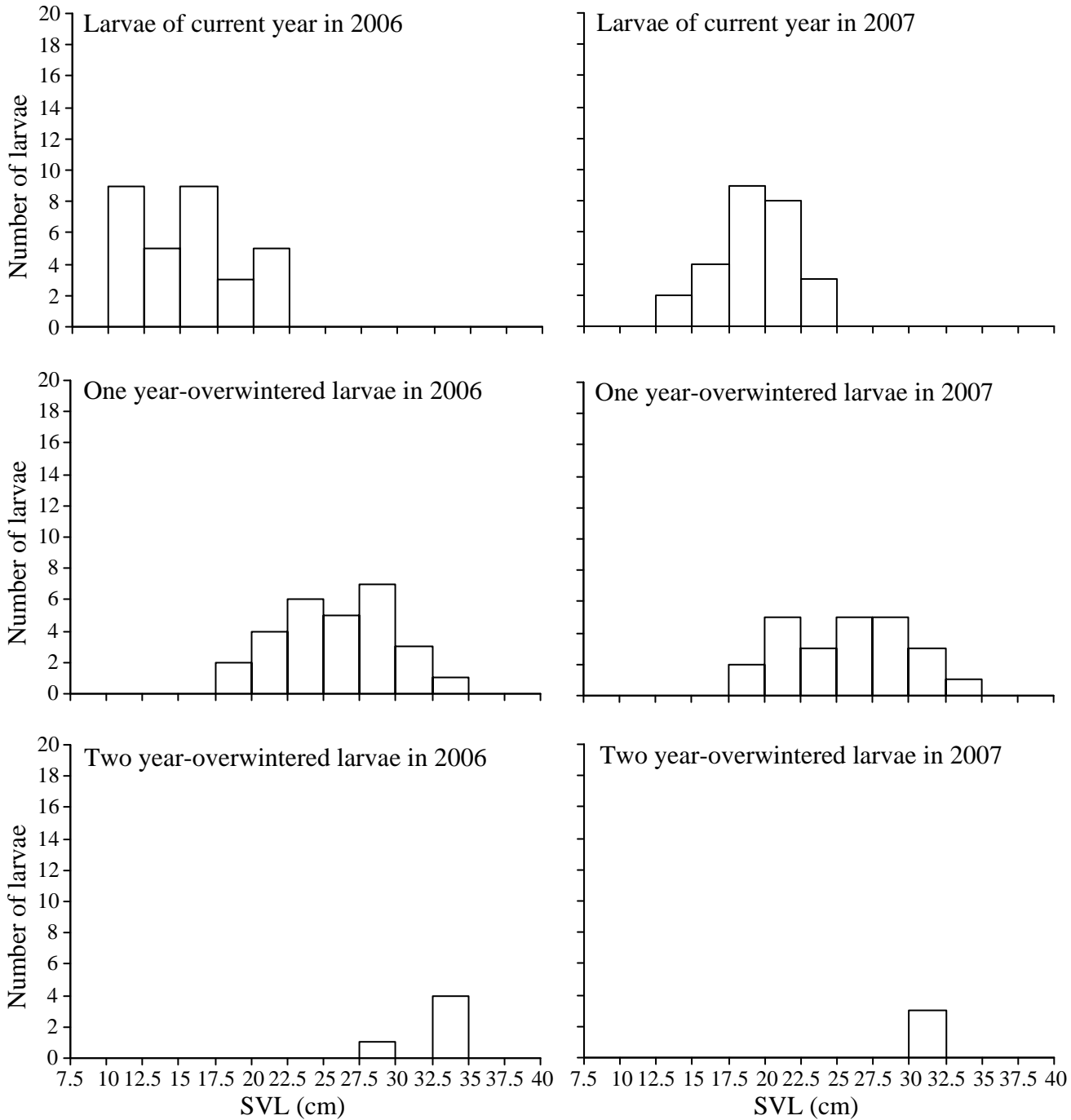


Figure 5

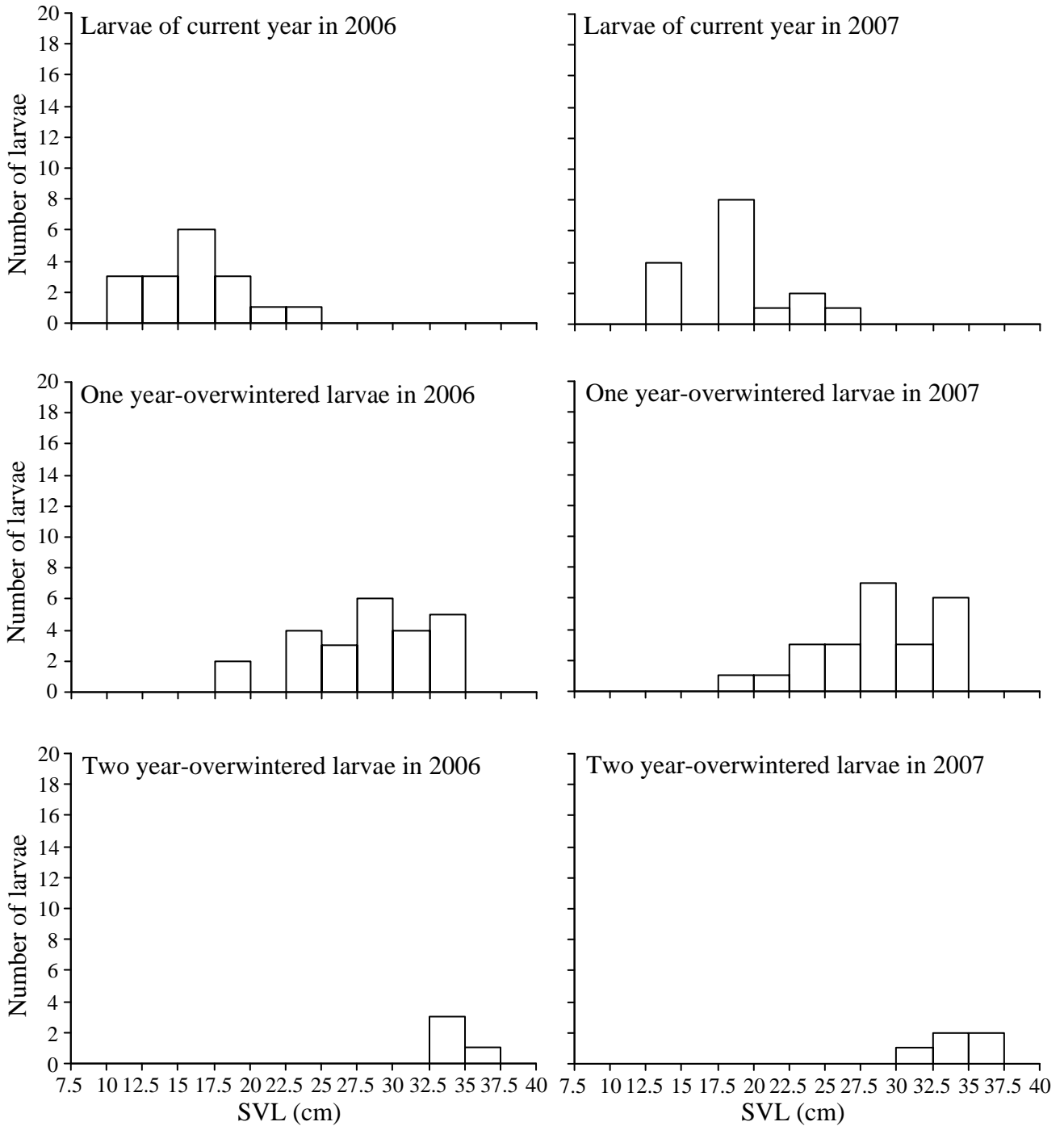
