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Diapause and quiescence in eggs of *Systellognatha* stonefly species (Plecoptera) occuring in alpine areas of Norway

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Keywords : Plecoptera, egg diapause, quiescence period, temperature dependence.

Eggs of the stoneflies *Diura bicaudata* and *Arcynopteryx compacta*, occurring in the middle-alpine vegetation belt, and *Dinocras cephalotes* not recorded above the sub-alpine, reacted differently to the same temperatures. Those of *D. bicaudata* and *A. compacta* had a diapause during the winter. *D. cephalotes* eggs went into a stage of quiescence when the water temperature was lowered from 16° C to 8° C or below. They remained alive in this stage for about a year, but this quiescence could be broken at anay time when the eggs were transferred to 16 and 20° C.

Freezing at -6° C followed by an increase in the water temperature led to a break in the diapause of *A. compacta* eggs after five months, but not of *D. bicaudata* eggs.

Eggs of *D. bicaudata* began to hatch after nine months independently of previous freezing followed by an increase in water temperature. Eggs of *D. bicaudata* held at a constant temperature of 1.5° C also began to hatch at the same time as those kept at 8° or 16° C. However, the point of 50 % hatching was much delayed at 1.5° C.

The egg diapause of *A. compacta* and *D. bicaudata* is an adaptation to areas with a severe climate typified by long cold winters and short summers. The quiescence period of *D. cephalotes* eggs enables the species to survive outside its normal distribution area.

Diapause et quiescence des œufs de Plécoptères *(Systellognatha)* des zones alpines de Norvège.

Mots clés : Plecoptera, diapause des œufs, quiescence, température.

Les œufs des Plécoptères *Diura bicaudata, Arcynopteryx compacta* et *Dinocras cephalotes* réagissent différemment aux mêmes températures. Ceux de *D. bicaudata* et *A. compacta* ont une diapause hivernale. Les œufs de *D. cephalotes* sont entrés en quiescence lorsque la température était abaissée de 16° C à 8° C ou au-dessous.

Dans ces conditions, ils sont restés vivants environ une année mais leur quiescence pouvait être stoppée à tout instant par un relèvement de la température à 16° et 20° C.

Un refroidissement à − 6° C suivi par une augmentation de température de l'eau a entrainé une levée de la diapause chez *A. compacta* après cinq mois, mais pas chez *D. bicaudata.*

Les œufs de *D. bicaudata* ont commencé à éclore après neuf mois, même s'ils étaient, comme dans le cas précédent, refroidis puis rechauffés. Les œufs de *D. bicaudata,* maintenus à une température constante de 1,5° C commencèrent aussi à éclore en même temps que ceux conservés à 8° ou 16° C. Cependant, le temps nécessaire à l'éclosion de 50 % des œufs était plus long à 1,5° C.

La diapause des œufs de *A. compacta* et *D. bicaudata* est une adaptation aux régions à climat rude caractérisé par un hiver long et froid et un été court. La période de quiescence des œufs de *D. cephalotes* permet à l'espèce de survivre en dehors de sa zone normale de distribution.

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1. Introduction

The eggs of a number of stonefly species, mainly *Systellognatha*, are known to have a diapause during the winter, (Khoo 1968, Lillehammer 1976, Saltveit & Lillehammer 1984). The eggs of other species have no obligatory diapause, but hatch after a shorter or longer period, depending upon the ambient water temperature, (Lillehammer 1976, 1986, Saltveit & Lillehammer 1984).

The factors which lead to the onset of a diapause are not yet fully understood, although Sweeney (1984) states that water temperature influences the pre-diapause, the length of the diapause and the duration of the post-diapausal development. Dowes (1965) states that, as an adaptation to the arctic environment, the initiation and control of the seasonal development cycle depends primarily on water temperature, the changes in photoperiods being ineffective under arctic conditions.

Those factors responsible for breaking the diapause, such as temperature, are investigated and discussed in the present paper. Experiments have been made to investigate the role of cooling and even freezing the eggs. For this purpose the eggs of two species of stonefly, *Arcynopteryx compacta* and *Diura bicaudata*, were studied. In Fennoscandia, the eggs of both species have an obligatory diapause during the winter (Lillehammer 1976).

In addition, eggs of *Dinocras cephalotes*, which do not develop at temperatures much below 12° C (Lillehammer 1987), were studied to find out whether a temperature-regulated period of quiescence occurs in this species.

