

## Supplement 5. A meta-study of growth rate and thermal responses during F-0 development in *Ischnura elegans*.

A series of publications from Robby Stoks' group at the University of Leuven, Belgium have examined effects of parameters of climate change and often pesticide exposure on mainly *I. elegans*. Here, larvae from different latitudes (S: France, 43–45°N and N: Sweden/Denmark, 55–57°N) were examined at different temperatures in a quite consistent manner. Taken together, the control groups provide some interesting and intriguing information on thermal responses relevant in the present context. Effects were evaluated as growth rates by weight in recently moulted F-0 larvae over a period of 4 to 13, most often 6–7 days, usually at 20 and 24°C. Photoperiod was mostly constant LD 14:10 from hatching, sometimes LD 16:8. Without a winter, LD 14:10, including Civil Twilights, is probably relevant for late August (S) or early September (N), with reservation for the effect of constancy. In one study a winter treatment was included before the experiment. Since seasonal regulation responses in *I. elegans* have never been explicitly studied, my analysis below is in many respects provisional.

The graphic summary for *I. elegans* (Figure S5) also contains some data from other temperatures and for *I. pumilio* and *Enallagma cyathigerum*, which are asynchronous species with similar phenology and voltinism as *I. elegans* (Dijkstra, 2006). It is clearly seen that there was a variation in growth rate by a factor of about three or four between experiments within each latitudinal group and temperature treatment. Generally, southern populations, assumed to be bi- or trivoltine, grew faster than northern ones, often ascribed to a regional adaptation to different levels of general time stress. Also, southern populations increased their growth rate between 20 and 24°C, with one exception (e). The supposedly semivoltine northern populations showed mixed results, with most experiments at these temperatures showing an inverse or essentially flat thermal response.

The high variation between closely similar experiments may have several explanations, including that of a variable diapause component. The growth measurements must, with a few probable exceptions (some in a, c, d and j), have been limited to the intermoult or pre-apolysis period, the time of hormonal orchestration of developmental delays. Since the intensity of a diapause component may vary with time, differences in the intervals of growth measurement could have contributed. Also, the experiments were derived from different populations, even within each latitudinal range. There are also differences in photoperiod, pre-treatment and feeding, although larvae in most experiments were reared from eggs at a constant photoperiod of LD 14:10.

Growth rates by weight in early F-0 are difficult to put into the context of development rate as seen in stadium durations. Growth operates to some extent independently of the morphogenetic component of larval development and F-0 is the stage of the fundamental morphogenetic transformation into the adult stage. A cross-reference with stadium durations was present in a couple of studies where early F-0 growth rates were supplemented with F-0 development times (experiment a, and also in l with Belgian larvae at 22°C only).

In the latter study (Debecker, Sanmartín-Villar, de Guinea-Luengo, Cordero-Rivera, & Stoks, 2016) the durations of F-0 at 22°C of Belgian *I. elegans* (l) and *I. pumilio* (m) were relatively similar (28 and 24 days, respectively, both somewhat slow; cf. below), but the growth rates day 1-10 in *I. elegans* was 0.0067 day<sup>-1</sup> and in *I. pumilio* 0.014. This suggests that *I. elegans* had an initial growth stagnation (diapause component) which was stronger than in *I. pumilio*, but both relatively brief, and that a fast pre-emergence development was mainly absent.

In experiment (a), the growth rates of the southern larvae was very high, and F-0 duration correspondingly short (20.3 days at 20°C, and 14.0 days at 24°C). Northern larvae were instead slower with an F-0 duration of 32.5 days at 20°C and 49.3 days at 24°C, correlating reasonably with the growth rates. In another study (n; see above the graph) only F-0 duration at 20°C was measured: southern 21.5 days (shortest duration 16 days), and northern 62.7 days.

I observed pre-emergence development after winter conditions in a few *I. elegans* from an apparently univoltine population in Lund (Nöbbelövs mosse, 55°N; Supplement 2 and 3) at 20°C, and long summer photoperiods (LD 20:4). F-0 duration was 18.8 days (range 17–20; N=4), close to the 20 days of the southern larvae. This northern population was partially represented in (d) and (g), with relatively low initial growth rates at 20°C. All the above short F-0 durations are in accordance with pre-emergence F-0 data also from northern populations of related species (Supplement 3), but low numbers are again partly problematic.

This suggests that the fastest southern larvae were pre-emergence and prepared for another generation, and that an initial and variable diapause component really has contributed to the generally high variation and the low initial growth rates particularly affecting northern larvae, but probably also southern ones in some experiments. This, and the often negative thermal responses of the northern larvae, suggest regulatory development and preparations for winter. An F-0 development time of 49 days in northern *I. elegans* at 24°C, and just 14 in southern larvae, and 12 days in northern pre-emergence *C. puella* (Supplement 3) is otherwise hard to explain. The relatively high growth rates in some northern larvae at 24°C (b) and in Belgian larvae at 30°C (j), and also the appearance of a small second generation in August among the normally univoltine specimens in outdoor rearing tanks in southernmost Sweden (55°N) during the hot summer of 2018 (E. Svensson, personal communication) suggest more similar pre-emergence thermal responses at different latitudes.

