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Articles

Invertebrates of Siberia, a potential source of animal protein for innovative food production. 1. The keelback slugs (Gastropoda: Limacidae)

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The use of terrestrial invertebrates occurring in Siberia as a source of nutrients is an innovative form of new quality food production in North Asia. The species available for this production should be qualified by necessary criteria; for example, they should be common in the region and easily obtainable, free from restriction or prohibition as rare or protected species, adapted to regional environmental conditions, and their bodies should be free from toxins and allergens. They should also be unpretentious in terms of housing, consumption of cheap and suitable feed which provides a satisfactory increase in biomass and contains necessary nutrients in the required ratio. Several local species of terrestrial molluscs and insects fit these criteria and have been were selected as model species, such as the yellow slug *Limacus flavus* (Linnaeus, 1758) which has been studied in detail. Individuals of this slug were collected from a subterranean vegetable store in the city of Novosibirsk, and reared for 5 months under laboratory conditions with different lighting, humidity and temperature. Standard vegetables for winter storage, carrot, cabbage and potato (the preferred ingredient), were provided to the slugs. The most effective factors for the development of body weight and size of the slugs were registered in the dark under moderate humidity and temperature. Average weight and length of slugs at the beginning of the experiment in March 2022 were 0.62 gram and 3.42 mm, and at the end of the experiment in August 2022 were 3.67 gram and 5.76 mm (respectively x 5.9 and x 1.7). Therefore, basement and underground cold premises lacking constant lighting and provided with potato waste as a feeding substrate appear to be optimal for raising and rearing this slug species; naturally this would be of particular interest for food production in regions with cold climate conditions.

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Keywords

Mollusca, Gastropoda, Limacidae, *Limacus flavus*, West Siberia, raisin, regional conditions.

Introduction

Edible invertebrate nutrients as source of food in the future

Detection of new resources of food containing a spectrum of nutrients, which are available and cheap to produce, are necessary to support terrestrial invertebrates capable of providing protein production for human consumption (Van Huis et al. 2013; Zielińska et al. 2015; Van Raamsdonk et al. 2017). The temperate climate zone of Eurasia, particularly South Siberia, is ideal for such an activity. Hence, a new project, the first of its kind in North Asia, entitled “Invertebrates of Siberia as a resource of animal protein for innovative food production”, was launched in the National Research Tomsk State University in 2022.

The potential of terrestrial invertebrate farming for food production

The use of terrestrial invertebrates for human consumption has been used for a long time, but only a few species of them have been domestically bred; for example, shell gastropods of the families Achatinidae and Helicidae (Thompson, Cheney 1996). These species are strongly invasive pests and widespread in countries with a warm climate. The exceptionally large size that these snails reach was one of characters attracted to human attention as a food source. As a result, farms have been adapted to produce snail ‘meat’ for restaurants and markets; it should be noted that the ‘domesticated’ slugs were formerly regarded as pests which damaged agricultural activity. At present, the mealworm (*Tenebrio molitor*) larvae and the adult desert locust (*Schistocerca gregaria*) are studied for their potential as food usage (Zielińska et al. 2015). They are common species occurring in high numbers, adapted to a range of environmental conditions, and although registered as pests, they are not problematic in terms of fodder plants or substrates.

Amongst terrestrial invertebrates only insects and molluscs are farmed (Thompson, Cheney 1996; Hanboonsong et al. 2013). Species raised, traded and consumed vary in different countries. Since 2021, the European Commission (EC) authorized only three species of insects for sale, farming and novel food consumption (Regulation (EC) No. 852/2004; EFSA 2015; Lahteenmaki-Uutela, Grmelova 2016). The EC Regulation no. 853/2004 defines edible snails as 5 species terrestrial gastropods: *Helix pomatia* Linnaeus, 1758, the Roman snail, or apple snail, lunar, La Vignaiola, the German "Weinbergschnecke," the French "escargot de Bourgogne" or "Burgundy snail," or "gros blanc"; *Cornu aspersum* (O. F. Müller, 1774) (= *Helix aspersa* Muller), also known as the French "petit gris," "small grey snail," the "escargot chagrine," or "La Zigrinata"; *Helix lucorum* Linnaeus, 1758, or Turkish snail, as well as tropical species of the family Achatinidae, including the well-known species *Achatina achatina* (Linnaeus, 1758), the Giant African Snail, and *A. fulica* (Férussac, 1821), the Giant East African Snail.

Such a restriction for the food industry is explainable by the strict order of “studies on bacteria, viruses, parasites and prions associated with food and feed insects, chemical contamination, allergens, processing, and the potential environmental impact of insect farms” (Belluco et al. 2013; Spiegel van der et al. 2013; Finke et al. 2015). No doubt, further development of invertebrate farming will solve these problems and significantly widen the list of edible species necessary for the generation new quality food products. Currently, a survey of new species with suitable life cycles and morphology is necessary (Van Huis et al. 2013; Van Huis 2013).

