

INFLUENCE OF SAMPLING DATE ON REPRODUCTION IN THE LAND SNAIL *HELIX*  
*APERTA* KEPT UNDER CONTROLLED CONDITIONS OF TEMPERATURE  
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RÉSUMÉ. — *Influence de la date d'échantillonnage sur la reproduction de l'escargot terrestre Helix aperta maintenu en conditions contrôlées de température et de photopériode.* — Les caractères reproductifs des escargots *Helix aperta* ont été étudiés sous quatre combinaisons de température et photopériode (20°C/16hL:8hD ; 20°C/8hL:16hD ; 15°C/16hL:8hD and 15°C/8hL:16hD). Trois échantillons ont été utilisés : Ech. 1 et Ech. 2 collectés à Annaba (Nord-Est Algérien) respectivement durant et après l'hibernation ; Ech. 3, précédemment analysé (données non publiées), collecté à Béjaïa (proche Nord-Est Algérien) durant l'estivation. Tous les escargots ont commencé à s'accoupler dès la 1<sup>ère</sup> semaine et à pondre durant la 3<sup>ème</sup> ou la 4<sup>ème</sup> semaine de leur mise en conditions de reproduction. Ech. 1 et Ech. 2 se sont distingués par leur plus courte période de reproduction (4-6 semaines) en comparaison avec Ech. 3 (5-7 semaines). D'une manière frappante, bien que les escargots collectés durant ou après hibernation (Ech. 1 et Ech. 2) se soient accouplés, dans la plupart des cas, à des taux plus élevés (56-87 %) que ceux échantillonnés durant l'estivation (32-92 %), leurs taux de pontes ont été dramatiquement plus faibles (6-25 % contre 12-80 %). Autrement dit, parmi les escargots qui se sont accouplés dans Ech. 1 et Ech. 2, seulement 11-36 % ont pondu, contre 38-87 % dans Ech. 3. Les nombres moyens d'œufs par ponte étaient plus élevés en Ech. 1 (293-323) et Ech. 3 (337-348) qu'en Ech. 2 (237-248) ( $P < 0.05$ ). Inversement, les poids moyens des œufs par ponte étaient plus élevés en Ech. 2 (17.5-17.8 mg) qu'en Ech. 1 (16.1-16.3 mg) et Ech. 3 (16.3-16.6 mg) ( $P < 0.05$ ). Après la période de reproduction, en conséquence des rendements reproductifs différentiels, les poids moyens des escargots ont très significativement augmenté en Ech. 1 et Ech. 2 ( $P < 0.001$ ) et significativement baissé en Ech. 3 ( $P < 0.05$ ). Les performances reproductives étaient plus affectées et les taux de mortalité plus élevés sous basse température et courte photopériode. La meilleure combinaison de ces deux facteurs était souvent 20°C/16hL:8hD, plus proche des conditions sur le terrain en automne, surtout durant la nuit, phase d'activité des escargots. Après la période de reproduction, la mortalité devenait de plus en plus élevée et les survivants moins actifs ou essayant de s'enfouir dans le sol des pots de ponte. Tous ces arguments plaident que les escargots *H. aperta* sont mieux adaptés à se reproduire en automne (jours décroissants et températures plus élevées) après une longue estivation stimulant la gamétogenèse qu'au printemps (jours croissants et températures basses) après l'hibernation inhibant la gamétogenèse.

SUMMARY. — Reproductive traits of *Helix aperta* snails were investigated under four combinations of temperature and photoperiod (20°C/16hL:8hD; 20°C/8hL:16hD; 15°C/16hL:8hD and 15°C/8hL:16hD). Three samples were used: Sample 1 and Sample 2 collected from Annaba (Northeastern Algeria) during and after hibernation respectively; Sample 3, previously studied (unpublished data), collected from Bejaïa (near Northeastern Algeria) during aestivation. All the snails began to mate on the first week and to lay on the 3<sup>rd</sup>-4<sup>th</sup> week of setting under conditions of reproduction. Sample 1 and Sample 2 were characterized by shorter reproduction periods (4-6 weeks) than Sample 3 (5-7 weeks). Strikingly, although most of the snails collected during and after hibernation mated at a high rate (56-87 %) in comparison with those collected during aestivation (32-92 %), their rates of egg-laying were drastically lower (6-25 % against 12-80 %).

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Among the snails that had mated, only 11-36 % laid eggs in Samples 1 and 2 against 38-87 % in Sample 3. The mean numbers of eggs per clutch were higher in Sample 1 (293-323) and Sample 3 (337-348) than in Sample 2 (237-248) ( $P < 0.05$ ). Inversely, the mean egg weights per clutch were heavier in Sample 2 (17.5-17.8 mg) than in Sample 1 (16.1-16.3 mg) and Sample 3 (16.3-16.6 mg) ( $P < 0.05$ ). After the reproduction period, as a consequence of differential reproductive yields, the mean weights of snails very significantly increased in Sample 1 and Sample 2 ( $P < 0.001$ ) and significantly decreased in Sample 3 ( $P < 0.05$ ). Reproductive performances were more affected and lethality rates higher under low temperature and short day photoperiod. The best combination of temperature and photoperiod was mostly 20°C/16hL:8hD, conditions closer to those in the field during autumn, especially overnight, the phase of snail activity. After the reproduction period, the lethality was becoming higher and higher and the surviving snails were less active or trying to burrow themselves into the soil of the egg-laying pots. All these arguments plead that *H. aperta* snails are better adapted to reproduce in autumn (decreasing days and higher temperatures) after a long aestivation stimulating gametogenesis than in spring (increasing days and lower temperatures) after hibernation inhibiting gametogenesis.