Performance of pre-final stadia were not recorded, but the present model suggests that the regulatory load should be relatively weak (long flight season) and distributed among approximately the last four stadia (cf. Figure 1b). However, the late-season photoperiod of LD 14:10 might allow an initially faster regulatory development in the smallest predictive sizes, but a later slow-down in bigger sizes. There is also a possibility that F-0 responses are not always ecologically relevant. A late stadium larva should either prepare for emergence or overwintering, and in F-0 the time for decision is running out. Responses may then be unrealistic when not encountering the winter they have been preparing for (see Supplement 1). After a previous winter treatment (d), F-0 growth in northern larvae was somewhat slow in comparison to southern ones, but like these they showed a positive thermal response. The

probable LD 14:10 photoperiod, here relevant for the end of March and subthreshold temperatures, could have produced an extreme low-time-constraint pre-emergence development. This raises the question about the difference between slow pre-emergence development and fast regulatory, where responses perhaps merge during shorter to intermediate photoperiods in persistently constant conditions. Such a response is perhaps present also in some other experiments. A development time of 32 days at 20°C (a), and 28 days at 22°C (l) might be reasonable here, but 49 days at 24°C (a), and 62 days at 20°C (n) in the season of emergence do not make sense from field observations (Figure 1b), and are in a diapause range. For comparison, LD 14.5:9.5 at 20°C could often re-induce a winter diapause after the first moult even in spring-collected larvae in the more slow-growing *Aeshna viridis* in southernmost Sweden (c. 55°N; Norling, 1971).

It would be interesting to know post-apolysis thermal responses, and how they vary with source latitude.