Slugs have never been used and studied as food supplement. Limacidae species are the largest slugs, which can reach the same size as Achatinidae and Helicidae, but lack a shell and and are richer in protein biomass. The synanthropic slug *Limacus flavus* (L.) is widespread and introduced into different countries, including North Asia (Castillo 2020). It has, for example, been found by us in an underground vegetable store in Siberia and questioned: would this species be effective for protein production when raised under simulated conditions? Thus, we initiated an experiment with the aim of defining the optimal parameters for environmental conditions that would provide rapid growth of biomass and slug development.

Material and methods

Slug rearing

Slug specimens examined by Roman Egorov in Moscow in June 2016 were identified as *Limacus flavus* (L.). On 31 March 2022, 10 juvenile specimens of this species were taken from an underground vegetable store in Novosibirsk and placed in five numbered plastic containers (1 liter vol.) with closely fitted covers to prevent air exchange. Containers 1 and 3 were maintained at 24–26˚C under daylight, containers 2 and 4 at room temperature and in darkness, and container 5 in a refrigerator at 10–12˚C in darkness. Containers 3 and 4 were also provided with viscose tissue saturated with water to increase humidity.

The size and weight of each slug were evaluated once a week, and its position on the bottom, wall or cover of the container was recorded. Individuals were placed in a petri dish with forceps and were scaled using an electronic balance (E-68, Russia). The length was defined with a ruler when the slug had straightened to its maximum length. Temperature and humidity were measured a thermohygrometer (DT-321, Russia). Food pieces from slug waste were cleaned and weighed by means of a Gosmeter VLTE–500 balance, Russia, and then substituted by fresh feed. Containers were washed with water lacking detergent and using a sponge to remove mucus too difficult to clean. Humidity less than 80% was prevented by spraying water, and humidity over 95% by ventilation of the container. Humidity was generated soleby by evaporation generated by the vegetables eaten by the slugs.

Slug feeding

Each container was supplied with several pieces of potato, carrot and cabbage, all of which had been washed with warm water to remove any pesticides dangerous to slugs that could have been applied during storage of the vegetables. Twice a week the vegetables were substituted, all pieces being weighed before and after substitution to determine the quantity consumed in terms of preference. After 1 July 2022, when it was revealed that potato was preferred by the slugs, only this vegetable was provided in the diet. The slugs did not feed on the surface of the potato, but gnawed holes equal to their body width in the tuber.

Slug hatching

Soil similar to that in natural habitats of slugs was collected on 20 May 2002 from dacha Polyanka in the suburbs of Tomsk and placed in containers to a depth of 1–2 cm. The first egg mass was recorded on 8 September 2022 in the container that was kept in the refrigerator under low temperatures. Three more egg masses were recorded on 12 September 2022, one in the container from the refrigerator, and two in the containers kept in the laboratory; each egg mass contained from 9 to 14 eggs, and 11 juvenile slugs were hatched on 4 October 2022, but all the remaining eggs perished.

Statistical analysis

R version 4.0.2 (R Core Team 2020) was used for statistical analysis of the slug parameters, and for multiple comparison the nonparametric statistics Kruskal-Wallis rank sum test (kruskal.test) (Kruskal, Wallis 1952) was applied. For evaluation of differences between groups, the Dunn's test (Dunn 1964; Dinno 2017) was used with correction to multiply comparisons of the Benjamini-Hochberg procedure(1995), applicable for independent tests. Linear regression was applied for analysis of slug growth, and data analysis with estimated graphs (Ho et al. 2019) was used to evaluate environmental influence on the growth of slugs growth during the experiment.

Results

In the winter of 2015, a large number of large slugs, dark brown-grey in colour and up to 10 cm length and c. 1 cm in width, had invaded a Novosibirsk vegetable store. These were found on walls and in potatoes, kept in bulk on sandy soil on the floor. People using this store provided information on the long-term occurrence of the slugs in the store. Several specimens were taken for species identification, and instructions were given on how to avoid a further spread of the slugs; today, this species, identified as *Limacus flavus* (Linnaeus, 1758), occurs in low quantity and only in small sizes in this vegetable store. Known as the cellar slug, the yellow slug, or the tawny garden slug, this species is also widespread in Europe (Sysoev, Schileyko 2009; Telebak et al. 2013); although it is known as strictly synantropic (Kappes, Schilthuizen 2014), its bionomy is poorly studied, and it is considered as a resident of meadows in wild nature and damp areas near or in gardens and in houses (such as cellars and bathrooms), and feeds on fungi, decaying matter or vegetables. Similar to most representatives of the family Limacidae, this species attains relatively large sizes c. 10–15 cm in length and 5–6 in gram weight. The occurrence of the *Limacus flavus* (L.) population in an underground vegetable store in Siberia demonstrated phenomenal ability to adapt to tough living conditions in North Asia. The air temperature in winter sometimes decreases up to –35–40˚C, while indoor temperature could be c. 0˚C, the air is infrequently ventilated, light almost lacking, and the only a limited range of vegetables are available to provide nutrition and development. Nevertheless, slugs successfully develop and breed there during a long period of time.

