Belgian Journal of Zoology

www.belgianjournalzoology.be

 ISSN 2295-0451

Research article

https://doi.org/10.26496/bjz.2026.203

Just in time? Flexibility in reproductive traits of *Vertigo antivertigo* (Gastropoda: Vertiginidae) according to population origin, hibernation characteristics, and photoperiod

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Abstract. This study investigates the reproductive traits of *Vertigo antivertigo*, focusing on oviposition timing and number of eggs laid across varying environmental conditions to evaluate the species' phenotypic responsiveness and adaptive capacity. Five experiments were conducted using snails from both geographically proximate and distant populations to assess the flexibility of reproductive traits in response to ecological factors such as habitat quality, hibernation conditions, and photoperiod. Significant inter- and intrapopulation differences were observed in response to microhabitat and hibernation conditions. Naturally hibernating snails initiated egg laying earlier and produced more eggs than those hibernating under laboratory conditions. Geographically distant populations differed in reproductive timing under laboratory conditions, suggesting local adaptation to seasonal constraints. Hibernation duration affected the onset of reproduction, supporting the role of endogenous rhythms in seasonal reproductive regulation. In contrast, photoperiod had no significant effect. Overall, the results highlight substantial phenotypic flexibility in reproductive traits of *V. antivertigo*, shaped by both passive environmental responses and adaptive processes, which may enhance resilience to ongoing climate change.

Keywords. Invertebrates, Mollusca, phenotypic responsiveness, phenology, climate change.

Książkiewicz Z. & Fajfer D. (2026). Just in time? Flexibility in reproductive traits of *Vertigo antivertigo* (Gastropoda: Vertiginidae) according to population origin, hibernation characteristics, and photoperiod. *Belgian Journal of Zoology* 156: 1–18. https://doi.org/10.26496/bjz.2026.203

Introduction

Organisms need to be flexible in how they respond to changing environments in order to survive (Padilla & Savedo 2013; Proćków *et al*. 2022). Some changes, like photoperiod, follow predictable daily and seasonal cycles (Bradshaw & Holzapfel 2007). However, most environmental factors are less predictable. Today, rapid and extreme environmental changes – driven by the climate crisis – make conditions even more uncertain (Schilthuizen & Kellermann 2014; Hoffmann & Bridle 2022), challenging how flexible organisms can be.

This flexibility is called phenotypic responsiveness, which helps individuals to survive and reproduce in unstable environments (Hildebrandt 2023). In a narrow sense, this can mean permanent changes to traits,

known as phenotypic plasticity. However, if the changes are reversible – if conditions return to normal and the organism reverts its traits – it is called phenotypic elasticity (Hildebrandt 2023). Sometimes, changes in an organism's traits are just passive responses to the environment (e.g., slower growth rate when food is scarce) and may not be adaptive (Hildebrandt 2023). Understanding which of these changes are ecologically important requires careful examination. The flexibility, both reversible and irreversible, is especially important for organisms that cannot move away from disturbed conditions. Thus, animals with limited dispersal often show high phenotypic responsiveness (Proćków *et al*. 2022). One such animal group is land snails. When faced with stress, they often hide in their shells and become inactive (Barker 2001). Consequently, their reproduction is strongly affected by environmental conditions (de Vaufleury 2001), and their reproductive traits often reflect phenotypic responsiveness (Madec *et al*. 2000).

In our study, we conducted a series of experiments to test how the minute land snail *Vertigo antivertigo* (Draparnaud, 1801) (shell up to 2.30 mm in height) adjusts its reproduction under different conditions. This species is found across most of Europe and is typical of wetland habitats (Pokryszko 1990a, 2003). It lives in leaf litter but also climbs plants like *Carex* sp., *Phragmites communis*, and *Typha* sp. up to 10–20 cm above the ground (Pokryszko 1990b; Hornung *et al*. 2003). *Vertigo antivertigo* is an iteroparous hermaphrodite (Pokryszko 1990a). Field studies in Poland (Myzyk 2011) showed that its reproduction began in mid-May. In the lab, these snails laid 1–2 eggs nearly every day from May to August. The breeding period in artificial conditions lasted 50–88 days, and their maximum lifespan in the laboratory was three years (Myzyk 2011). In species of *Vertigo*, mating seems to be a rare event, and eggs are either laid following copulation or via self-fertilization (Pokryszko 1992).

The aim of our study was to examine how factors such as geographical origin, hibernation duration, habitat differences and photoperiod affect reproduction in *Vertigo antivertigo*, defined here as the timing of egg laying and egg output (number of eggs laid). Climate change is expected to extend the growing season and may alter the behaviour and life-history strategies of hibernating species. Specifically we tested: (1) whether snails from geographically close and distant populations differed in oviposition timing and egg output under natural and laboratory hibernation conditions; (2) whether hibernation conditions (natural vs laboratory) affected reproductive traits within the same population; (3) how variation in hibernation duration influenced reproduction; and (4) how different photoperiod regimes affected oviposition timing and egg output, given that variation in hibernation duration may result in emergence under different day–night regimes. Finally, we discuss our results in the context of phenotypic responsiveness.

Material and methods

Site descriptions and material collection

Snails used in this study were collected in Poland, a country located within the temperate climate zone. Individuals were collected manually (directly from plants, usually from sedges Fig. 1D) from three sites (Figs 1A–C, 2), representing two distinct geographical regions. Two sampling sites were located in lowland areas (Sites L1 and L2, Wielkopolska Region), and one in a mountainous region (Site M1 – Bieszczady Mountains). Meteorological data were sourced from nearby synoptic stations: Poznań-Ławica station (52°24′59.6″ N, 16°50′04.6″ E) for L1 and L2 (located 4.28 km and 4.14 km from the sites, respectively), and Solina-Jawor station (49°23′59.7″ N, 22°28′05.9″ E), 19.60 km from site M1. Detailed climate profiles are provided in the Appendix.

Lowland sites

Site L1

Coordinates: 52.426278° N; 16.771917° E; elevation 98 m a.s.l.; location: western Poland, Greater Poland Lake District; collection date: 10 March 2023.

Site L1 (Fig. 1A) is a small urban forest park located within the western part of the Poznań agglomeration. The area is moderately used for recreation, with walking trails nearby. It is surrounded by residential buildings to the east and north and by farmland to the southwest.