(a) Asari



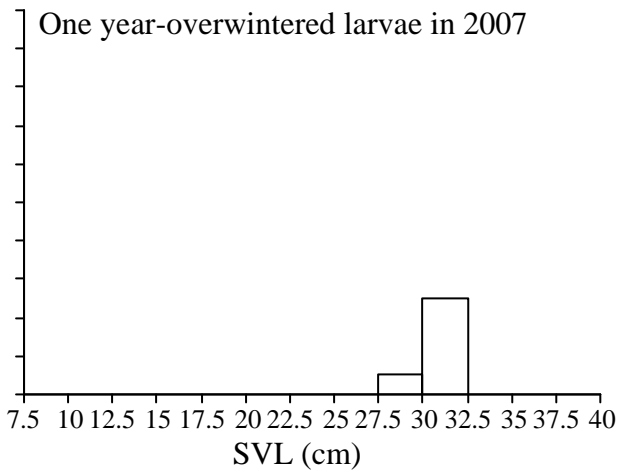
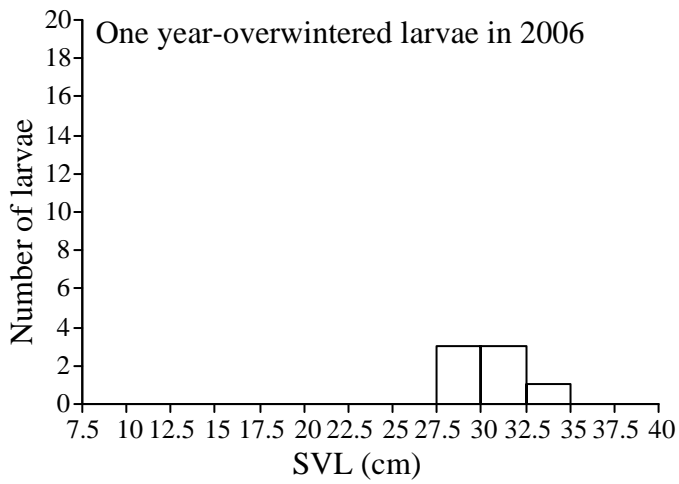