Therefore, *Limacus flavus* (L.) can be considered as a model invertebrate species to study for its potential to produce food protein for human consumption under regional environmental conditions.

Limacus flavus **(Linnaeus, 1758)**

Figs 1 a, b.

Limax flavus Linnaeus, 1758

Limax (Limacus) flavus Linnaeus, 1758

Limacus flavus (Linnaeus, 1758)

Material. **West Siberia, *Russia:*** City of Novosibirsk, Shamshurina str., undersground vegetable store, N55°01', E82°55', March 2022, leg. S.E. Tshernyshev – 10 specimens.

Distribution. Widespread in Europe, from Italy, France and Spain in the south to Austria, Great Britain, Germany, Macedonia, Bulgaria and Ukraine, Caucasus of Russia in the north, introduced into many countries and recorded from North and South Africa and Madagascar, China, Australia and New Zealand, North and South America. This is a first record of the species from West Siberia. (Mienis 2004; Agudo-Padron 2011; Chernyshova 2011; Balashev et al. 2013; Telebak et al. 2013;

Magomedov et al. 2014; Neiber 2017; Castillo 2020; Balashev, Markova 2021; Kotsur 2021)

Figure 1. *Limacus flavus (L.) under investigation: a, upper side, b, under side or pedal side. Scale bar 100 mm.*

Laboratory observations of *Limacus flavus* (L.) life preferences

The following topics were investigated in the laboratory: (1) what kind of vegetables are preferable for a slug diet, (2) what parameters of humidity, temperature and lighting are optimal for slug development, and (3) what size and weight of slugs can be reached under laboratory conditions?

Selection of feeding substrate for consumption by the slugs

The slugs were taken from a population developed on traditional crops (potato, carrot and cabbage) in an underground vegetable store. Only once did the slugs taste the carrot, and cabbage was occasionally tasted; often it remained intact, but the weight of decayed parts in a mucous mass were difficult to measure. Potatoes were clearly the preferred diet, the slugs eating the middle part of the tuber and ignoring peel. Sometimes slugs used food pieces as a place to hide. From July 2022 onwards all slugs received a mono-diet of potato; this did not have a negative effect on slug growth, but food consumption increased (Figure 2). The conversion to a mono-diet was more significant in container no.5 kept in the refrigerator (Figure 3).