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The terrestrial gastropod pulmonates are dependent on the abiotic factors that govern their life cycle. Most of these organisms observe periods of rest when their environment becomes hostile. Their growth and reproduction are strongly affected by their biotope conditions (Gomot *et al.*, 1982; Laurent *et al.*, 1984; Gomot & Deray, 1987).

Since the mid-60s, many researchers have taken an interest in studying the influence of environmental factors, in particular temperature and photoperiod, on the reproductive activity of land snails. The studies dealing with the effect of photoperiod on reproduction in helioid snails have yielded very divergent results from species to species. Thus, in *Cornu aspersum* (Stephens & Stephens, 1966; Charrier, 1980; Bailey, 1981; Enée *et al.*, 1982; Bonnefoy-Claudet *et al.*, 1987; Aupinel & Daguzan, 1989; Gomot *et al.*, 1990) and *Helix pomatia* (Gomot, 1990), it has been demonstrated that reproduction is stimulated by long-day photoperiods. However, in *Cepaea nemoralis*, reduction in reproduction activity is induced by both short-day and long-day photoperiods, with a more pronounced negative effect of the latter (Hunter & Stone, 1986).

Furthermore, the experiments of Gomot *et al.* (1986) and Griffond *et al.* (1992) on *Cornu aspersum* have clearly revealed that temperature has a very net effect on the multiplication and the development of the male line cells. On the other hand, in *Cornu aspersum aspersum* (Gomot *et al.*, 1989), *Helix pomatia* (Gomot, 1990) and *Cornu aspersum maxima* (Jess & Marks, 1998), temperature and photoperiod have been proved to exert a combined action on reproductive activity, with the stimulating long-day photoperiods compensating the negative effects of low temperatures.

Very few studies have been dedicated to *Helix aperta* Born (1778) (= *Cantareus apertus* Born, 1778). So, most of the eco-physiological aspects of its biology are not well known. This helioid species, native to the Mediterranean basin, is mentioned in the south of France, Italy, Turkey, Cyprus and North Africa (Kerney & Cameron, 1979; Schütt, 2001). According to Sacchi (1955, 1958), when temperatures are incompatible with its activity (below 7°C or beyond 27°C) or when relative humidity is outside its optimum (75-95 %), the *H. aperta* snail becomes inactive (i.e. enters endogenous aestivation or hibernation) and lives in a state of metabolism slowdown; this land mollusc is known as the “burrowing snail”, coming up above ground only by rainy weather; by dry conditions, it burrows 7-15 cm deep into the ground and aestivates in a thick whitish operculum until rain softens the soil. *H. aperta* snails are poikilotherms (heterotherms) because their internal temperature varies with the temperature of their external surroundings; they are also eurytherms because they withstand strong variations in temperature of their external and internal environments.

The reproduction of *H. aperta* snail is rather poorly documented. Giusti & Andreini (1988) reported that individuals of this species, collected in autumn and in spring close to Orbetello and Castelnuovo Berardenga (Siena, Tuscany, Italy) and maintained under laboratory conditions similar to those in the field for continuous observations over three years (1982-1984), reproduce preferentially in autumn from early October to mid-December. In a recent work, de Vaufléury & Gimbert (2009) noted that reproduction of *H. aperta* snails, collected in late

summer at Bazina (Tunisia), is stimulated by short-day photoperiods (6 hours of Light – 18 hours of Dark) but inhibited under long-days (18 hours of Light – 6 hours of Dark). In addition, Tafoughalt-Benbellil *et al.* (2009) collected in autumn a sample of adult *H. aperta* snails at Bakaro (Bejaïa, Eastern Algeria) and bred them for several generations under controlled conditions; they have observed that, in snails of the fourth generation, the reproduction is strongly affected by photoperiod length and, moreover, temperature and photoperiod have interactive effects on both the number and duration of matings and layings, with a predominant influence of photoperiod.

In the present study, we aim to assess how time of sampling (during or after hibernation) affects the reproductive performances of *Helix aperta* snails in comparison with data on conspecific snails collected during aestivation.

## MATERIALS AND METHODS

### GEOGRAPHIC ORIGIN OF SNAILS AND TIME OF SAMPLING

Two samples of adult snails identified as belonging to *Helix aperta* were collected at the same spot in the vicinity of the University of Annaba (Annaba, Northeastern Algeria). The first sample (Sample 1) was collected on the 3<sup>rd</sup> week of February 2007; at this time of year, the subjects were still inactive, i.e. in hibernation and hidden in the ground with shell blocked up by a thick whitish operculum; the snails were dug out of the soil using a small pick. The second sample (Sample 2) was drawn at the end of hibernation, on the fourth week of March 2007; the snails were collected early in the morning, by a rainy day; at the moment of sampling, the snails were active, crawling on the ground. Unpublished data on snails collected in September 2005 at Bakaro (Bejaïa, Northeastern Algeria) (Sample 3) were included in the present analysis. These unpublished data were obtained under exactly the same experimental conditions as for Sample 1 and Sample 2. At the moment of collect, the snails were still under aestivation, burrowed deep in the soil.

For the three samples, the snails presented roughly the same shell sizes, indicating a same stage of maturity. The meteorological characteristics of the sites of study are given in Table I.