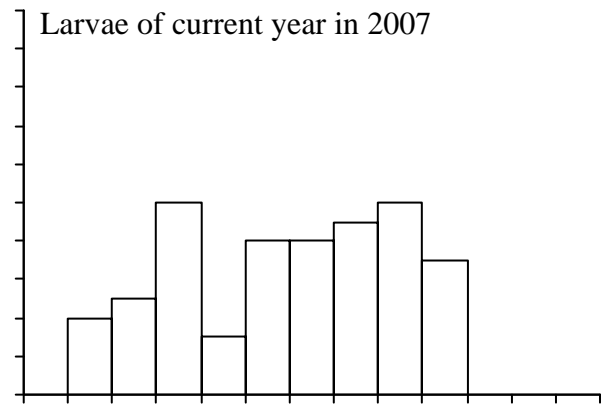
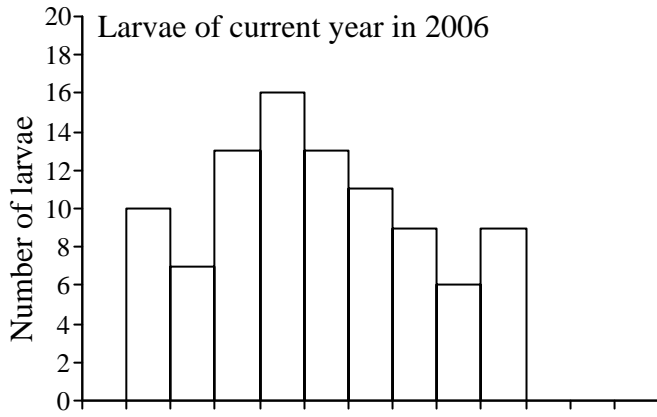