The site is largely shaded by mixed forest dominated by Scots pine (*Pinus sylvestris* L.), with clusters of silver birch (*Betula pendula* Roth), pedunculate oak (*Quercus robur* L.), common ash (*Fraxinus excelsior* L.), and black alder (*Alnus glutinosa* (L.) Gaerth.). The central part contains an unshaded, temporary pond that partially dries out during summer. The site's semi-open layout and topography suggest it was once a larger pond, now reduced in size. The pH of the waterlogged soil was measured at 6.75.

Site L2

Coordinates: 52.42517° N, 16.77378° E; elevation: 97 m a.s.l.; location: Greater Poland Lake District; collection dates: 29 October 2020, 14 April 2022, 10 October 2022, 10 March 2023.

Located about 160 meters southeast of L1, Site L2 (Fig. 1B) shares similar surroundings. It is a mid-forest meadow surrounded mainly by black alder and silver birch, with a relatively open centre. Historically a wetland, the area has gradually transformed into a semi-wet meadow over the past two decades. The soil pH was recorded at 7.3.

Mountain site

Site M1

Coordinates: 49.36447° N, 22.73336° E; elevation: 567 m a.s.l.; location: Bieszczady Mountains, Southeastern Poland; collection date: 13 October 2022.

Site M1 (Fig. 1C) is located approximately 500 km southwest of L1 and L2. It is classified as a bog-spring ecosystem, traversed by the Maksymivs'ki and Syhavka rivers. The site is open and surrounded

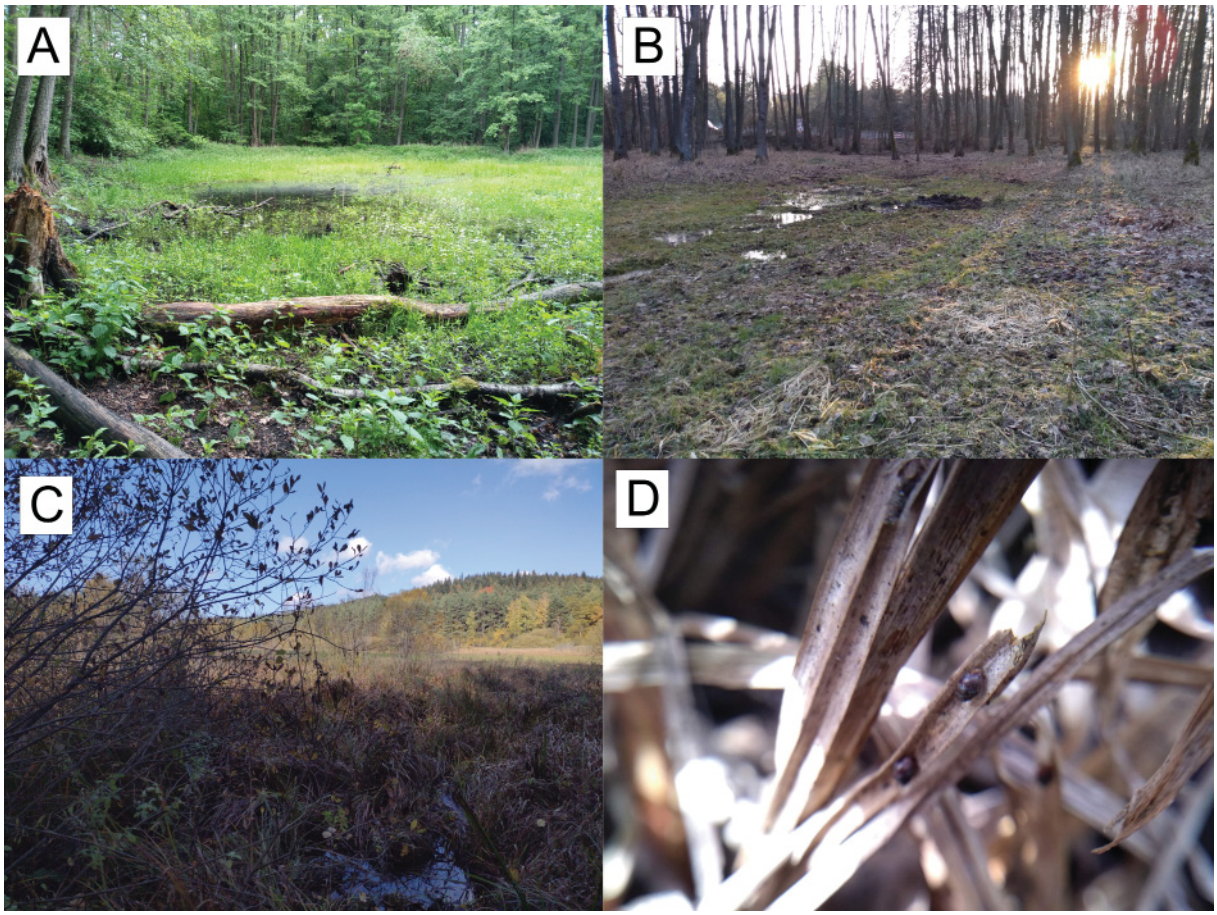

Figure 1 – **A**. Site L1 - temporary pond in the lowlands. **B**. Site L2 - forest sedge meadow in the lowlands. **C**. Site M1 - bog-spring in the Bieszczady Mountains. **D**. *Vertigo antivertigo* individuals on a sedge leaf.

Figure 2 – Map showing the three study sites, which are indicated by orange dots: L1 & L2 (lowland sites in the Wielkopolska Region, proximal to Poznań) and M1 (montane site in the Bieszczady Mountains, proximal to Sanok; urban centres are shown with yellowish boundaries). The map illustrates the administrative divisions of Poland into voivodeships, whose nomenclature is shown in blue letters.

by mixed forest, primarily composed of European spruce (*Picea abies* L.), with common aspen (*Populus tremula* L.) and willows present in wetter zones. The pH of waterlogged soil was 7.5.

Laboratory cultures

Active period

After manual collection, snails were transported to the laboratory in ventilated plastic cups (120 ml) containing cotton and litter for food and moisture. In the lab, individuals were housed separately in ventilated Petri dishes (5 cm diameter) containing water-saturated cotton pads, dolomite dust (as a calcium source), and leaf litter (primarily from sedges and alder trees) as food and shelter. The dishes were stored in larger ventilated transparent plastic containers (22 × 14 × 9 cm) to maintain stable rearing conditions (17°C, 12:12 h light/dark photoperiod, except in the photoperiod-specific experiments).

