Supplement 2. Methods and notes on unpublished experiments

Experiments on Coenagrion larvae

All own *C. puella* derive from a small (c. 3×2m), vegetation rich, mostly exposed pond at 58.56°N, 16.58°E. During a yet unpublished field study, covering 2011-2019 (cf. Figure 1c), samples were used for experiments a couple of times. Some of the results are presented in Figure 6 and Supplement 3.

On 10 October 2014 larvae were collected and subsequently stored in darkness at 2-6°C, and occasionally fed with crustaceans, mayfly larvae or *Tubifex*. Lack of suitable food items might have caused some amount of underfeeding during storage. Experiments were started on 20-21 November, when larvae of a head width from c. 1 mm and upwards were brought to 20°C and photoperiods of LD 20:4 and 16:8. They were reared in 30-60 ml vials with c. 2cm of water, placed in water baths, and measured in the same way as described for *Lestes sponsa* by Norling (2018), but food was mostly *Tubifex*, supplemented with *Cloeon* larvae, and only for smaller larvae *Artemia* metanauplii were used as a supplement. Inspection was performed daily.

Also, some overwintering larvae of *I. elegans* and *E. cyathigerum* were collected from Nöbbelövs mosse, Lund (55.74°N, 13.15°E) and reared during these 20°C experiments on *C. puella* with identical methods. Some results are mentioned in the text and in Supplement 3.

Experiments at 24 and 28°C on *C. puella* were performed twice, on overwintering larvae in F-4(5) to F-2, collected on 10 October 2016, and on 27 April 2017. Those from October were stored like the previous ones, and experiments were initiated on 22 February, and larvae were successively acclimated during 1-2 days, depending on final temperature. Those from April were used directly without storage after a brief acclimation period.

The 24° experiments were performed in a slightly rebuilt and temperature-controlled cooling box (Rubicson 12V, 4A, volume 24 liters) in heating position, with white LED:s as light source. The bottom was covered with two centimeters of water, with a wet sheet of cellulose sponge covering the walls hit by the heated airstream from the fan. This largely, but perhaps not entirely, eliminated evaporation cooling, which was no problem in the water baths. Larvae were also here kept in 30-60 ml vials but standing on a grid above the water level. Food was mainly *Tubifex*.

The 28°C experiments took place in another rebuilt smaller cooler (Jula Hamron 604-023, 12v, 2.5-3.5A, volume 5 liters) in heating position, where the Peltier element was attached to an aluminum tray forming the bottom and lower walls. This was used as a temperature-controlled water bath, where six 100 ml vials could be placed, each with c. 2 cm of water. Due to the small air volume and the absence of a fan, aeration capillaries were used to provide oxygen. As before, LED:s were used as a light source. Small larvae (<F-2) sometimes had to be kept together in one vial.

At the two higher temperatures feeding and inspection were performed twice daily, at about 8.00 and 20.00. Inspections inevitably caused some transient cooling.

Since the latter experiments were rather crude high temperature tests, three larvae (in F-2, F-1 and F-0, respectively) were transferred directly from 24°C to 28°C on the moulting occasion to compensate for mortality in the limited space at the higher temperature, and in the April 2017 experiment many larvae started moulting very soon after reaching experimental temperatures. This did not allow previous acclimation for many specimens, but similar results suggested that this had a limited effect, not affecting conclusions. Overall, the high rate of development at these higher temperatures soon after winter conditions did not allow for long-term acclimation compared to constant-condition experiments from the egg stage.

Both published (Norling, 1984a: table 2) and unpublished details in the data set from old experiments on *C. hastulatum* during 1973 are also presented in Figure 6 and Supplement 3. The methods and collecting site are described in Norling (1984a). Photoperiods and numbers of own specimens shown in Figure 6 are shown below.

C. hastulatum, fast pre-emergence F-1 (LD 19.3:4.7) 15 and 20°C, N=4 and 4, respectively.

C. puella, fast pre-emergence F-1 (LD 20:4) 20, 24 and 28°C, N= 10, 11 and 8, respectively.

Slow pre-emergence F-1 (LD 16:8) 20°C, N=15.

Regulatory development (LD 20:4) 20°C was recorded in 8 specimens, but supernumerary moulting produced 22 intervals mainly within the approximate sizes of F-3 and F-2. No later regulatory stadia were finished before termination.