TABLE I

*Meteorological characteristics of the study sites. Precipitation values (P), maximal (M) and minimal (m) temperatures were recorded from January through December near the sites of study (Data from the Meteorological Stations of Bejaïa and Annaba, Algeria). The mid-month day lengths were estimated using sunrise and sunset times in the area (Calendar Talantikit, undated, Ed. Talantikit, Bejaia, Algeria)*

Months	Bejaia 2005			Annaba 2007*			Day length (h:mn)
	P (mm)	M (°C)	m (°C)	P (mm)	M (°C)	m (°C)	
January	165.5	13.9	4.6	35.0	13.7	4.7	9:58
February	167.5	13.6	5.9	51.0	12.9	5.6	10:52
March	60.4	17.3	9.2	204.0	16.8	8.2	11:58
April	41.9	20.3	11.9	27.6	21.1	10.9	13:10
May	7.7	24.3	14.5	42.1	24.8	14.8	14:11
June	0.0	27.6	18.0	52.2	27.4	17.6	14:43
July	0.4	29.9	21.3	3.0	29.6	21.2	14:30
August	6.1	29.8	20.5	3.0	29.5	19.9	13:38
September	28.2	28.0	18.5	33.0	28.2	18.4	12:29
October	31.7	26.1	16.5	102.0	25.8	16.3	11:20
November	107.0	20.5	11.4	84.0	20.7	11.3	10:17
December	172.4	16.5	8.1	125.4	16.3	7.9	9:42

\* The temperature values in 2006 were very similar to those in 2007

### REARING AND MONITORING TECHNIQUES

Two 2.5x4.0x5.5 m rooms were used for the experiments, one at 20°C and the other at 15°C. The temperatures were controlled by means of 12000 BTU air-conditioners (Samha, Setif, Algeria). In each room, an opaque screen served to separate between the two photoperiods used (16hL:8hD and 8hL:16hD, in hours (h) of light (L) and dark (D)). The light was provided using daylight neons with an intensity of 50–100 lux. The neons were connected to an electronic chronometer clock set to automatically control the photoperiods.

We used the 'soiless' breeding technique developed by Daguzan (1981) and adopted after him by many other authors (e.g. Enée *et al.*, 1982; Laurent *et al.*, 1984; Gomot & Deray, 1987) and also in heliciculture to raise the *Cornu aspersum* snail, another ubiquitous helicid living beside *Helix aperta* in Algeria.

All the experimental snails were housed in polythene containers with 3600 cm<sup>3</sup> volume at a density of 50 snails/m<sup>2</sup>. A wet absorbent paper was placed on the floor of the containers to maintain humidity. During the experiment, the relative humidity oscillated between 90-95 %. Pots of 10 cm diameter and 8 cm height filled with wet light soil were placed in each container for egg-laying. Each sample was split into four groups. The groups were of 16 snails in Sample 1 and Sample 2, and 25 snails in Sample 3. The groups are coded A<sub>1</sub>, B<sub>1</sub>, C<sub>1</sub> and D<sub>1</sub> for Sample 1 (collected in February, during hibernation), A<sub>2</sub>, B<sub>2</sub>, C<sub>2</sub> and D<sub>2</sub> for Sample 2 (collected in March, after hibernation) and A<sub>3</sub>, B<sub>3</sub>, C<sub>3</sub> and D<sub>3</sub> for Sample 3 (collected in September, during aestivation). Sample 1 and Sample 2 snails were set to reproduction immediately after collect; those of Sample 3, before to be set to reproduction, were kept under optimal rearing condition (as described below, at 20°C/16hL:8hD) for three weeks. For each sample, the four groups of snails were simultaneously submitted to different combinations of temperature and photoperiod as follows: Groups A<sub>i</sub> (20°C, 16hL:8hD), Groups B<sub>i</sub> (20°C, 8hL:16hD), Groups C<sub>i</sub> (15°C, 16hL:8hD), and Groups D<sub>i</sub> (15°C, 8hL:16hD).

In order to determine the possible influence of weight on reproduction, all the snails involved in the study were individually weighed at the beginning and the end of the experiment, using an electronic balance with 0,01 g precision; this would also be useful to assess weight variation during the experiment. Before any weighing operation, each snail had been washed to remove any excrements or food possibly imprisoned in the pedal plate folds. In order to discern the individuals, they were marked with adhesive labels.

Throughout all the experiment, the animals were fed with the commercial product "*Helixal*" (Etablissements Chays, France) developed by Gomot-de Vaufléury (2000). To nourish the snails, 50 g of food were provided in each container on Petri dishes. Three times a week, at the same time, the containers were cleaned, the food renewed and the absorbent paper changed. The boxes location in the rearing room was changed every day.

To follow the matings and egg-layings, observations were made twice a day: the first early in the morning (8 a.m.) and the second in the afternoon (8 p.m.) (The laying operation lasts more than 12 hours). To avoid confusion, as soon as a snail was observed in an egg-laying position, the pot was removed to another container and replaced. Each clutch was identified by its parentage, date of laying, number of eggs, date of hatching and number of young hatched. Twice a week, the eggs were collected using a teaspoon and their numbers recorded; 30 % of the eggs of each clutch were individually weighed with an accuracy of 0.001 g. All the eggs were then incubated at 20°C in Petri dishes (9 cm diameter and 1.5 cm height). To maintain humidity during all the incubation period, a layer of wet absorbent paper was placed in the bottom of the Petri dishes. To aerate the eggs, the lids of the Petri dishes were perforated with 10 small holes (1 mm diameter). After hatching, the numbers of the newly emerged snails were counted. Each week, the number of dead animals in each group was monitored. The length of reproduction period was estimated by recording the beginning of matings and the end of egg-layings. After the last clutch, the snails were kept under observation for 5 weeks during which no mating occurred but rather inactivity and mortality drastically increased.