Snails were monitored approximately every two weeks. During each check, new eggs were counted, litter was partially replaced and sprinkled with water. In laboratory conditions, snails were kept solitary; thus, eggs laid were probably the result of self-fertilization. If juveniles were found, they were transferred to separate dishes. Once a month, the cotton pads were replaced, while some litter was retained to preserve microbial communities.

Hibernation protocol

Hibernation in the laboratory was induced by placing snails under constant, low-temperature conditions at 4°C, a temperature known to effectively trigger dormancy in snails (see, e.g. Griffond *et al*. 1992). Laboratory snails hibernated for several months, from October to March (Fig. 3) corresponding to the

TABLE 1

Experimental options (Exp. opt.), hibernation conditions (Hib. cond.), and populations (Pop.) used. The "Short" column lists abbreviations used throughout the text.

Exp. opt.	Hib. cond.	Short	Pop.
Interpopulation differences	Wild hibernation	**IePD-WH**	L1, L2
Interpopulation differences	Lab hibernation	**IePD-LH**	M1, L2
Intrapopulation differences	Wild vs lab Hibernation	**IaPD-WL**	L2
Impact of hibernation Length	Lab hibernation	**HL-impact**	L2
Impact of photoperiod	Wild hibernation	**PP-impact**	L2

natural hibernation (hereafter also referred to as wild hibernation) period in temperate climates. The exact duration varied among experimental groups (details provided below).

Before hibernation, each snail was placed in an individual glass test tube (1.5 cm diameter, 10 cm height) containing water-saturated cotton, dolomite, and sedge leaves. The tubes were sealed with cotton plugs to allow ventilation and maintained under standard rearing conditions for 7 days to enable

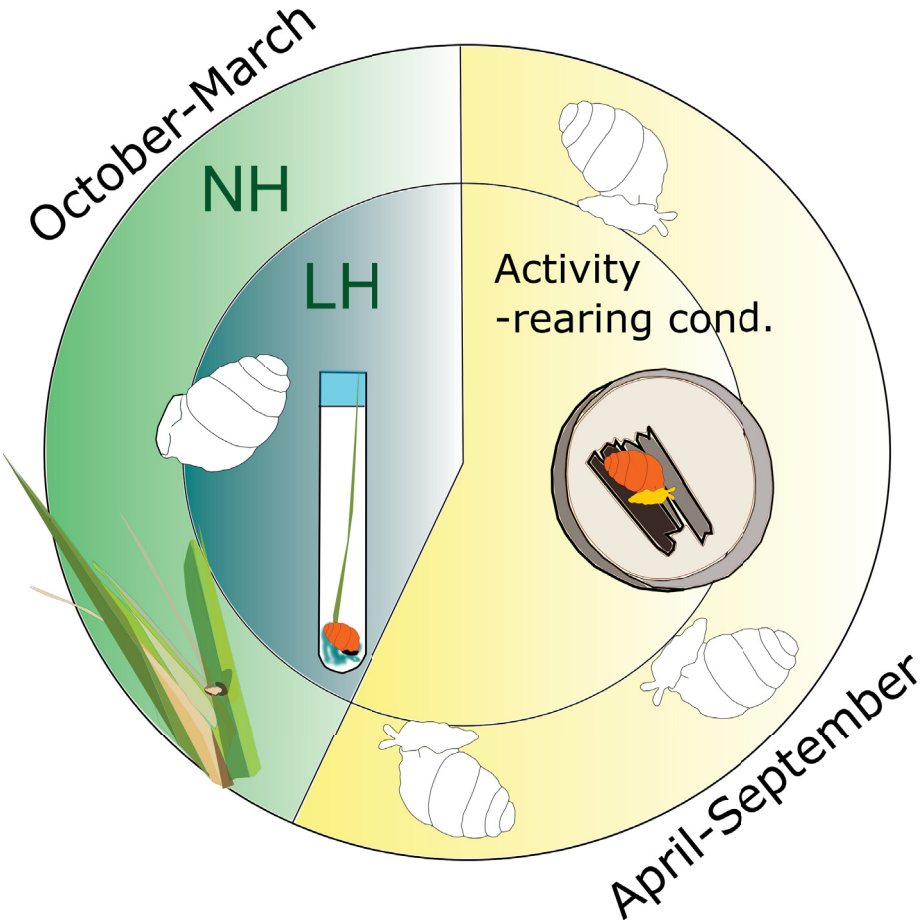

Figure 3 – Details of the experimental procedures, as well as the activity period (April–September) in rearing conditions for snails that had been hibernating under lab and natural conditions (October–March). Abbreviations: NH = natural hibernation; LH = lab hibernation.

acclimatization. After this period, the tubes were transferred to a dark cold room at 4°C. Once a month, the plugs were moistened with cooled, boiled tap water to prevent desiccation.

To terminate hibernation, the test tubes were first sprinkled with water and transferred to 10°C (photoperiod 12:12) for 3 days. Subsequently, the snails were transferred to Petri dishes and returned to standard rearing conditions (17°C, photoperiod 12:12).

Experiments

Due to limited availability of individuals, the number of experiments was constrained by the number of snails collected. Only adult individuals – defined as those with fully developed shells and apertural barriers – were used. All snails were collected as adults from their natural habitats (as described in the "Sites Description and Material Collection" section). A total of 314 snails were used across all experiments. However, only individuals that survived the entire experimental treatment were included for statistical analyses (see Table 2 for details).