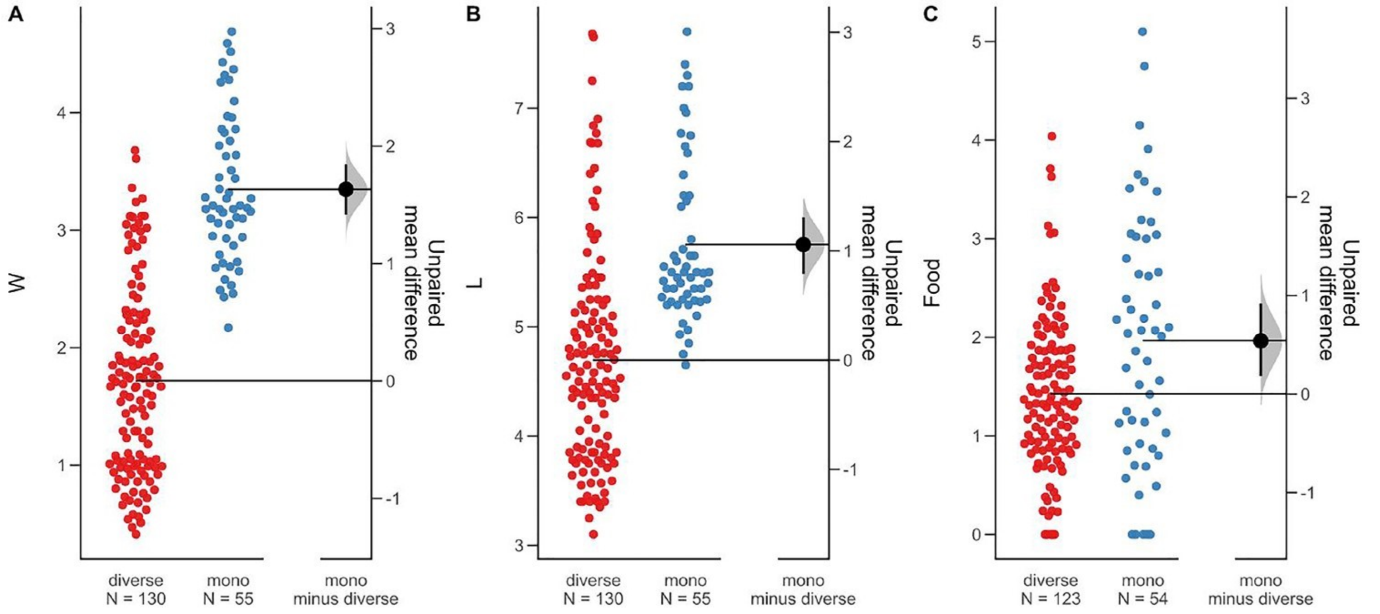

Figure 2. *Alteration of weight (A) and length of body (B) and quantity of eaten potato (C) according to slug diet.*

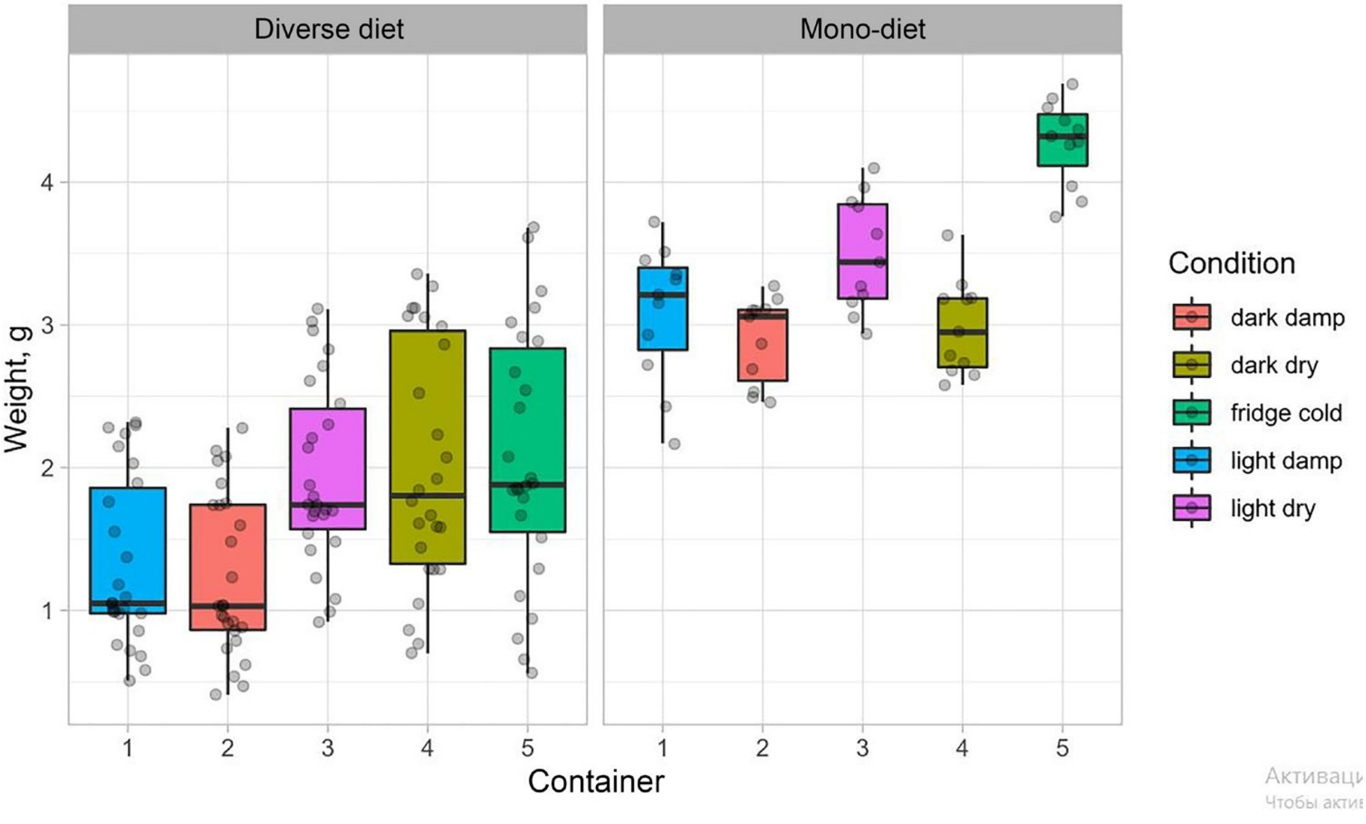

Figure 3. *Changes of slugs weight on diverse diet and mono-diet.*

Optimal conditions for slugs development

Analysis of slug development in containers 1–4 kept at an indoor temperature of 26.1°C and container 5 maintained in a refrigerator at 12.1°C showed significant differences; higher growth was recorded in the latter, but the average quantity of consumed feed under different temperature parameters was equal (Figure 4).