(b) Jouzankei



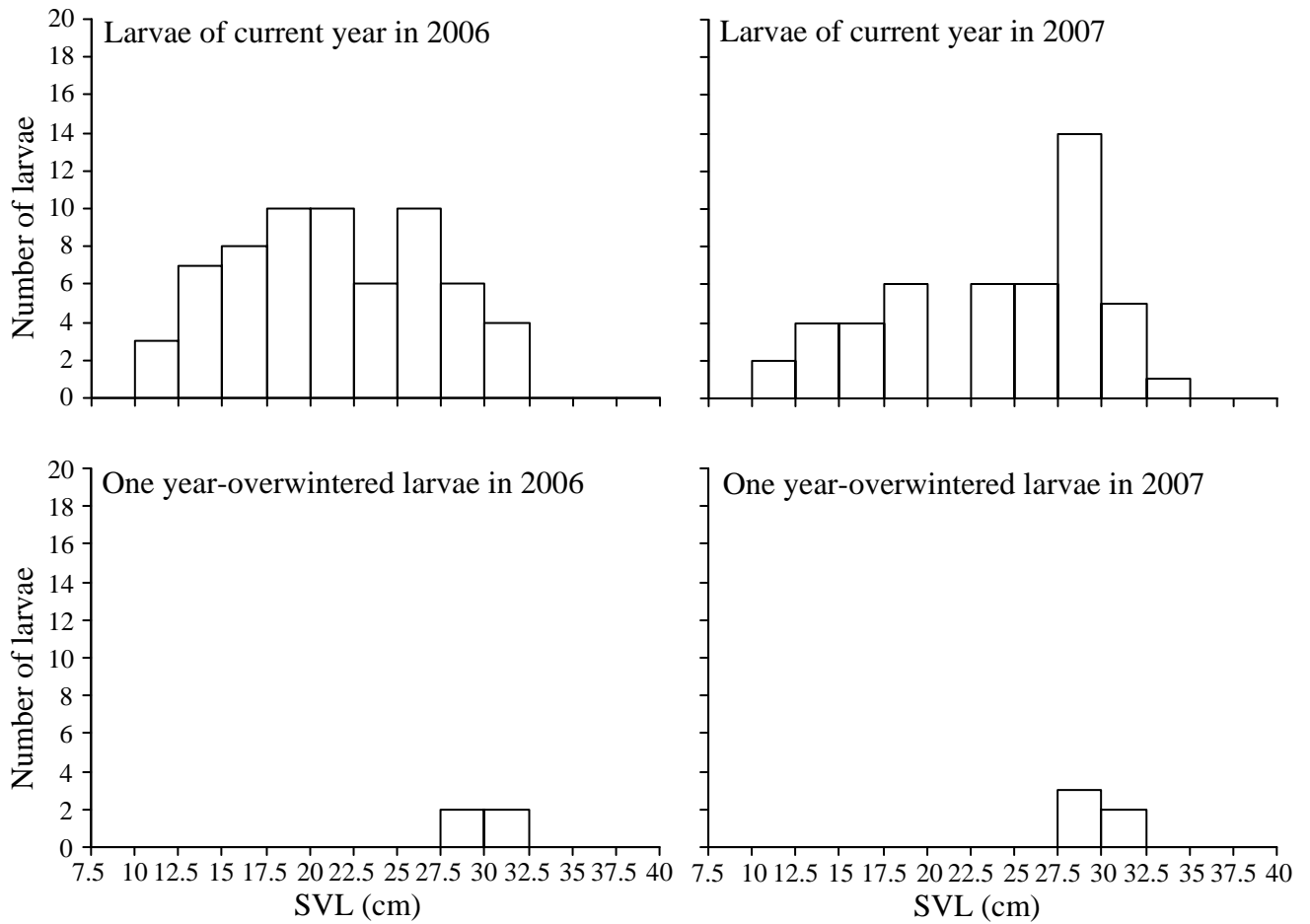
(c) Konuma