## STATISTICAL ANALYSIS

The data were subjected to statistical evaluation using Statistica 5.5 (StatSoft Inc., 1999). The mean weights of snails of the different groups and the mean number of eggs per clutch were expressed as means ± standard deviation (M±SD). The differences between the mean weights of snails in the different groups, mean weights of snails within each group at the start and the end of reproduction period, mean numbers of eggs per clutch and mean egg weights per clutch were tested using Anova, Student (t) or Lsd tests. For comparison of numbers of matings and numbers of layings between the corresponding groups of samples the chi-square ( $\chi^2$ ) test was applied. The Pearson's correlation coefficient served to estimate the relation between numbers of matings and numbers of egg-layings, as well as the relation of wet weight of snails with each of numbers of eggs per clutch and egg wet weights. Comparison between proportions test was used to compare the various percentages reported in the results.

## RESULTS

The main results and their statistical evaluation are condensed in Figures 1 & 2, and Table II.

### MATING AND EGG-LAYING ACTIVITIES

For all the three samples, the mating activity began on the first week after setting to reproduction; it lasted 5 weeks for all groups of Sample 1 (collected in February) and a week shorter at 20°C for Sample 2 (collected in March) (Fig. 1). The longest (6 weeks) and the shortest (2 weeks) periods of mating activity were observed in Sample 3 (collected in September) at 20°C/16L:8D and 15°C/8L:16D respectively (Tab. II, line 3).

The first clutch was laid on the 3<sup>rd</sup> week (at 20°C) or the 4<sup>th</sup> week (at 15°C) after setting to reproduction for all groups, including Sample 3 (Fig. 1 and data not shown). The laying activity lasted 3-4 weeks for most groups. The longest period of laying activity (5 weeks) was