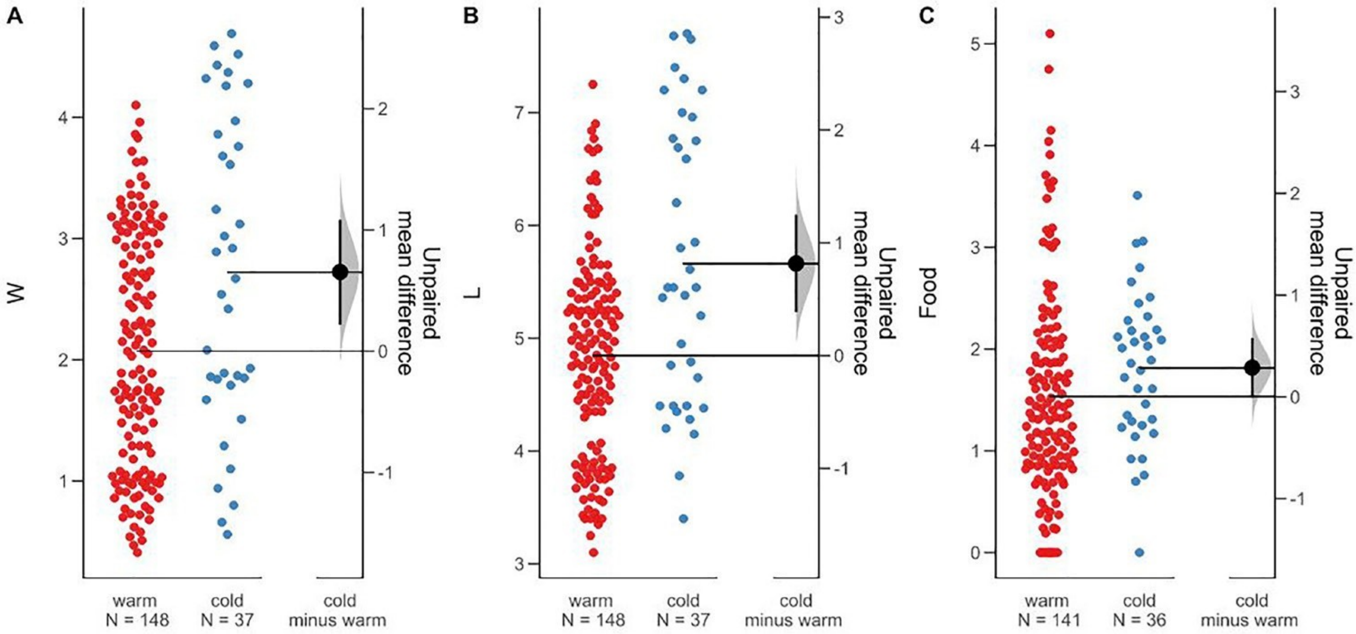

Figure 4. *Alteration of weight (A) and length of body (B) and quantity of consumed food (C) according to environmental temperature.*

During the experiment the humidity in the containers were recorded; the results show only small differences for the different containers (Table 1), but varied for the different months (Figure 5).