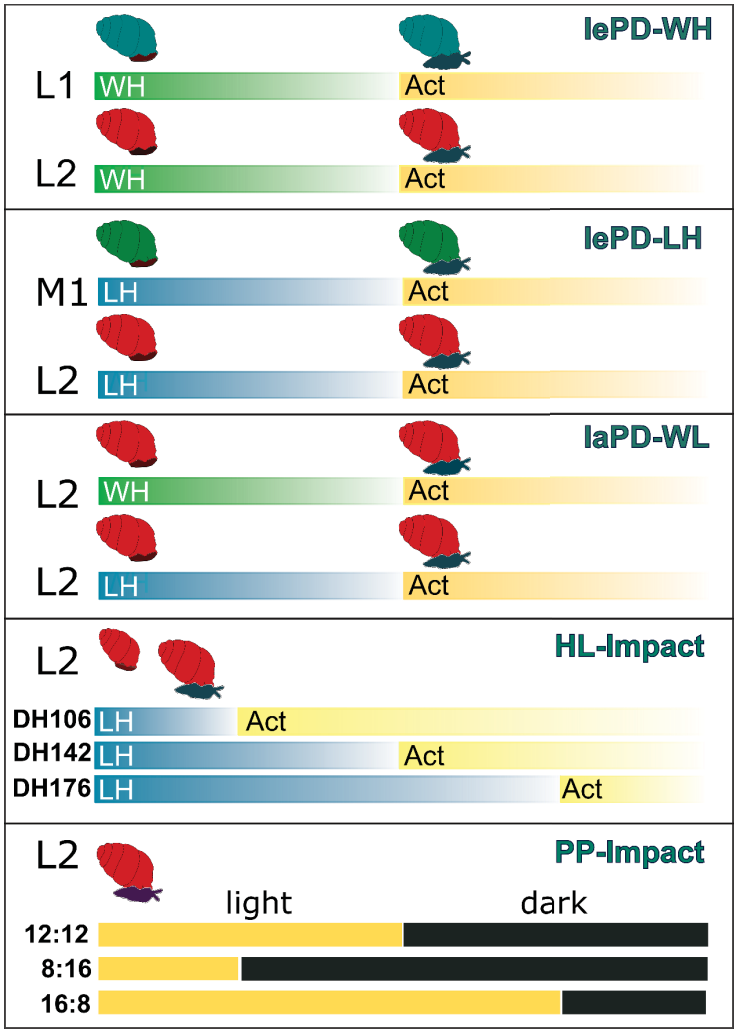

Figure 4 – Visualization of the design of experimental options testing the flexibility of reproductive traits in *Vertigo antivertigo*: IePD-WH, IePD-LH, IaPD-WL, HH-impact, PP-impact. Populations: lowland L1 and L2, mountain M1. The colour of the shell indicates their origin: L1 = blue; L2 = red; M1 = green. Experimental abbreviations are provided in Table 1. Abbreviations: WH = wild hibernation; LH = laboratory hibernation; Act = activity.

TABLE 2

Summary statistics for snails that survived till the end of experimental treatments: L1, L2, M1 = three study sites; COND = the experimental conditions (Wild = wild hibernation; Lab = laboratory hibernation; DH = days of hibernation, providing the number of days; LD = light:dark, including the hour ratios); N_0 = initial number of individuals; N = number of individuals that survived till the end of the experiment and were included in the analyses; Min = min number of eggs per individual; Max = maximum number of eggs per individual; SUM = total number of eggs laid by all individuals within a given treatment; Mean = mean number of eggs per individual; SE = standard error; SD = standard deviation.

POP	COND	N_0	N	Min	Max	SUM	Mean	SE	SD
L1	**Wild**	94	56	0	13	159	2.839	0.379	2.840
L2	**Wild**	55	47	0	14	256	5.447	0.570	3.905
L2	**Lab**	16	16	0	6	38	2.375	0.632	2.527
M1	**Lab**	39	29	0	10	66	2.276	0.468	2.520
L2	**DH106**	25	13	3	12	83	6.385	0.797	2.873
L2	**DH142**	25	17	0	20	154	9.059	1.675	6.905
L2	**DH176**	25	15	0	14	81	5.400	0.804	3.112
L2	**LD12:12**	11	8	0	8	25	3.125	1.060	2.997
L2	**LD16:8**	12	8	0	7	19	2.375	0.944	2.669
L2	**LD8:16**	12	10	0	8	32	3.200	1.083	3.425

Experimental set-up: detailed information

A schematic overview of the experimental set-up is shown in Fig. 4.

IePD-NH: interpopulation differences: natural hibernation

Hypothesis 1: Snails from two geographically close populations (L1 and L2) will show similar oviposition timing and egg output due to comparable macroclimatic conditions.

Experimental goal: To assess whether reproductive traits (oviposition timing and number of eggs) differ between geographically close populations L1 and L2. Individuals were collected from their natural environment in March 2023 while hibernating under natural conditions (L1: n = 94; L2: n = 55). Immediately after collection, each snail was placed in an individual Petri dish and exposed to rearing conditions (see "Laboratory cultures" section).

IePD-LH: interpopulation differences: lab hibernation

Hypothesis 2: Snails from geographically distant populations (lowland L2 and mountainous M1) will differ in oviposition timing and/or egg output after exposure to identical laboratory hibernation conditions.

Experimental goal: To compare reproductive traits between populations L2 and M1 under laboratory hibernation. Snails were collected on October 2022 (L2: n = 16; M1: n = 39), acclimatized for 7 days (10°C, darkness), and then subjected to laboratory hibernation. Hibernation was terminated in March 2023, after which individuals were transferred to rearing conditions.

IaPD-WL: intrapopulation differences: natural vs lab hibernation

Hypothesis 3: Within population L2, snails hibernating under natural conditions will differ in reproductive traits from those hibernating under laboratory conditions.

Experimental goal: To assess the effect of hibernation conditions on reproduction within population L2. Two groups were used: (1) laboratory-hibernated individuals (n = 16), collected in October 2022 and subjected to laboratory hibernation; and (2) naturally hibernated individuals (n = 55), collected in March 2023, immediately after winter dormancy.

HL-impact: impact of hibernation length

Hypothesis 4: Variation in the duration of laboratory hibernation affects reproductive traits within population L2.

Experimental goal: To test the effect of hibernation duration on oviposition timing and egg output. Snails (n = 75) collected from L2 in October 2020 were assigned to three groups differing in hibernation length: 106, 142, and 176 days (n = 25 each). After hibernation, individuals were transferred to standard rearing conditions.

PP-impact: impact of photoperiod

Hypothesis 5: Photoperiod regimes influence reproductive traits within population L2.

Experimental goal: To assess the effect of photoperiod on reproduction. Snails (n = 35) collected in April 2022 were divided into three groups exposed to different light:dark cycles (12:12; 8:16; 16:8) and monitored until October, 2022.

Statistical analysis

Differences in the number of eggs laid by individuals under experimental conditions were analyzed using a one-way randomized ANOVA with the RundomPro 3.14 software (Jadwiszczak 2009). Repeated measures analysis of variance (ANOVA) was used to assess the oviposition time pattern in experimental groups. The assumption of sphericity was tested by Mauchly's test, followed by Greenhouse-Geisser adjustments if Mauchly's test proved insignificant. Statistical analyses were conducted using Statistica 13.3 software.