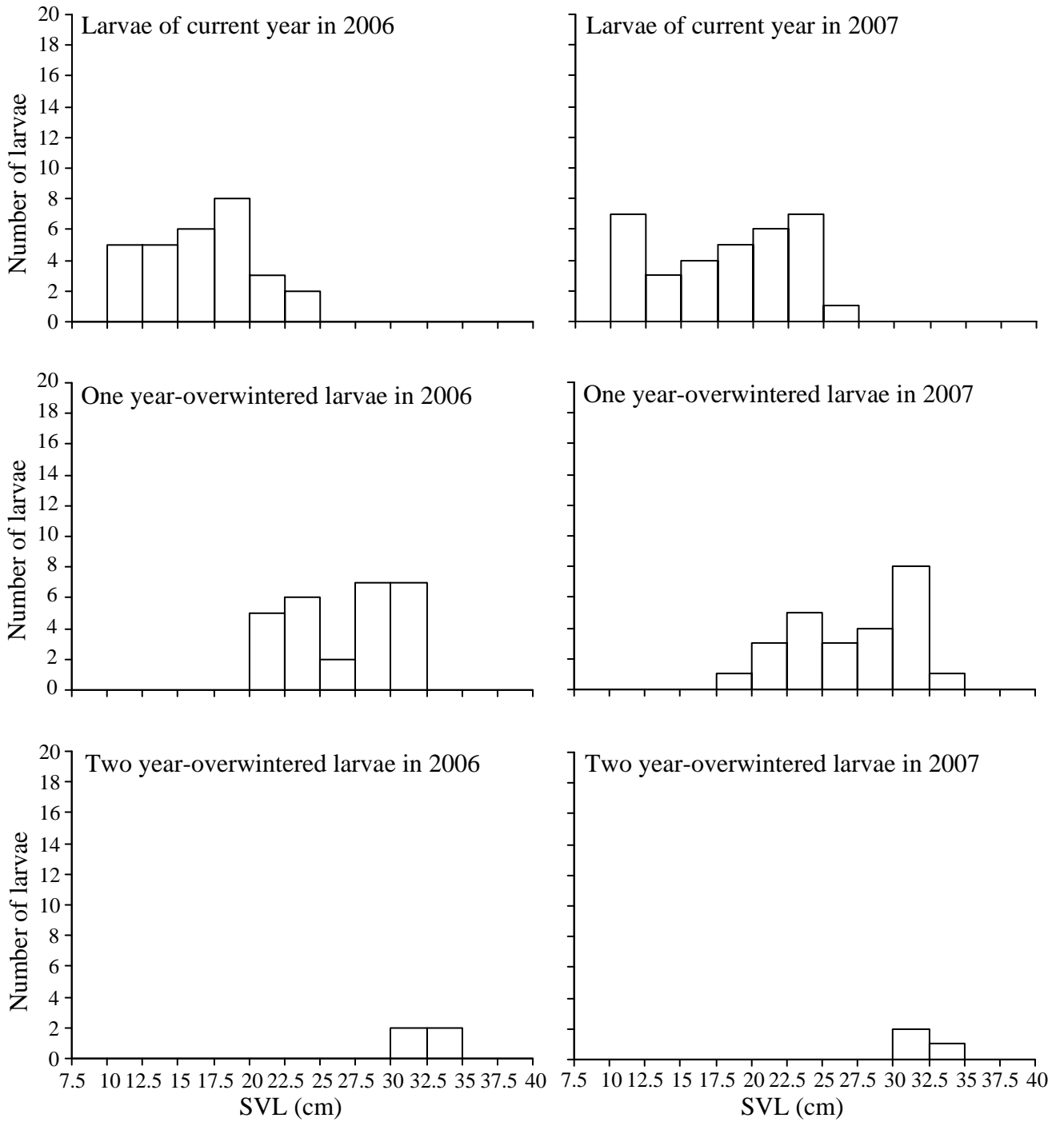
(d) Tomaru



(e) Teine1



(f) Teine2



(g) Teine3