Container No.	1	2	3	4	5
Humidity in the container, %	91.0	92.1	92.5	92.5	90.7

Table 1. *Average air humidity inside the containers*

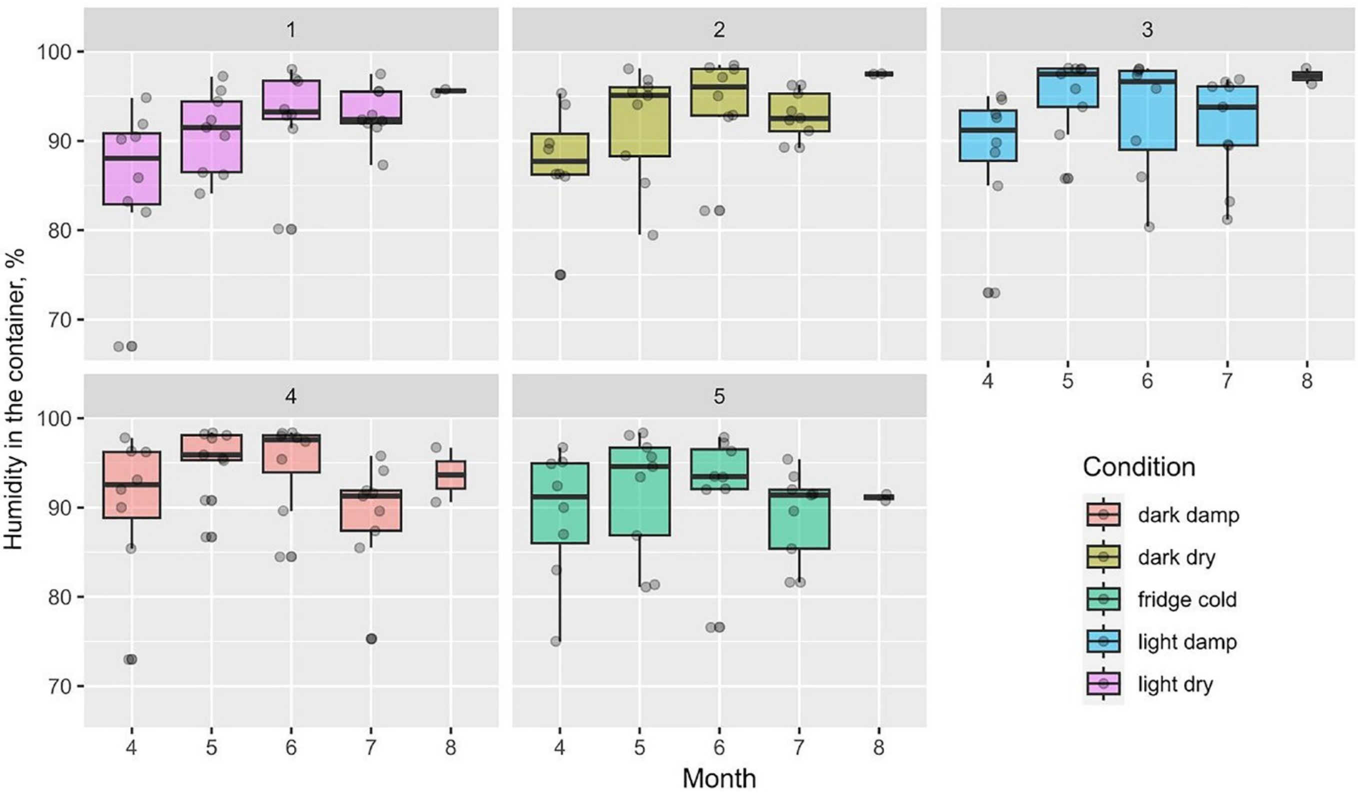

Figure 5. *Air humidity inside containers 1–5 under different conditions of maintenance over the months of observation.*

Changes in slug activity in the containers influenced by different conditions of maintenance were recorded (Pearson's Chi-squared test, df = 8, p-value = 0.017). More actively crawling slugs were in conditions of refrigerator and permanent damping of the container bottom, and less active or inactive were those in the other containers. The most preferable places for the slugs were provided by the cover of container and soil at the bottom of the container. More effective average growth was registered in slugs kept in the refrigerator and lacking light. Considerably more slowly developed slugs were in containers 1 and 2, which lacked dampness in the bottom of the container. The lighting did not impact on slug growth (Figure 6).

Living conditions in the different containers significantly influenced the growth of the slugs (Kruskal-Wallis test, p-value < 0.001; group comparison Dunn's test), as presented in Table 2.

Container	p-value (Weight)	p-value (Length)
3-1	0.0274	0.0014*
3-2	0.0083*	0.0007*
4-1	0.0500	0.0008*
4-2	0.0163*	0.0004*
5-1	0.0051*	0.0001*
5-2	0.0011*	0.0000*
*alpha = 0.05, Reject Ho if p <= alpha/2		

Table 2. *Significance level of differences by weight and length of a slug's body under different conditions of maintenance*

The average weight and length of slugs at the beginning of the experiment in March 2022 was 0.62 gram and 3.42 mm, and at the end of the experiment in August 2022 these parameters were 3.67 gram and 5.76 mm. Regular development of slug sizes during five months of the experiment resulted in an increase in their weight by 5.9 times and length by 1.7 times. Mass relation from length of body is linear and described by the equation: $W = 0.92 \times L - 2.4$, (adjusted R-squared: 0.80, F-statistic: 742.6, p-value: < 0.0001). Body weight increased evenly in all containers, except in container no. 4 which decelerated in July (Table 3). In mid-June a litter of soil was provided on the bottom of all the containers; a comparison of slug development showed that that average weight and length increased, and the quantity of food consumed before and after the soil litter input did not change (Figure 7).