Experiments on Aeshna juncea

Larvae in the experiment in Figure 7, and the extended version in S2b, were collected on 10 August 1974 in a small pond (diameter c. 4m) in a forest edge about 50m south of the later *C. puella* site. The variable life history and seasonal regulation of this population is partly published and discussed by Norling (1984 c, p.137-138, also figures 6 and 8 therein, and Figure 1h in the present paper). Larvae were divided into six groups of c. 12-14 larvae of as similar size and sex distribution as possible for the different treatments. The larvae used here varied between F-8 and F-6 at the start (see Figure S2a). They were brought into experimental conditions on 13 and 14 August (LD 13:11, 16:8 and 19.3:4.7 at both 20 and 25°C). Rearing equipment and procedure have been described elsewhere (Norling, 1971, 1976, 1984b). Feeding and records of moults were, with few exceptions, performed daily. Only stadia with a head width above 2 mm, entered after at least one week in experimental conditions were evaluated. In the group of larvae with early development (F-7 to F-4), not all stadia were present due to different start sizes and, in LD 13:11, most larvae were still remaining in this range at the end of experiments after six months due to diapauses.

After entry into F-0 most specimens were used for diapause termination transfer experiments to other photoperiods, but only after some time, when diapause could be confirmed by a halt in eye morphogenesis. Non-diapause specimens could thus be identified and retained, but a relevant F-0 duration could neither be recorded for diapause specimens, nor for late non-diapause specimens.

Also some F-1 and F-0 larvae in pre-emergence development from standard winter experiments at 20°C during 1973-4 on this and nearby populations (collection 26-29 October; start 14 November 1973) are added in (a) and (g) in Figure 7 and S2b. The group at 25°C was partly four non-diapause F-0 specimens from the August-74 experiment presented here (see above) and three specimens from a dedicated spring experiment in 2016 (collection 12 May, start 18 May; by-catch of *C. puella*). The equipment for the latter experiment was identical to that described above for *C. puella* at 24°C.

Extended discussion of the results on A. juncea in Figure 7 and S2b

Unspecified references in the text below are to the common elements (a-i) in these Figures.

On many occasions, the results from the different sizes and treatments in the August -74 experiment on *A. juncea* seems composite with a cluster of fast, essentially nondiapause larvae, and a group of diapause larvae, often spanning a wide and variable range of stadium durations, overlapping with the nondiapause ones. These two groups occur in different proportions, e. g. all except one is diapause in (a), and all are non-diapause or very weakly diapause in (e) and (g), and there are different mixtures in (b) and (d). The fastest nondiapause larvae were consistently faster at 25°C, but overall differences in fast development are not statistically significant in any separate case, if the pre-emergence F-0 in (g) are excepted. The diapause ones are extremely variable, also between groups.

The long-term constant conditions during c. 6 months could be expected to be unnatural, perhaps making interpretations uncertain. One specimen at LD 19.3:4.7 and 25°C showed an unexpected pre-emergence like and essentially non-diapause development from a start in F-7 all the way to emergence in 97 days (the fastest specimen in (a) and (d)). Also a few other specimens in this high-temperature long-day treatment were completely non-diapause in F-0 after earlier F-1 diapauses, and seemed then to develop as pre-emergence (Table above graphs). Perhaps this and some other unexpectedly short moulting intervals could be examples of a previously observed "diapause exhaustion" during constant conditions, when a strong diapause is temporarily followed by a stadium with no or only low-intensity diapause (see also Supplement 1). Perhaps connected with this, a persistently high temperature might displace the peak regulatory long-day diapause intensity to an earlier stage (compare the two temperatures in F-2 (d), F-1 (a) and F-0 (above graphs); see also Norling, 1976 for substages in F-0 *L. dubia*).

A likely bias in Figure 7 is that the slowest larvae did not reach the later stadia during the experiment; consequently, those big larvae present have a background of faster development. Another weakness is that, as in *Coenagrion*, some comparisons are made between different experiments performed at different times.

In Figure S2b it can be seen that F-3 (m-o) seems transitional between early and regulatory development, but the low numbers of larvae make conclusions uncertain.

In early development, the single moulting intervals in S2b (j-l) show that many larvae also here displayed a mixture of variable diapause and non-diapause intervals as discussed above.

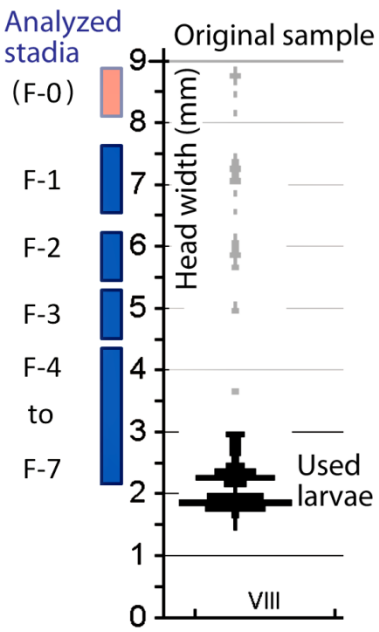

Figure S2a. Size distribution of the original sample and the size range of stadia in the *A. juncea* experiments.

Figure S2b. Extended version of Figure 7, also showing F-3 and the duration of single moulting intervals in early development. The labeling of the new parts is just added to the old labels, which remains unchanged.