2. Materials and Methods

The experiment included 400 eggs of *Arcynopteryx compacta*, 800 eggs of *Diura bicaudata* and 780 eggs of *Dinocras cephalotes*. Mature males and females of the three species were caught in the field and kept in the laboratory until eggs had been deposited. *A. compacta* and *D. bicaudata* were both taken from the same biotope, an outlet stream at Valdresflya in the Jotunheimen mountains of central Norway at about 1 400 m a.s.l. *D. cephalotes* was caught in a river in Øystre Slidre, about 50 km south of Valdresflya and about 300 m a.s.l.

Eggs of *A. compacta* and *D. bicaudata* were placed in water at 12° C in a temperature cabinet for a period of 60 days, while they developed and entered into the diapause.

The development of the eggs of *D. cephalotes* was started at 16° and then stopped, by placing them in water at 8° C. Some eggs of this species were also first kept at a temperature of 4° C and then transferred to 20° C for further development.

Egg volumes were calculated from the formula $V = ab^2 \pi/6$, where a is the egg length and b the egg width. The measurement of both eggs and nymphs were made under a stereomicroscope, to an accuracy of ± 0.01 mm. The mean egg volumes of *A. compacta* and *D. bicaudata* were respectively $613 \times 10^5\ \mu m^3$ and $339 \times 10^5 \mu m^3$, the respective ranges being 418×10^5 - $723 \times 10^5\ \mu m^3$ and 298×10^5 - $339 \times 10^5 \mu m^3$. The mean egg volume of *D. cephalotes* was $433 \times 10^5\ \mu m^3$, the range 431×10^5 - $439 \times 10^5 \mu m^3$. The body length of newborn nymphs of *A. compacta* varied from 1.28 to 1.67 mm, and those of *D. bicaudata* from 1.16 to 1.52 mm.

3. Results

3.1. Dinocras cephalotes

After being kept for 20 days in water at 16° C, the eggs were then transferred to water at 8° C (Table 1). Development stopped and the eggs went into a temperature dependent state of quiescence. After periods of 56, 221 and 324 days respectively, three batches of eggs were taken out and placed in water at 20° C. All three groups required about the same number of days to complete the incubation period, irrespective of the length of time they had been kept at 8° C.

In addition one batch of eggs was kept at 4° C, one at 1° C, and one was even frozen into a block of ice at - 6° C for seven days. After being transferred to water at 20° C they required 31, 32 and 30 days, respectively, before they hatched, a result similar to that recorded for those kept initially at 8° C. They were not influenced by the low temperature and freezing. Altogether 290 eggs placed at 4° C for 20 days just after deposition did not develop at all when subsequently kept at water temperatures of 16°, 20° and 24° C for 150 days. A temperature of 4° C seems to be lethal to eggs at that early stage of development.

Table 1 : Degree of hatching success of eggs of *Dinocras cephalotes* when kept at different water temperatures for different periods of time, (N) number of eggs.

	N	No. of days kept at the different temperatures (°C)						Hatching success
		8°	4°	1°	-6°	16°	20°	
1	128						33	100
2	250	76						0
3	120	107						0
4	85	153						0
5	91	56					31	96
6	15	221					32	66
7	140	324					35	93.4
8	36	49	7				31	95
9	55	114		16			32	100
10	28	151			7		30	100
11	26	300				10	28	50

3.2. Diura bicaudata

Ten batches of eggs were placed in a temperature cabinet at 12° C shortly after deposition on 21 June 1984. On 26 August head capsules with eye pigmentation were recognizable. Some other body structures were also visible. The heads all pointed downwards. At this stage they entered into a diapause. On 13 November all the eggs were placed in water at 1.6° C, and the temperature reduced to 0.5° C by 12 December. On 27 December, in an attempt to break the diapause, some batches of eggs were frozen at - 6° C for seven days, and thereafter placed in water at 8° C (Table 2). No hatching occurred within three months, although 10 eggs reached the terminae stage, i.e. the stage just prior to hatching, shortly after the freezing. A repetition of this experiment, made between 18-25 January yielded the same result, although a greater percentage of the eggs terminated this time (30 out of 45). None of these eggs hatched, even when kept to 20 June.

A third experiment, in which the eggs were only transferred from 0.5° C to water at 8° C on 8 January, without being frozen, gave the same results as the two previous ones.