Incrementation of body weight by months in containers 1, 2, 3 and 4 on average was 0.6 gram, and in container 5 – 0.9 gram. Incrementation of body length in containers 1 and 4 was 0.6 mm, in containers 2 and 3 – 0.4 mm, and in container 5 – 0.9 mm. Maximum incrementation of weight and length of slug's body was registered from May to June increased two-three times in some containers after providing a soil substrate to the bottom of the containers. Increases in body parameters in June and July were higher than in the spring months (Table 4) except for those slugs in container 4 which demonstrated a decrease.

Container	**Character**	**Month**					
		3	4	5	6	7	8
1	W, g	0.51	0.82	1.14	2.12	2.98	3.59
	L, mm	3.10	3.63	3.71	4.89	5.40	5.63
2	W, g	0.41	0.74	1.13	1.96	2.85	3.11
	L, mm	3.40	3.77	4.08	4.75	5.14	4.86
3	W, g	0.92	1.40	1.83	2.75	3.41	3.90
	L, mm	3.85	4.70	4.72	5.79	5.43	6.18
4	W, g	0.70	1.20	1.91	3.10	2.94	3.18

	L, mm	3.35	4.24	4.85	6.72	5.73	5.35
5	W, g	0.56	1.23	2.03	3.14	4.20	4.61
	L, mm	3.40	4.27	5.06	6.17	7.05	6.80

Table 3. *Average sizes of slugs under experiment during March – August 2022*

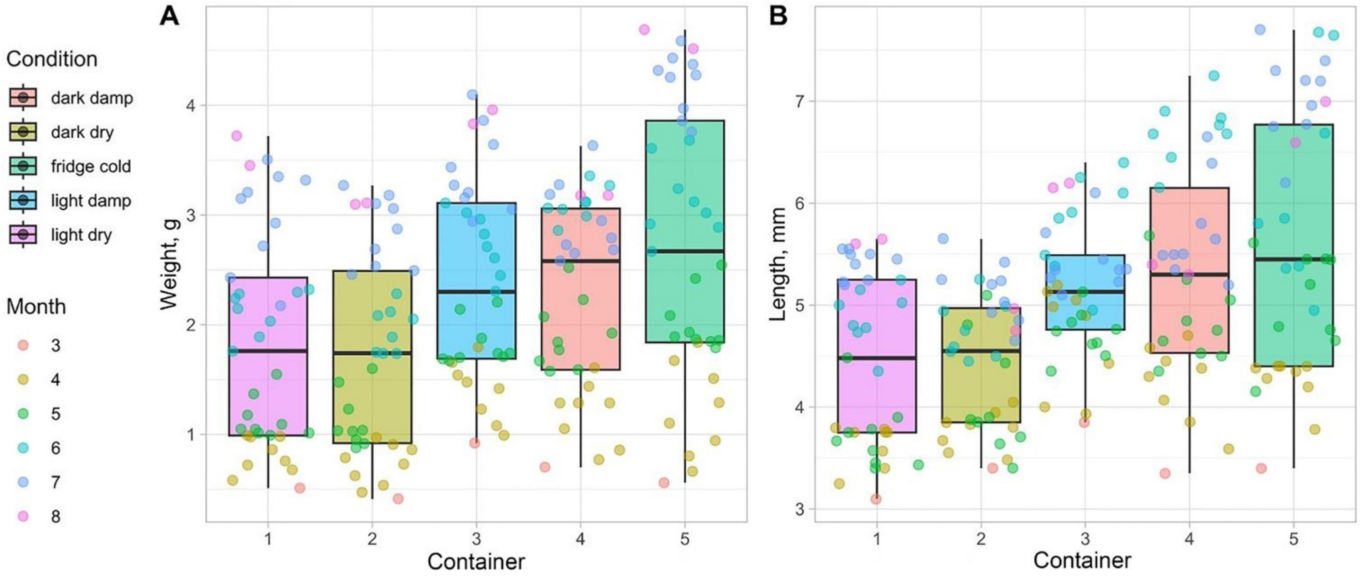

Figure 6. *Average weight (A) and length (B) of slug body under different conditions of maintenance in May-August 2022.*

Month / container No.	1		2		3		4		5	
	W, g	L, mm	W, g	L, mm	W, g	L, mm	W, g	L, mm	W, g	L, mm
3-4	0.31	0.53	0.33	0.37	0.48	0.85	0.50	0.89	0.67	0.87
4-5	0.33	0.08	0.39	0.31	0.43	0.02	0.71	0.61	0.80	0.78
5 -6	0.98	1.17	0.83	0.67	0.92	1.07	1.19	1.87	1.12	1.11
6-7	0.86	0.52	0.89	0.38	0.66	-0.36	-0.16	-0.99	1.06	0.88
7-8	0.61	0.22	0.26	-0.28	0.49	0.74	0.24	-0.38	0.40	-0.26
Mean	0.62	0.58	0.61	0.43	0.62	0.40	0.56	0.59	0.91	0.91