Results

Of the 321 snails initially exposed to experimental conditions, 219 survived. Egg mortality was not observed, as the number of eggs matched the number of newly hatched snails. However, due to the small dimensions of eggs (0.55–0.78 mm diameter, mean 0.648 mm; Myzyk 2011) and their transparency, it is possible that some eggs were missed, especially fresh eggs from early developmental stages. Summary statistics for the number of eggs laid in each treatment are shown in Table 2.

IePD-WH: interpopulation differences: wild hibernation

Hypothesis 1 was rejected. One-way ANOVA showed significant differences in the number of eggs laid by wild-hibernating snails from the two spatially close sites, L1 and L2. Snails from site L2 laid significantly more eggs than those from site L1 ($F = 15.381$; $p < 0.001$; Fig. 5A). Repeated measures ANOVA also revealed a statistically significant difference in the timing of oviposition between individuals from L1 and L2 ($F = 5.577$; $p < 0.001$; Fig. 6A). In both groups, the first eggs were recorded on day 8. The L1 group had its highest egg production on day 8, while the L2 group reached its peak on day 19, after which the number of eggs gradually decreased. The L1 group had an additional peak of egg production on day 43, and the last eggs were recorded on day 143, whereas the last eggs for the L2 group were noticed on day 59.

IePD-LH: interpopulation differences: lab hibernation

Hypothesis 2 was partially confirmed. One-way ANOVA did not reveal significant differences in the number of eggs laid by snails from the two geographically distant sites, L2 and M1 ($F = 0.016$; $p = 0.903$;

Fig. 5B). However, repeated measures ANOVA showed a significant difference in oviposition timing between the two groups ($F=4.214$; $p=0.001$; Fig. 6B). The M1 group began reproduction earlier and completed it sooner than the L2 group. Both groups displayed two oviposition peaks: M1 on days 16 and 64, while the number of laid eggs of L2 individuals peaked on days 46 and 78. The first eggs in both groups were laid on day 16, with the last eggs being recorded on day 78 for M1 and on day 97 for L2.

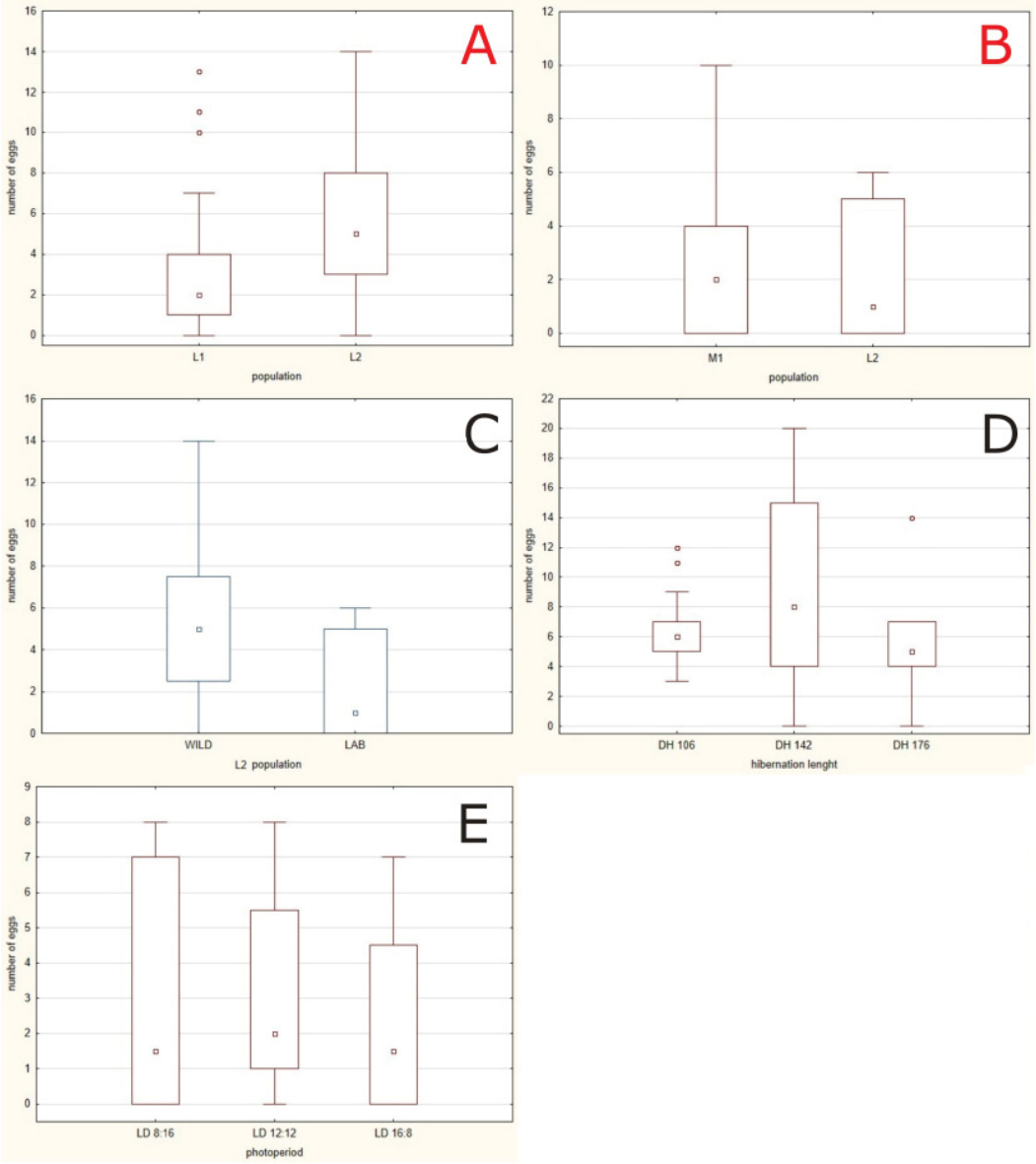

Figure 5 – Number of eggs laid by individual snails. **A**. The two lowland populations in proximity (L1 and L2), hibernating under natural conditions. **B**. The two geographically distant populations from the mountains (M1) and lowlands (L2) hibernating under lab conditions. **C**. The lowland population L2, hibernating under different conditions (natural vs lab). **D**. The lowland population L2 being exposed to different lengths of hibernation periods: DH 142=142 days of hibernation; DH 176=176 days of hibernation. **E**. The lowland population L2 being exposed to different light:dark cycles (12:12; 8:16; 16:8). Squares inside the boxes indicate the mean values; ratio: 25%–75%; whisker=range of non-outliers; circles denote outliers. Red letters statistically significant results while black letters show non-significant results.