However, in a fourth experiment after freezing on 14 March for one month, and then being exposed to full daylight for 8 hours, all the eggs hatched successfully during the latter half of April when transferred to water at 8° C. The same result was also recorded when eggs on 29 April were transferred from 1.5° C to water of 8° and to 16° C at 2 May without having been exposed to daylight. Those eggs which had been kept at a constant water temperature of 1.5° C from 20 February also began to hatch at the same time. The only difference noted was in the duration of the hatching period. At 16° C the hatching was completed in 6-13 days, while at 1.5° C hatching took 34-73 days.

On 12 June, one batch of eggs was transferred from 1.5° C to 8° C, and then to 16° C on the 15th. By 20 June, 200 nymphs had hatched successfully.

3.3. Arcynopteryx compacta

The eggs were placed in a temperature cabinet at 12° C shortly after deposition on 12 July 1984. They entered into the diapause at a much earlier stage than the eggs of *D. bicaudata*, but head capsules were not clearly visible. The same experiments were made with the eggs of this species as already described for *D. bicaudata*. The results obtained were different.

After freezing at - 6° C from 27 December 1984 to 3 January 1985, the eggs then developed very fast when placed at 8° C and the nymphs hatched after 18 days (Table 3). Freezing on 18 January yielded a similar result, complete hatching success after an incubation period of 19 days at 8° C. The eggs were observed to shrink in size during freezing.

A control experiment made on 8 January 1985 in which eggs were transferred from 0.5° C to 8° C without freezing, resulted in no hatching taking place after three months.

Freezing for one month from 14 March to 17 April also resulted in full hatching success after 12 days at 16° C.

In a control experiment made from 20 February to 29 April eggs were transferred from 1.5° C to 8° C and then to 16° C on 2 May. After one day at full daylight, the majority of eggs failed to hatch, only two did so.

In June, one batch of egg was transferred to 8° C on the 12th and to 16° C on the 15th. 123 nymphs had hatched by the 24th.

Table 2 : Eggs of *Diura bicaudata* deposited on 12-15 July 1984 and placed in water of 12° C on the same day, transferred to a water temperature of 1° C on 13 November 1984 and of 0.5° on 12 December 1984. (N) number of eggs.

	N	Subjected to temperatures of -6°C	1.5°	8°C	12°C	16°C	50% hatching at: (1985)	Hatching period (in days)	Duration of hatching (days)	Hatching success %
1	82	27.12		3.1			-	-	0	0
2	45	18.1		25.1			-	-	0	0
3	18			8.1			-	-	0	0
4	16	14.3		17.4		19.4	29.4	12	2	93.6
5	20		20.2	29.4		2.5	10.5	79	5	95
6	221		20.2	12.6		15.6	20.6	120	6	94.1
7	123		20.2				25.6	125	42	93.8
8	348		20.2				28.6	128	41	97.0
9	199		20.2				28.6	128	73	99.3
10	13		20.2				1.8	169	42	84.6

Table 3 : Eggs of *Arcynopteryx compacta* deposited on 10-12 July 1984 and placed in water of 12° C on the same day. They were transferred to a water temperature of 1° C on 13 November 1984 and then to 0.5° C on 12 December 1984. (N) number of eggs.

	N	Subjected to temperatures of -6°C	1.5°	8°C	12°C	16°C	50% hatching at: (1985)	Hatching period (in days)	Duration of hatching (days)	Hatching success %
1	19	27.12		3.1			21.1	18	9	90
2	20			3.1			-	-	0	0
3	24	17.12		25.1			14.2	19	4	83.3
4	44			8.1			-	-	0	0
5	38	stayed at 0.5°C					-	-	0	0
6	16	14.3		17.4		19.4	29.4	12	2	93.6
7	25		20.2	29.4		2.5	10.5	68	3	8
8	30		20.2	12.6			13.6	113	8	3.3
9	140		20.2	15.6			24.6	124	13	98
10	28		20.2				7.7	137	24	100
11	17		20.2				9.7	1 9	34	100

At a constant temperature of 1.5° C two egg batches began to develop during early May and the nymphs were recognizable. By 20 May. They began to hatch in late June, over a period of 24-34 days, but the overall hatching success was low. 38 eggs kept at 0.5 C all the time, they did not hatch, even in June.