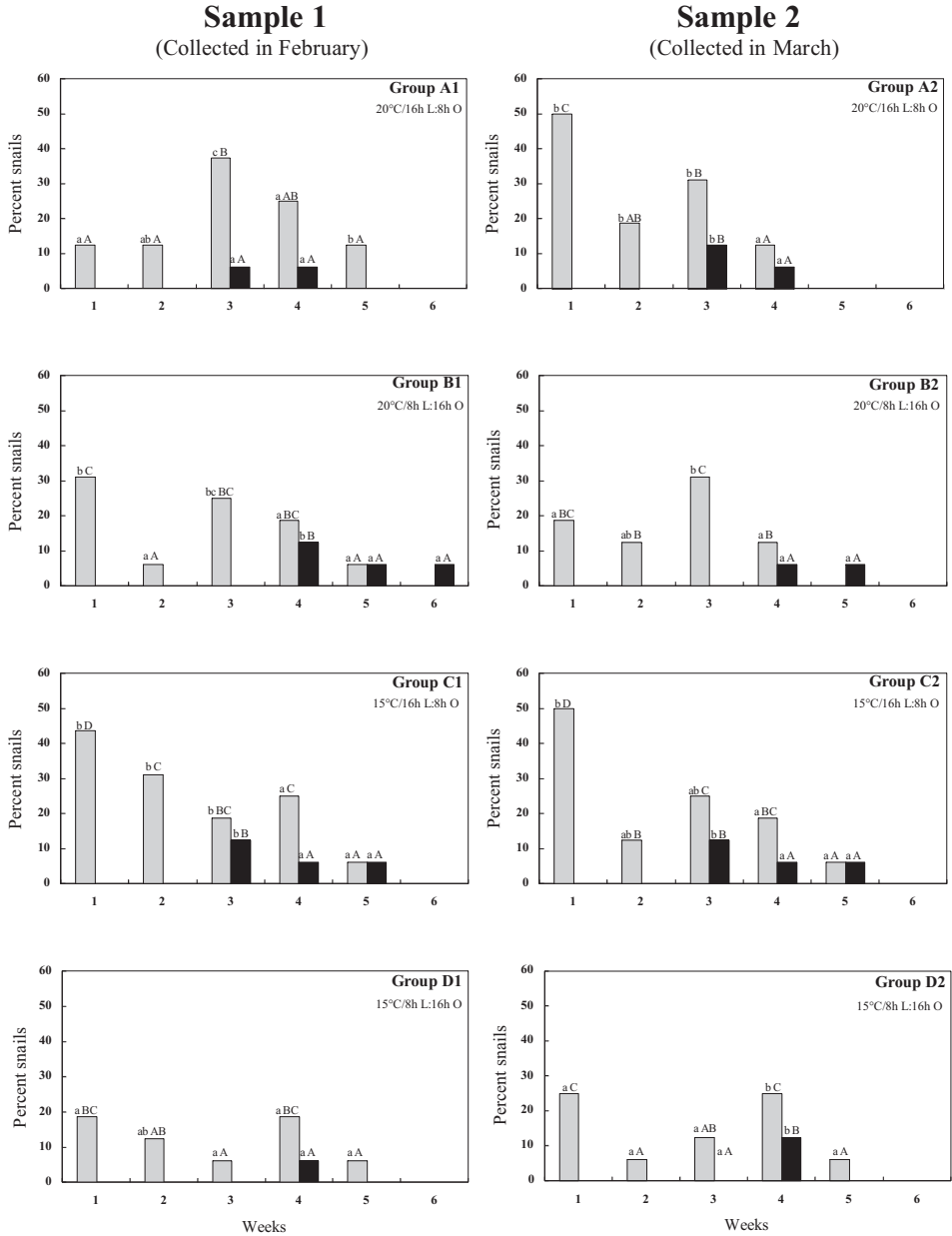


Figure 1. — Mating (grey) and egg-laying (black) rates per week after collect in *Helix aperta* snails sampled in February (Sample 1) and March (Sample 2) under four controlled combinations of temperature (°C) and photoperiod (in hours (h) of light (L) and dark (D)). Within each sample, the vertically corresponding values with different small letters are significantly different ( $P < 0.05$ ); likewise, for matings and egg-layings separately, the capital letters compare all the values within a couple of corresponding groups taken as a whole; those percentages with different capital letters are significantly different ( $P < 0.05$ ).

observed in Sample 3 at 20°C/16L:8D, and the shortest (1 week) in Sample 1 and Sample 2 at 15°C/8L:16D (Fig. 1 & Tab. II, line 7).

The numbers of matings and layings per snail ranged from 0-2 and 0-1 respectively for all groups (Fig. 2) except for Sample 3 in which these numbers ranged from 0-3 and 0-2 res-

TABLE II

Compared reproductive activities of *Helix aspersa* snails collected at different dates. Sample 1 and Sample 2 were collected at Annaba in February 2007 (during hibernation) and March 2007 (after hibernation). Sample 3 was collected at Bejaia in September 2005 (during aestivation). For each sample, the codes A<sub>i</sub>, B<sub>i</sub>, C<sub>i</sub> and D<sub>i</sub> represent the four snail groups submitted to different combinations of temperature and photoperiod respectively; each of the four groups was of 16 snails in Sample 1 and Sample 2, and 25 snails in Sample 3. For each line, the small letters under values compare in increasing order all the twelve groups (columns); values with same small letters are not significantly different ( $P > 0.05$ ). For each column (each group), the capital letters compare the mean weights of snails before (line 14) and after (line 15) the reproduction period; the mean values with different capital letters are significantly different ( $P < 0.05$ )

Descriptive traits	Sample 1 (Collected in February 2007)				Sample 2 (Collected in March 2007)				Sample 3 (Collected in September 2005)			
	A <sub>1</sub> (20°C/L) <sup>1</sup>	B <sub>1</sub> (20°C/D) <sup>2</sup>	C <sub>1</sub> (15°C/L) <sup>1</sup>	D <sub>1</sub> (15°C/D) <sup>2</sup>	A <sub>2</sub> (20°C/L) <sup>1</sup>	B <sub>2</sub> (20°C/D) <sup>2</sup>	C <sub>2</sub> (15°C/L) <sup>1</sup>	D <sub>2</sub> (15°C/D) <sup>2</sup>	A <sub>3</sub> (20°C/L) <sup>1</sup>	B <sub>3</sub> (20°C/D) <sup>2</sup>	C <sub>3</sub> (15°C/L) <sup>1</sup>	D <sub>3</sub> (15°C/D) <sup>2</sup>
1. Percent of snails that mated (%)	87.50 b	68.75 b	87.50 b	56.25 b	87.50 b	62.50 b	81.25 b	62.50 b	92 c	48 a	84 b	32 a
2. Number of matings snail <sup>-1</sup> ±SD	1±0.5 abc	0.9±0.7 abc	1.25±0.7 c	0.63±0.6 a	1.12±0.6 bc	0.75±0.7 ab	1.12±0.7 bc	0.75±0.7 ab	1.52±0.7 b	0.56±0.6 a	1.28±0.7 b	0.32±0.5 a
3. Duration of mating activity (weeks)	5	5	5	5	4	4	5	5	6	4	5	2
4. Time between mating and egg-laying (days)	20	20	20	20	20	20	20	20	-	-	-	-
5. Percent of snails that laid eggs (%)	12.5 a	25 a	25 a	6.25 a	18.75 a	12.5 a	25.00 a	12.5 a	80 c	32 ab	64 b	12 a
6. Number of clutches snail <sup>-1</sup> ±SD	0.12±0.3 a	0.25±0.4 a	0.25±0.4 a	0.06±0.2 a	0.19±0.4 a	0.12±0.3 a	0.25±0.3 a	0.12±0.3 a	0.84±0.5 c	0.32±0.5 ab	0.64±0.5 b	0.12±0.3 a
7. Duration of laying activity (weeks)	2	3	3	1	2	2	3	1	5	3	4	2
8. Total duration of reproduction activity (weeks)	5	6	5	5	4	5	5	5	7	6	6	5
9. Mean number of eggs ±SD clutch <sup>-1</sup>	313±8.5 bcd	302.0±14.4 bc	292.7±11.4 b	323.0±0.0 bcde	247.7±4 a	237.5±6 a	241.5±6 a	237.0±14 a	347.7±27 e	344.2±31 de	340.6±19 cde	337.3±41 cde
10. Mean egg weight (mg)	16.3±0.1 a	16.1±0.3 a	16.1±0.2 a	16.2±0.3 a	17.6±0.2 c	17.5±0.3 bc	17.5±0.3 c	17.8±0.2 c	16.6±1.2 b	16.4±1.2 ab	16.5±0.9 b	16.3±1.2 ab
11. Time of incubation (days)	13	13	13	13	13	13	13	13	13	13	13	13
12. % of successful hatching	79 a	85 a	88 a	83 a	84 a	86 a	87 a	85 a	84 a	86 a	85 a	78 a
13. Mortality rate during reproduction period (%)	25 a	31.25 b	25 a	37.5 b	18.75 a	25 a	25 a	37.5 b	28 a	8 a	12 a	20 a
14. Mean weights ± SD (g) of snails at start of experiment	7.2±0.9 aA	7.3±0.9 aA	7.4±0.8 aA	7.1±0.8 aA	9.3±1.5 bA	8.7±1.0 bA	9.3±1.3 bA	9.2±0.8 bA	14.4±2.0 cdB	14.2±1.9 deB	14.4±1.9 eA	14.3±1.6 fB
15. Mean weights ± SD (g) of snails after reproduction	12.6±0.5 bcB	12.2±1.0 bcB	12.8±1.1 cdB	13.1±1.0 deB	13.1±0.8 deB	12.9±0.7 deB	12.7±1.0 cdB	12.5±1.2 bcB	12.2±1.3 bcA	12.1±1.2 bA	13.8±1.1 fA	11.3±1.1 aA