IaPD-WL: intrapopulation differences: natural vs lab hibernation

Hypothesis 3 was confirmed. One-way ANOVA showed significant differences in the number of eggs laid by snails from the same population but hibernating under different conditions (wild vs laboratory) ($F = 8.085$; $p = 0.005$; Fig. 5C). Snails that overwintered under natural conditions laid significantly more eggs than those that hibernated in the laboratory. Repeated measures ANOVA also revealed a significant difference in oviposition timing between snails hibernating under natural and laboratory conditions ($F = 9.136$; $p < 0.001$; Fig. 6C). The group under natural-hibernating conditions had a single pronounced

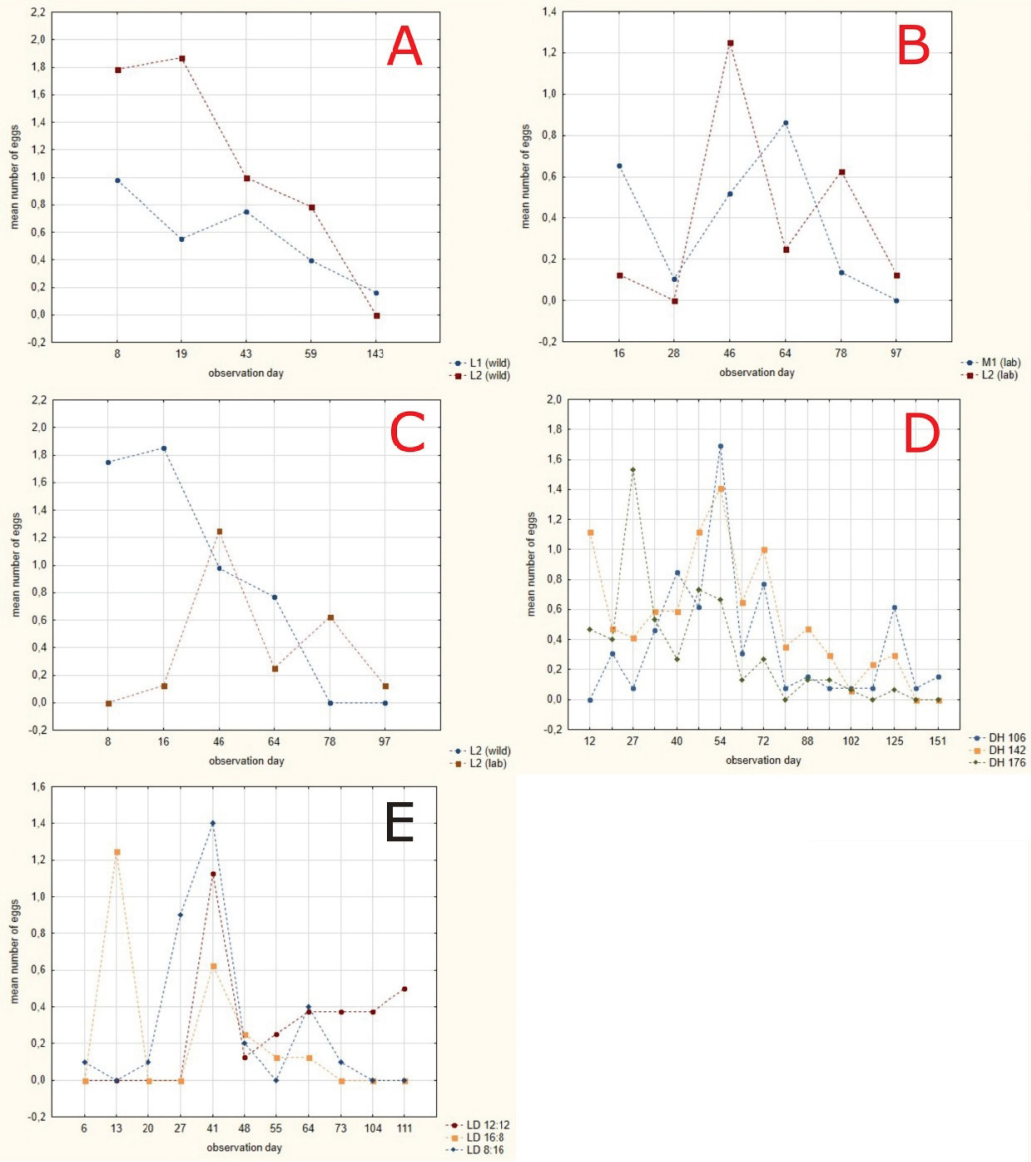

Figure 6 – Oviposition timing. **A**. The two lowlands populations in proximity (L1 and L2), hibernating under natural conditions. **B**. The two geographically distant populations from the mountains (M1) and lowlands L2, hibernating under lab conditions. **C**. The lowland population L2, hibernating under different conditions (natural vs lab). **D**. The lowland population L2 being exposed to different hibernation lengths of hibernation periods: DH 142 = 142 days of hibernation; DH 176 = 176 days of hibernation. **E**. The lowland population L2 being exposed to different light/dark cycles (12:12; 8:16; 16:8). Squares inside the boxes indicate the mean values; boxes represent the interquartile range (25–75%); whiskers indicate the range of non-outlier values; circles denote outliers. Red letters indicate the graphs that depict statistically significant results while black letters show non-significant results.

peak, after which egg production gradually decreased. The lab-hibernating group began laying eggs later (day 16 for lab vs day 8 for wild) and exhibited two peaks (days 46 and 78).

HL-impact: impact of hibernation length

Hypothesis 4 was partially confirmed. Statistical analysis did not show significant differences in the number of eggs laid by snails exposed to different lengths of hibernation ($F = 2.784$; $p = 0.070$; Fig. 5D). However, repeated measures ANOVA indicated a significant difference in oviposition timing among the groups ($F = 1.762$; $p = 0.006$; Fig. 6D). The DH 106 group, which hibernated for the shortest time (106 days), started laying eggs later (day 19) than the DH 142 and DH 176 groups (first eggs recorded on day 12). The DH 106 group also had the longest oviposition period, with the last eggs being recorded on day 151, compared to day 125 for both DH 142 and DH 176. For DH 142, intense oviposition began 12 days after hibernation was terminated, with a peak on day 54. Egg production of the DH 106 group also peaked on day 54, while DH 176 reached its peak earlier, on day 27.