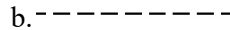











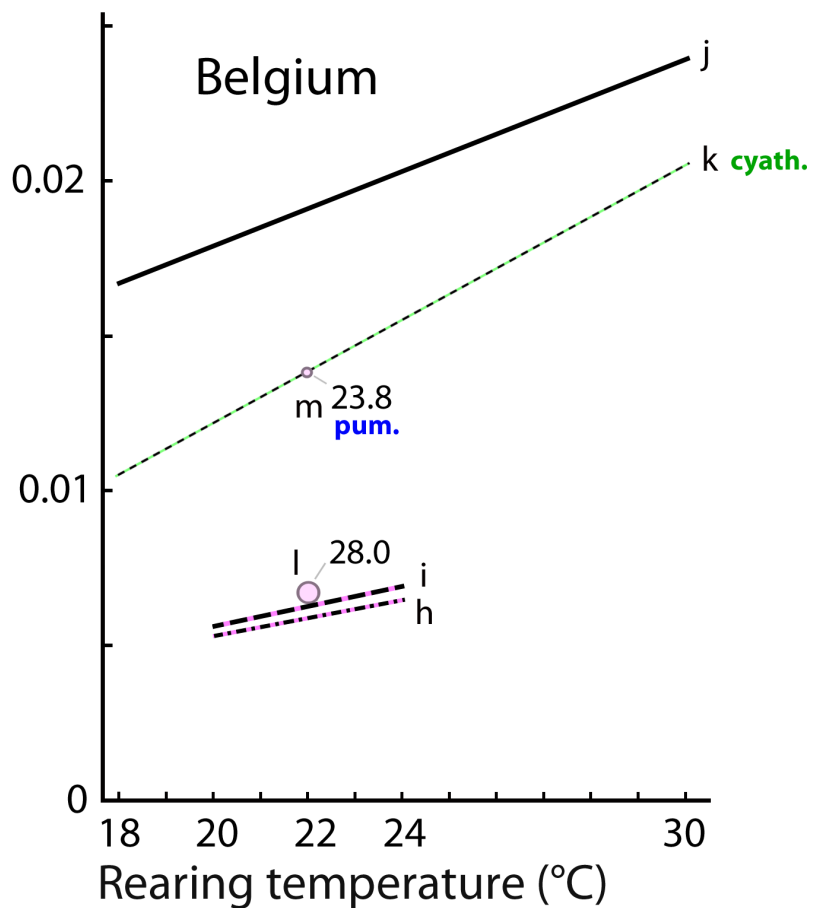
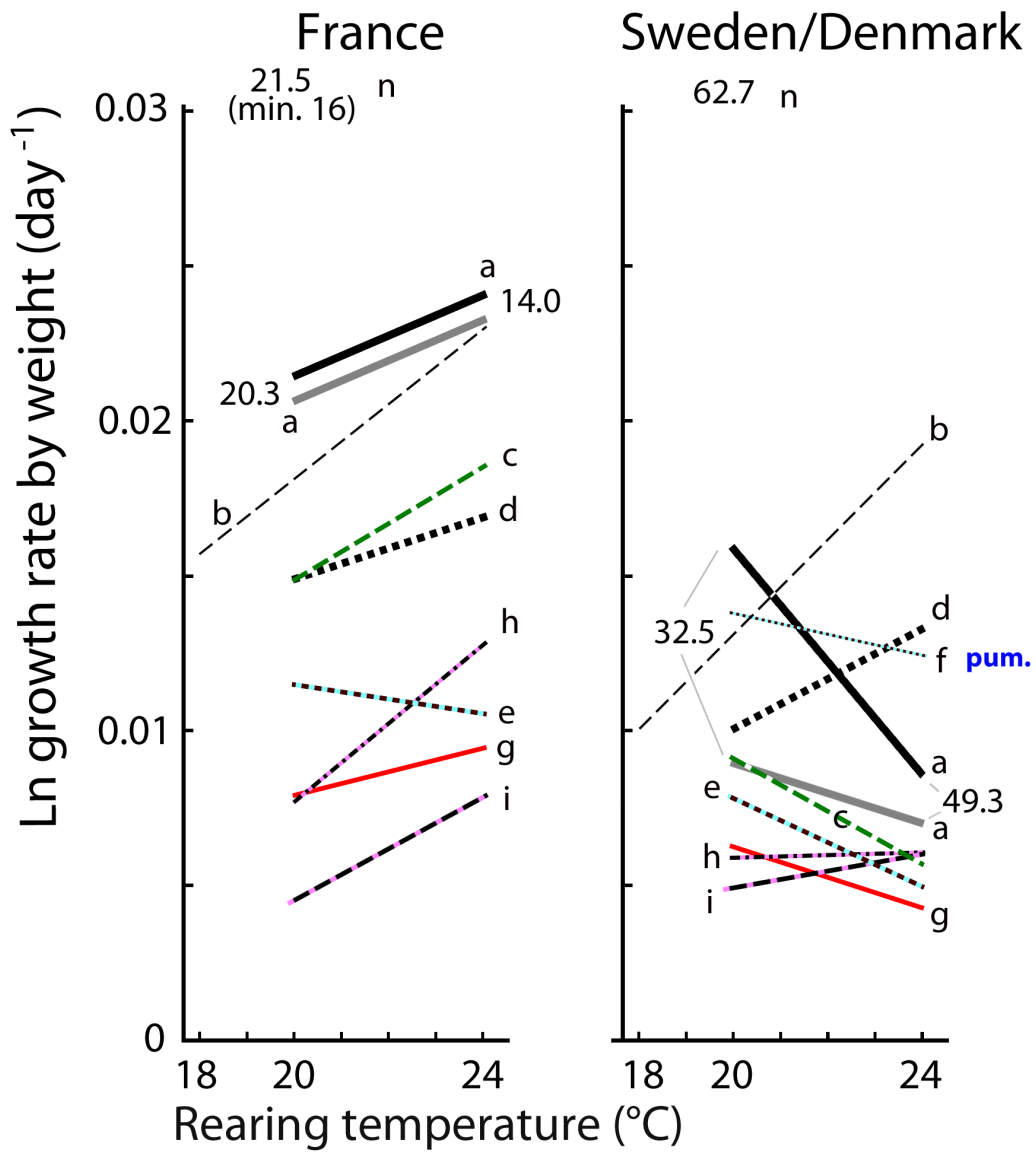
Symbol	Species	Period	Photoperiod	Comments	Ref.
a. 	<i>I. elegans</i>	6 days	14:10	-	1
a. 	<i>I. elegans</i>	+4 days	14:10	additional growth period	1
b. 	<i>I. elegans</i>	4 days	14:10 (?)	-	10
c. 	<i>I. elegans</i>	10 days	14:10	-	3
d. 	<i>I. elegans</i>	13 days	14:10 (?)	Belgian winter	12
e. 	<i>I. elegans</i>	7 days	14:10	possibly low food	7
f. 	<i>I. pumilio</i>	7 days	14:10	possibly low food	9
g. 	<i>I. elegans</i>	7 days	14:10	2 competing larvae	8
h. 	<i>I. elegans</i>	6 days	16:8	s. pop. 45°N; + Belg.	4
i. 	<i>I. elegans</i>	6 days	16:8	s. pop. 45°N; + Belg.	5
j. 	<i>I. elegans</i>	7 days	?	Belgium only	11
k. 	<i>E. cyatigerum</i>	7 days	?	Belgium only	11
l. 	<i>I. elegans</i>	9 days	16:8	Belg., 22°C only	2
m. 	<i>I. pumilio</i>	9 days	16:8	Belg., 22°C only	2
n. (numerals above graphs)	<i>I. elegans</i>	-	14:10	F-0 time at 20°C only	6

Figure S5 (graph on next page). Initial F-0 growth rates, mainly for *I. elegans*, from different populations and at different temperatures. In the graph, also F-0 development time is shown for two studies, and at the top of the graph from one study at 20°C and LD 14:10 without recorded growth rates (Janssens, Dinh Van & Stoks, 2014). In the legend above, symbols are mainly arranged after decreasing growth rate at 20°C, France. Shown after the symbol: the period of growth measurements in days, started soon after entry into F-0; photoperiod (Light:Dark); other comments; source reference.



## References to the meta study

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