<sup>1</sup> L: 16 hours of light and 8 hours of dark (Light days); D: 8 hours of light and 16 hours of dark (Dark days).

pectively in Group A<sub>3</sub> (20°C/16hL:8hD) and from 0-1 in Group D<sub>3</sub> (15°C/8hL:16hD) (data not shown). In both Sample 1 and Sample 2, the modal values of numbers of matings and numbers of layings were 0 and 1 respectively (Fig. 2). In contrast, in Sample 3, the modal values of

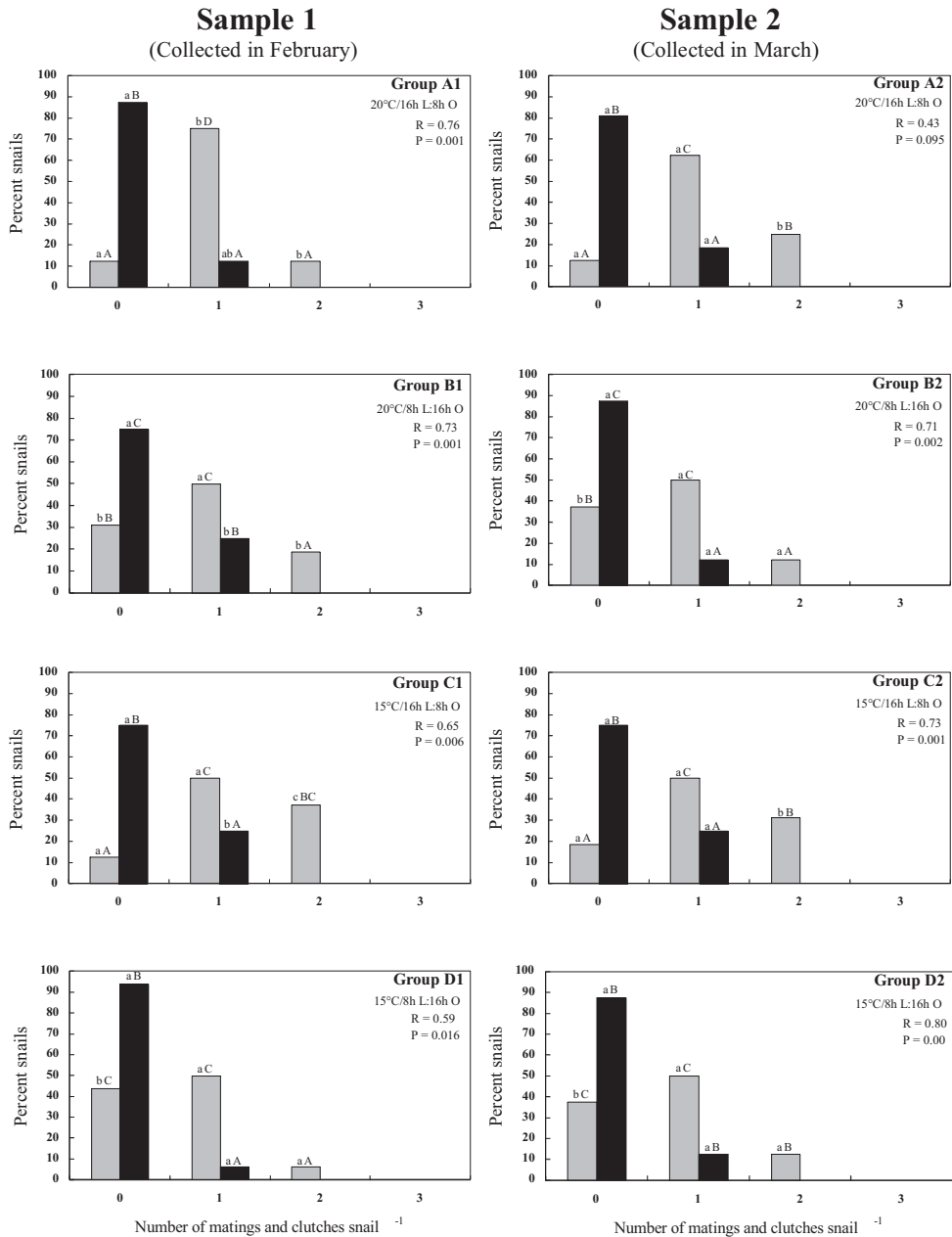


Figure 2. — Numbers of matings (grey) and clutches (black) variation in *Helix aperta* snails collected in February (left) and March (right) under four controlled combinations of temperature (°C) and photoperiod (in hours (h) of light (L) and dark (D)). Within each sample, the vertically corresponding values with small letters are significantly different ( $P < 0.05$ ); likewise, for each pair of horizontally corresponding groups, separately for the matings and clutches, the values with different capital letters are significantly different ( $P < 0.05$ ). R and P values mentioned in each group box are those of Pearson's correlations and their significance levels between the numbers of matings and the numbers of clutches within the group.