PP-Impact: impact of photoperiod

Hypothesis 5 was rejected. One-way ANOVA did not reveal any significant differences in the number of eggs laid by snails exposed to different light:dark cycles (12:12; 8:16; 16:8) ($F = 0.184$; $p = 0.834$; Fig. 5E). Similarly, repeated measures ANOVA showed no significant differences in oviposition timing among the experimental groups ($F = 1.286$; $p = 0.190$; Fig. 6E).

Discussion

This study investigated the reproductive traits of *Vertigo antivertigo*, focusing on oviposition timing and the number of eggs laid under varying environmental conditions (egg output). Our aim was to assess the flexibility of these traits in response to different ecologies. We conducted five experiments including snails from both geographically close and distant populations (Fig. 4). Given the ongoing impacts of climate change, we also examined how these snails respond to variations of hibernation, including hibernation duration, which may in turn affect the photoperiodic conditions snails experience after emerging from hibernation, to better understand their adaptive capacity. Our results demonstrate that both interpopulation and intrapopulation differences significantly influence reproductive outcomes, shaped by local habitat conditions, hibernation parameters, and climatic conditions.

Despite experiencing the same macroclimatic hibernation conditions, notably, snails from two geographically close populations (L1 and L2), showed significant differences in reproductive timing and number of eggs laid (IePD-WH experiment). These differences likely reflect passive responses to microclimate. The L1 site, characterised by periodic flooding and more acidic soil, probably limited access to food resources and calcium which is critical for calciphilic *V. antivertigo* (von Proschwitz 2003). In contrast, the L2 population benefited from more stable, litter-rich conditions, potentially enabling a better nutritional status (e.g., according to Skoog (1978), food quality can influence the number of eggs laid by snails) and earlier onset of reproduction. These findings suggest that habitat quality, even at a fine spatial scale, plays a critical role in shaping reproductive traits in *V. antivertigo*.

Intrapopulation comparisons between snails having hibernated under natural or laboratory conditions (IaPD-WL experiment) further emphasized the importance of the natural environment on reproductive traits. Naturally-hibernated snails exhibited higher fecundity and advanced reproductive timing compared to lab-hibernating. This result could be due to intermittent winter activity triggered by natural temperature and humidity fluctuations, enabling occasional feeding and maintenance of physiological responsiveness during the winter (e.g., Książkiewicz-Parulska & Pawlak 2017; Lipińska *et al*. 2025). In

contrast, constant lab conditions may suppress such activity, leading to lower fitness and reproductive output. The absence of natural stimuli – such as variable temperatures, humidity, diverse food sources and food supply in general (perhaps affecting microbiome quality), and light cues – likely reduces the physiological readiness for reproduction in lab-hibernated snails (Jeppesen 1977; Skoog 1978; Barker 2001). These findings indicate that observed differences reflect habitat quality rather than adaptive plasticity.

Comparisons of geographically distant populations (L2 and M1; IePD-LH experiment) under standardized laboratory conditions revealed no significant difference in reproductive output derived from the number of eggs laid. This outcome may in part reflect the differences in sample sizes between the two populations – 16 individuals from L2 and 29 from M1. Notably, although the L2 group was smaller, all individuals survived hibernation and had the opportunity to reproduce. In contrast, more than a quarter of the 39 initially collected individuals from M1 did not survive the hibernation period (Table 2). These patterns suggest that differences between populations may exist, and a more balanced or larger sample size for each population might provide additional insights. However, obtaining larger numbers from the L2 site was constrained by the limited availability of individuals during collection. The considerable geographical distance and distinct environmental characteristics of the two sites likely influence population traits. This is reflected not only in the number of individuals collected but also in their overwintering survival. Importantly, our results revealed significant differences in oviposition timing: snails from the mountainous M1 site began reproduction earlier and ceased it sooner than their lowland L2 counterparts. This pattern likely reflects adaptations to the shorter growing season at higher elevations (Sulikowska-Drozd *et al*. 2013, 2019; Cameron 2016). These results suggest population-specific phenotypic plasticity, possibly involving physiological adjustments, such as regional differences in cold tolerance or reproductive scheduling aligned with local phenology (Lipińska *et al*. 2024).

Hibernation duration (HL-impact experiment) was also found to affect reproductive timing but not the number of eggs laid, indicating that physiological readiness and recovery post-hibernation are sensitive to the length of dormancy. Snails undergoing longer dormancy resumed reproduction earlier than those with shorter hibernation periods, indicating that internal physiological changes during hibernation may prime individuals for post-dormancy reproduction. Climate change is causing increased annual temperatures and lengthening the growing season by 10–20 days in recent decades (Linderholm 2006). Consequently, the number of days suitable for hibernation is decreasing. Few studies have examined the effect of hibernation duration on gastropod reproduction. Çelik *et al*. (2022) found that in *Cornu aspersum* (Gastropoda, Helicidae), egg production depended on both hibernation conditions and dormancy length. The elastic response to hibernation length and conditions could provide a fitness advantage by allowing early reproduction when conditions are favourable, while also preventing premature breeding after insufficient rest. These results emphasize the role of endogenous rhythms in regulating reproductive readiness, particularly in seasonal environments.

Finally, the photoperiod (PP-impact experiment) had no detectable effect on either reproductive output or timing. Although the photoperiod is a known regulator of reproduction in many gastropods (Bohlken & Joosse 1981; Hunter & Stone 1986; Otchoumou *et al*. 2007; Benbellil-Tafoughalt *et al*. 2009; Cameron 2016), its influence in *V. antivertigo* appears limited. In our experiments, snails in longer light conditions (16:8) started reproduction slightly sooner than those in other groups, but the differences were not statistically significant. This lack of response could reflect the species' adaptation to a climate where light cues no longer reliably indicate suitable reproductive conditions due to climate change. Alternatively, small sample sizes may have limited our ability to detect subtle effects.