Table 4. *Incrementation of weight and length of slugs body by months*

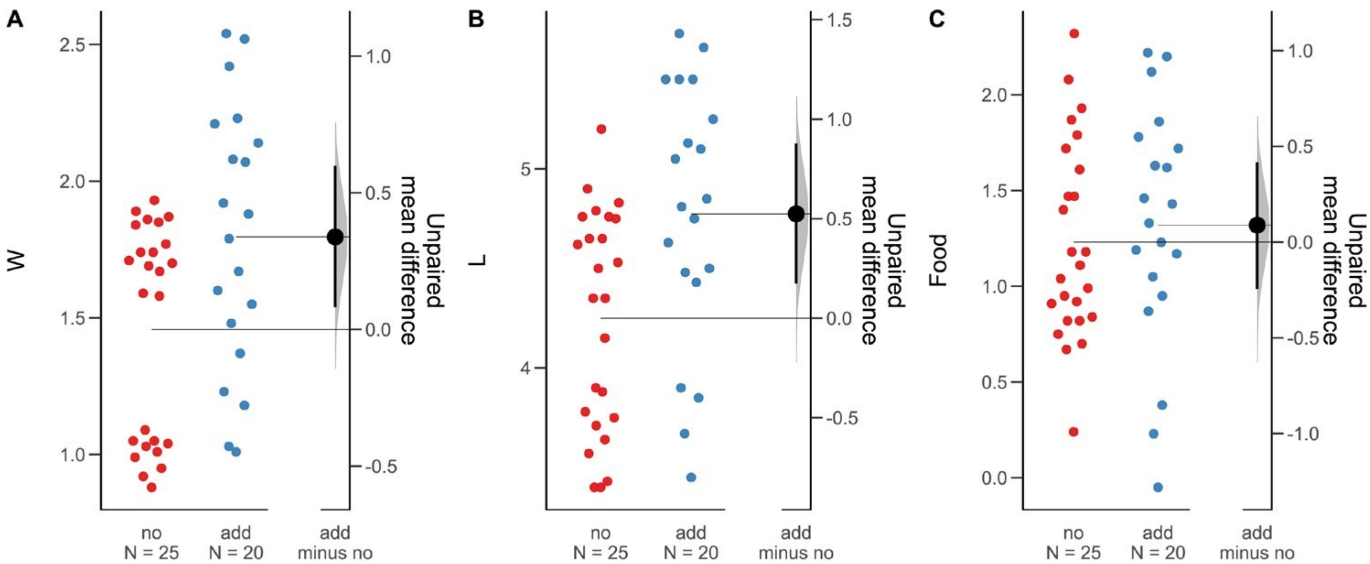

Figure 7. *Alteration of weight (A) and length (B) of slug's body and quantity of food consumed (C) after providing soil substrate on the container bottom during June.*

Conclusions

The present study to breed *Limacus flavus* (L.) under laboratory conditions in Russia follows that by Mohamed and Ali (2013) in Egypt who showed that slug body weight increased from hatching until the seventh month and reached an average maximum weight of 3.51 ± 0.86 gram. In our experiment, slugs reached 3.67 gram on average, but in 5 months. Probably, slug growth was more effective in our experiment which provided a mono-diet of potato tuber, kept under cool and damp environmental conditions (12°C, c. 90% humidity & in darkness) to each slug maintained in a 0.5 litre container.

The results show that *Limacus flavus* (L.)is an undemanding species in terms of its feeding substrate and environmental conditions. Juvenile and adult slugs prefer potato tubers and successfully developed on a mono-diet. In the course of our experiment, the slug's growth was more successful at 12°C than at 26˚C and in darkness. Humidity was maintained at c. 90%. Similar conditions are typical of underground vegetable stores or other storage facilities in Siberia, being very effective under frost climate conditions of a long winter season. Maintenance and service of underground storage are not expensive, since no permanent warmth or illumination are needed. Raising slugs under these storage conditions could be considered as a prospective business for northern regions undergoing long-term cold climatic conditions.

The prospective use of slug biomass *Limacus flavus* (L.) in a practical economic manner requires further investigation, since its muscular tissue is rich in animal protein, a product that is necessary for human food and animal feed. An inexpensive method of employing a slug's biochemical content would open up new methods in farming for animal protein production.

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