numbers of matings and numbers of layings were 1 and 2 respectively under long day photoperiod, and 0 under short day photoperiod (data not shown). In most cases, the numbers of matings were significantly correlated with the numbers of layings ( $P < 0.05$ ).

Strikingly, even though the snails collected in February and March (Samples 1 and 2 respectively) showed a rather high mating activity in comparison with those collected in September (Sample 3) (Tab. II, lines 1 & 2), their laying performances were drastically lower (Tab. II, lines 5 & 6). Among the snails that had mated, the proportion of those that laid eggs was of only 11-36 % in Samples 1 and 2, and as much as 38-87 % in Sample 3. Otherwise, in Sample 1 and Sample 2, although most snails had mated at least once, most of them did not lay eggs; but in Sample 3, most snails mated and laid at least once except under short day photoperiod. The long day photoperiod was significantly ( $P < 0.05$ ) more stimulating of mating and egg-laying activities; the best combination of temperature and photoperiod was 20°C/16L:8D and the worst was 15°C/8L:16D (Tab. II, lines 1, 2, 5 & 6).

#### CLUTCH AND EGG SIZES

The snails collected in February and September (Sample 1 and Sample 3 respectively) laid significantly more eggs per clutch than those sampled in March ( $P < 0.001$ ) (Tab. II, line 9). Significant differences between groups were revealed within Sample 1 and Sample 3 only, but not within Sample 2 (Tab. II, line 9). The average range of variation in eggs numbers was of 31 eggs in Sample 1 and only of 10 and 11 eggs in Sample 2 and Sample 3 respectively.

The eggs laid by snails collected in March and September were in average significantly heavier and more heterogeneous than for snails sampled in February ( $P < 0.001$ ) (Tab. II, line 10). In addition, there was a strong negative correlation between weights of eggs and numbers of eggs per clutch ( $P < 0.001$ ) in all groups but no significant dependence was detected between weights of snails and weights of eggs ( $P = 0.473$ ).

#### MORTALITY RATES AND BODY WEIGHTS VARIATION

During the reproduction period, especially at low temperature and short day photoperiod, the mortality rates were higher and more heterogeneous in Sample 1 (25-37.5 %) and Sample 2 (18.75-37.5 %) than in Sample 3 (8-28 %) (Tab. II, line 13). During the five weeks following the reproduction period, the mortality was becoming higher and higher and the surviving snails were inactive or trying to burrow themselves into the soil of the egg-laying pots.

Just after the reproduction period, the mean body weights of snails very significantly increased ( $P < 0.001$ ) for Sample 1 and Sample 2, and significantly decreased ( $P < 0.05$ ) for Sample 3 (Tab. II, lines 14 & 15).

## DISCUSSION

#### MATINGS AND LAYING ACTIVITIES

It is from late December to late March in winter, and early May to late September in summer that temperature conditions did not fit the optimums of *H. aperta* snails (7-27°C) (Tab. I and data not shown). So, approximately, Sample 1 and Sample 2 were collected after two and three months of hibernation respectively; and Sample 3, after 4-5 months of aestivation. Despite these differences, all the three samples began to mate on the first week and to lay on the 3<sup>rd</sup>-4<sup>th</sup> week. It appears from this that hibernation or aestivation durations do not influence times of mating and egg-laying activities in *H. aperta*. In *H. aspera* (*Cornu aspersum*), for instance, after a month and a half of hibernation, the mating activity is delayed by 7 weeks; after six months of hibernation, matings take place earlier and the first clutch is laid after 7 weeks; after a year of hibernation, mating activity occurs right after the waking, and the first clutch is observed after only 3 weeks (Bonnefoy & Deray, 1984).

The snails collected during or after hibernation were very similar for their mating and egg-laying activities (Tab. II, lines 1-8). Although they had, in most cases, mated more



than those collected during aestivation, they strikingly laid far less clutches (Tab. II, lines 1, 2, 5, 6). Obviously, the problem resided in some aspect of their gonad physiology; the meteorological conditions (likely temperatures and photoperiods) during hibernation did not favour gametogenesis or egg fertilization processes; these seemed to be stimulated rather during aestivation. During hibernation of snails, neuro-endocrine modifications are induced, with negative repercussions on cytological activities (gametic multiplication and differentiation) of the ovotestis (Bouillon, 1956) depending on both temperature (Gomot *et al.*, 1986; Gomot & Deray, 1990) and photoperiod (Gomot & Griffond, 1993), the main factors controlling the sexual behaviour. The cerebral centres responsible of this kind of neuro-endocrine regulation of reproduction activity are the caudo-dorsal cells of the cerebral ganglia in *Lymnaea stagnalis* (Geraerts & Bohlken, 1976), the bag cells of the abdominal ganglia in *Aplysia californica* (Kupfermann, 1967; Arch, 1976) and the dorsal bodies in *Helisoma* (Saleuddin & Khan, 1981) and *Cornu aspersum* (Saleuddin *et al.*, 1983).