Conclusions

This study highlights the complex interplay of environmental and physiological factors shaping reproductive traits in *Vertigo antivertigo*, emphasizing the species' phenotypic responsiveness to both macro- and microhabitat variability. Differences in oviposition timing and egg output among populations – even those that are geographically close – underscore the significant influence of local habitat conditions. Our findings suggest that *V. antivertigo* exhibits notable flexibility in reproductive traits, likely as a result of both passive environmental effects and population-specific adaptation. Evidence from interpopulation comparisons supports the hypothesis that life-history traits, such as timing of reproduction, may evolve in response to regionally distinct climatic pressures, reflecting adaptive responses to local seasonality. Furthermore, the impact of hibernation duration on reproductive timing, but not output, points to an endogenous mechanism priming snails for post-dormancy reproduction – an elastic response that may become increasingly important under ongoing climate change. While photoperiod showed limited influence in our experiments, the overall reproductive plasticity observed suggests that *V. antivertigo* possesses a degree of adaptive capacity, mediated through phenotypic flexibility, that may buffer the species against environmental variability and shifting climatic conditions.

Acknowledgments

The authors would like to thank Michał Rybak for statistical support during the manuscript preparation.

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Manuscript received: 10 December 2024
Manuscript accepted: 16 April 2026
Published on: 22 April 2026
Branch editor: Alice Dennis

APPENDIX

Weather conditions in studied areas

The meteorological data obtained from the archive database of the Institute of Meteorology and Water Management, National Research Institute. They contain weather conditions recorded by synoptic stations during the month of material collection from the sites and for the 12 months prior (for the second column it is 11 months prior, for the table clarity). The last row in the tables is the month of the material collection (in the second column, it is the penultimate row – material in this case was collected in March 2023). For the purposes of this work, the following factors were given: monthly: 1) minimum temperature; 2) maximum temperature; 3) mean temperature; 4) total rainfall; 5) maximum height of snow cover; 6) number of days with snow cover.

APPENDIX TABLE 1

Monthly minimal, maximal and mean temperature [°C] for the study sites in lowlands (L1 and L2) and mountains (M1). The years from which the data come from are indicated in brackets. The data is divided into two-time intervals - separated by a double line in the middle of the table.

	L1 & L2 (2020–2021)			**L1 & L2 (2022–2023)**			**L1 & L2 (2021–2022)**			**M1 (2021–2022)**			
	Min.	**Max.**	**Mean**	**Min.**	**Max.**	**Mean**	**Min.**	**Max.**	**Mean**	**Min.**	**Max.**	**Mean**	
Apr.	-3.9	24.7	9.8	-5.8	20.4	7.9	-1.3	25.2	10.3	-2.1	21.0	7.6	Oct.
May	0.2	24.1	12.0	3.0	27.6	14.9	-1.4	15.5	5.7	-2.5	15.5	4.8	Nov.
Jun.	6.0	31.9	18.5	5.7	36.1	19.9	-15.8	12.3	0.0	-13.0	6.1	-2.1	Dec.
Jul.	9.2	32.4	19.3	9.2	37.1	20.2	-11.2	12.2	1.7	-13.6	9.0	-1.5	Jan.
Aug.	10.2	33.1	21.0	9.0	35.9	22.1	-3.6	11.0	4.3	-8.3	9.4	1.5	Feb.
Sep.	3.6	29.9	15.6	2.0	25.2	13.4	-7.8	19.0	4.3	-13.2	20.2	1.1	Mar.
Oct.	2.8	23.9	11.0	-1.3	24.8	12.0	-5.8	20.4	7.9	-4.8	20.8	5.7	Apr.
Nov.	-4.5	15.5	6.2	-8.9	15.4	4.8	3.0	27.6	14.9	0.2	25.3	13.2	May
Dec.	-3.9	11.3	2.4	-11.6	15.6	1.3	5.7	36.1	19.9	5.9	32.3	18.2	Jun.
Jan.	-15.9	12.0	-0.5	-5.5	15.8	3.4	9.2	37.1	20.2	7.1	34.5	18.6	Jul.
Feb.	-17	18.1	-0.4	-8.4	11.3	2.2	9.0	35.9	22.1	8.6	30.1	18.6	Aug.
Mar.	-8.0	21.9	4.1	-7.5	17.5	4.8	2.0	25.2	13.4	1.6	25.1	11.9	Sep.
Apr.	-4.2	19.2	6.6	-4.0	21.8	8.4	-1.3	24.8	12.0	-1.5	20.9	10.2	Oct.

APPENDIX TABLE 2

Monthly maximal height of snow cover [cm] and number of days with snow cover at the sites (L1, L2, M1) during the material collection and the months prior.

	L1 & L2 (2020–2021)		**L1 & L2 (2022–2023)**		**L1 & L2 (2021–2022)**		**M1 (2021–2022)**		
	Max. height	**No. of days**	**Max. height**	**No. of days**	**Max. height**	**No. of days**	**Max. height**	**No. of days**	
Apr.	0	0	0	0	0	0	0	0	Oct.
May	0	0	0	0	0	0	4	2	Nov.
Jun.	0	0	0	0	8	12	18	30	Dec.
Jul.	0	0	0	0	4	8	29	25	Jan
Aug.	0	0	0	0	0	0	38	18	Feb.
Sep.	0	0	0	0	0	0	2	5	Mar.
Oct.	0	0	0	0	0	0	5	1	Apr.
Nov.	0	0	6	3	0	0	0	0	May
Dec.	2	1	1	1	0	0	0	0	Jun.
Jan.	12	14	6	6	0	0	0	0	Jul.
Feb.	6	12	0	0	0	0	0	0	Aug.
Mar.	1	1	1	5	0	0	0	0	Sep.
Apr.	1	1	0	0	0	0	0	0	Oct.

APPENDIX TABLE 3

Monthly precipitation [mm] for the sites (L1, L2, M1) during the material collection and the months prior.

	L1 & L2 (2020–2021)	**L1 & L2 (2022–2023)**	**L1 & L2 (2021–2022)**	**M1 (2021–2022)**	
Apr	2.6	36.3	25.0	2.1	Oct
May	47.5	22.6	38.1	50.9	Nov
Jun	51.3	63.4	36.5	83.1	Dec
Jul	65.3	22.7	46.1	77.7	Jan
Aug	60.9	52.8	57.2	41.4	Feb
Sep	37.4	31.6	2.1	39.3	Mar
Oct	41.0	28.3	36.3	69.0	Apr
Nov	11.8	16.3	22.6	36.9	May
Dec	31.5	35.2	63.4	62.2	Jun
Jan	53.5	53.3	22.7	122.9	Jul
Feb	35.0	40.1	52.8	54.8	Aug
Mar	22.1	51.4	31.6	189.4	Sep
Apr	33.7	36.8	28.3	66.6	Oct