4. Discussion

Eggs of *Diura bicaudata* and *Arcynopteryx compacta* entered into a diapause at a water temperature of 12° C. Development of the *Dinocras cephalotes* eggs was only stopped by placing them in water of 8° C after being kept at 16° C. The differences between them are : While the eggs of the first two species have an obligatory diapause, the eggs of *Dinocras cephalotes* have a temperature dependent period of quiescence at a water temperature of 8° C or below. The water temperature could be lowered to 1.0° C, and the eggs even frozen into a solid block of ice at −6° C for six days without any harm. At 8° C they were successfully kept alive from July one year to June the following year. This means that at localities where suitable egg development temperatures occur for only a shorter period, the eggs of this species are able to remain quiescent for a whole year if necessary. This ability would appear to be very important for the species to be able to maintain local populations outside its optimal area of distribution, such as in high altitudes, and north of the polar circle (Lillehammer 1985 a).

The two species with eggs requiring an obligatory diapause react differently to changes in water temperature, While the eggs of *D. bicaudata* seem to be little influenced by a lowering of water temperature, and even by freezing, early in winter, the diapause lasts for 8-10 months independent of changes in temperature including freezing. The eggs of *A. compacta* however, could be taken out of the diapause relatively early (December and January) by freezing the eggs for a period. Such freezing seems to induce a more rapid development when the eggs are afterwards placed in water of higher temperatures.

However, eggs of *A. compacta* held at a constant temperature of 1.5 C began to develop during the first half of May and then required about the same time for full development as those of *D. bicaudata.* In May and June the eggs of both species hatched when kept at constant temperatures, even at 1.5° C. An increase in the water temperature during the postdiapausal period of eggs of *Diura bicaudata* seems greatly to shorten the time required for full development. These two species mainly occure in different types of habitats. In southern Fennoscandia *D. bicaudata* occurs in lakes with water temperatures between 0° C and 4° C during the winter, whereas *A. compacta* occurs in streams that are frozen during the greater part of the winter. Eggs of the latter species therefore seem to be more dependent on water temperature for full hatching success, while eggs of *D. bicaudata* go through a time dependent development phase which may last for a longer period.

The type of egg development of each of the three species of stonefly has individually adapted to the particular enviromental factors of its habitat. The eggs of *D. cephalotes* can enter into a state of egg quiescence for up to a year. Although it is a warm stenotherm species, which originated in the Mediterranean (Zwick 1981), it is also able to live at high altitudes such as in Ovre Heimdalen, in central Norway (Lillehammer & Brittain 1978) and at high latitudes, in northern Fennoscandia — habitats in which two summers are required for egg hatching in some years. The eggs of the other two species, which are the two most abundant species found in the alpine areas of Fennoscandia, seem to be well adapted to their habitat by entering into a diapausal state throughout their first winter, at low water temperatures and then utilising the entire warm period of about three months for nymphal growth. This fits with Khoo's (1968) suggestion that a diapause is an adaptation to a very cold climate and that the occurrence of non-diapausal eggs of *Diura bicaudata* in Lake Bala in England is a recently evolved feature. It is thought that over a greater part of Norway both *D. bicaudata* and *A. compacta* occur within the areas of optimal water temperature for these two species. This fits also with the concept of geographical thermal equilibrium, proposed by Vannot & Sweeney (1980), who state that for successful reproduction aquatic insects should live within the regions of their optimal temperature. However, *D. cephalotes* does occur outside its thermal optimum area, at high altitudes and in northern Fennoscandia. Its eggs therefore need to be specially temperature adapted to such habitats by tolerating a long period of quiescence necessary to adapt to the annual temperature regime so that the nymphs wil hatch at a suitable time for subsequent mating and reproduction of adults.

For two of the species *Dinocras cephalotes* and *Arcynopteryx compacta* the changes in water temperature seem to be the most important factor for regulating egg development. Dowes (1965) statement, that the control of the seasonal development cycle under arctic condition depends primarily on the water temperature, seems to well apply to those species studied here. Both *Arcynopteryx compacta* and *Diura bicaudata* live in the middle alpine zone, which climatically closely parallels arctic conditions.

Plasticity in life cycle occurs in all three species studied. *Dinocras cephalotes* is able to go into an egg quiescence when the water temperature is low, as in high altitudes during most of the year. The egg development can then be prolonged from three months to one year. The quiescence of the eggs of *D. cephalotes* occurs also in the Pyrenean populations (Berthélemy *in litt.*, unpublished results). *Diura bicaudata* can either have only diapausing eggs, as in this study, or have both diapausing and non-diapausing eggs (Khoo 1968). *A. compacta* which occur in the Pyrenees (Berthelemy 1966), can have an prolonged nymphal growth period producing a three year life cycle (Lavandier 1979) or in the case of Mountain lake outlet biotopes a two year life cycle (Lillehammer 1985 b).

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