Our observations are in full agreement with those reported by Giusti & Andreini, (1988) on *H. aperta* snails collected from Siena (Tuscany, Italy) and kept under controlled conditions imitating those in the field for 3 consecutive years (1982-1984); it resulted that *H. aperta* snails mate preferentially from early October to mid-December. Similar observations have also been made on *Theba pisana* in Italy (Giusti & Andreini, 1988) as well as *Theba pisana* and *Cernea virgata* in Australia (Baker, 1991).

De Vaufléury & Gimbert (2009) have noted that *H. aperta* snails, collected in September at Bazina (Tunisia), lay eggs under short-day photoperiod (6hL:18hD) only, they do not lay eggs at all under long-day (18hL:6hD) although they have mated normally; this suggests that the extreme long day photoperiod of 18hL:6hD is gametogenesis inhibitory. When acclimatized to the laboratory environment over 4 generations under conditions as in the present study, *H. aperta* snails from Bakaro (Bejaïa, Algeria) are reproductively 7-16-fold more efficient, especially under 20°C/16hL:8hD (Benbellil-Tafoughalt *et al.*, 2009). These higher reproductive performances are probably the product of a physiological plasticity of the process of reproduction as in *Cornu aspersum* (Madec, 1988).

The significant positive correlation between numbers of matings and egg-layings ( $P = 0.00-0.09$ ) (Fig. 2) indicates that the snails that laid more clutches were those that mated more frequently. A similar observation has also been reported on laboratory *H. aperta* snails (Tafoughalt-Benbellil *et al.*, 2009), *Arianta arbustorum* (Baur, 1988; Baur & Baur, 1992) and *Cornu aspersum* (Madec *et al.*, 1998, 2000).

#### NUMBERS OF EGGS PER CLUTCH, EGG WEIGHTS AND RATES OF HATCHING

The snails collected in March (Sample 1) were those that laid significantly ( $P < 0.05$ ) less eggs per clutch than those collected in February (Sample 2) and September (Sample 3) (Tab. II, line 9). Consequently, this resulted in significantly ( $P < 0.05$ ) heavier mean egg weights in Sample 2 than in Sample 1 and, at a lesser degree, Sample 3 (Tab. II, line 10). Otherwise, there was a strong negative correlation between numbers of eggs per clutch and the mean egg weights ( $P < 0.01$ ): the larger were the numbers of eggs per clutch, the lighter were, in average, the individual egg weights of a clutch.

*H. aperta* snails collected in September at Bazina (Tunisia) have shown a lower mean egg number per clutch (279), lighter mean egg weights (12.6-14.8 mg) and a shorter time of eggs incubation (12 days) (Vaufléury & Gimbert, 2009). This means that the extreme short day photoperiod (6hL:16hD) used by the authors is even more stressing than 8hL:16hD used in the present study (Tab. II, lines 11-14). However, the laboratory acclimatized *H. aperta* snails have produced far heavier mean egg weights (26-27 mg) even when the numbers of eggs per clutch are high (306-340 eggs/clutch) (Tafoughalt-Benbellil *et al.*, 2009).

In spring, the Mediterranean helioid species, such as *Helix lucorum* (Staikou *et al.*, 1988) and *Helix texta* (Heller & Ittiel, 1990), tend to reduce their eggs number per clutch (70-80 eggs/clutch) and compensate it by an increase in eggs weight. This is in relation with youthful mortality during aestivation because heavier eggs have higher nutritive contents, as in *Arianta arbustorum* (Baur, 1994), leading to larger youthful and better survival during the long aesti-

vation (May-September) under Mediterranean climate. In Western Europe too, where winter survival is the principal factor in the helioid populations dynamics, several authors have also noted a reduction in eggs number per clutch accompanied by an increase in eggs weights during the autumnal season (Wolda & Kreulen, 1973; Pollard, 1975; Oosterhoff, 1977; Peake, 1978; Caïn, 1983; Cowie, 1984; Madec *et al.*, 1998, 2000).

#### MORTALITY RATES AND BODY WEIGHTS DURING THE REPRODUCTION PERIOD

The higher mortality rates and lower reproductive performances under 15°C/8hD:16hD in most cases (Tab. II, lines 1-3 & 5-7) suggest that *H. aperta* snails prefer the 20°C/16hL:8hD combination of temperature and photoperiod closer to the autumnal conditions, especially overnight, their phase of activity (Tab. I).

In regard to body weights, if we assume the genetic identity of the snails, the differences between samples observed for this trait before the reproduction period can be attributed to differences in amounts of food ingested before the reproduction period (Tab. II, line 14).

The snails sampled during or after hibernation had significantly increased in body weight and those collected during aestivation had more or less sensibly decreased in weight (Tab. II, line 15). This is simply because, in the first case, the snails reproduced very little and tended more to consume food (in prevision of survival and optimized gametogenesis during aestivation ?) whereby their significant increase in weight; in the second case, the snails that had already stocked much energy occupied themselves much more to reproduce and therefore sensibly decreased in body weight.

All these arguments on mortality rates and body weights, and those gathered above regarding reproductive performances plead that *H. aperta* snails are better adapted to reproduce in autumn (decreasing days and higher temperatures) after a long aestivation stimulating gametogenesis than in spring (increasing days and lower temperatures) after hibernation inhibiting gametogenesis.

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