

# SHOW

A m s t e r d a m 2 0 2 3

Simultaneously **H**ermaphroditic **O**rganisms **W**orkshop

30<sup>th</sup> - 31<sup>st</sup> March 2023

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## 1. Program

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March 29th

18:00 Pre-meeting drink / dinner

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March 30th

09:00 Opening

09:15 **Discussion 1 Primer:** “How and why to use our SHOW expertise network to tackle big questions” by Steven Ramm, Joris Koene and Yumi Nakadera

09:30 **Discussion 2 Primer:** “How and why hermaphrodites affect each other’s reproduction” by Cristina Lorenzi, Chiara Benvenuto and Cynthia Norton

09:45 Presentation: **Christina Maciel** (Online) - Protandric simultaneous hermaphroditism in *Lysmata ankeri* – reproductive behaviour, implication for aquaculture and evolutionary questions

10:30 Break

11:00 Presentation: **Steven Ramm** - Heatwave impacts on flatworm reproduction: are they sex function-specific?

11:45 Presentation: **Mario Gordillo** - Thermoresistance in the hermaphroditic Cuban painted snail *Polymita muscarum* (Gastropoda: Cepolidae): implications in its conservation

12:30 Lunch at EMW

13:30 **Discussion 1 Introduction**

13:45 Presentation: **Lara Cifola & Julia Piza** (Online) - Intraspecific trait variation in the land snail *Rumina decollata* & Distribution and origin of introduced populations of the land snail *Rumina decollata* (Linnaeus 1758) in Argentina

14:30 Break

15:00 **Discussion 1 + Recap/Summary/Future plans**

16:30 **Discussion 2 Introduction**

16:45 Lab tour (optional)

17:30 Walk to boat departure

18:00 Boat dinner

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March 31st

- 09:00 Presentation: **John Hutchinson** - The diversity of mating behaviours in *Lehmannia* slugs
- 09:45 Presentation: **Heike Reise** - Does the penial gland secretion influence egg production in *Deroceras* slugs?
- 10:30 Break + Poster presentation
- 10:45 **Discussion 2 + Recap/Summary/Future plans**
- 12:15 Lunch at EMW
- 13:15 Presentation: **Elisa Jeanne** - As search for sexual cues in hermaphrodites
- 14:00 Presentation: **Yumi Nakadera** - Identifying seminal fluid proteins via transcriptomics in a simultaneously hermaphroditic snail species
- 14:45 Closing: Reflection/Future plans
- 15:00 End!
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## 2. General information

### Venue

The auditorium for our workshop is in the O|2 building on the VU campus (**De Boelelaan 1108**, 1081 HZ Amsterdam, <https://goo.gl/maps/WLQTbB13taCnJUNHA>). The campus can be reached easily by public transport (e.g. tram 5, 24 and 24, metro 50 and 51) and is a 10-minute walk from Station Zuid. See <https://vu.nl/en/about-vu/more-about/o2-lab-building>.



### Pre-meeting drinks & dinner

We will have a pre-meeting drinks and dinner at De Ysbreeker at **Weesperzijde 23** (<https://www.deysbreeker.nl/>, <https://goo.gl/maps/ounUK3dBzEmvcTCo8>). We are looking forward to welcoming you there from 18:00 onwards!

### Lunch

During SHOW, we will have lunch at the room next to the auditorium, called Eat Meet Work (EMW). We will prepare sandwiches by ourselves. Hope you like them!

### Conference (Boat) dinner

We arranged a boat dinner (<https://smidtje.nl/>, <https://goo.gl/maps/Xq2yCwnxZAFxuKLy7>)! At the end of 30<sup>th</sup> March, we will walk together to the dock, located at **Locatellikade 1**. It takes ca. 20 min by foot from campus. Once everyone is on board, we will have a canal cruise, drinks and dinner! At the end of dinner, we will be brought back to the same location.

### Wifi

If you have an eduroam account, it should work seamlessly at VU. Alternatively, we will also have wifi codes available that will work on campus.

### Zoom (for online participants)

SHOW 2023

<https://vu-live.zoom.us/j/92704453350?pwd=cWFMN2pDNXZNaxJUR0MyQ2hiU1JuUT09>

Meeting ID: 927 0445 3350

Passcode: 873478

## **Fee**

In order to make SHOW2023 possible we will ask in-person participants a workshop fee of 75 Euros. The preferred mode of payment will be via Paypal, for which you will receive a link during the meeting. A smaller financial contribution by the online participants will be appreciated. Alternative payment methods via Tikkie (for Dutch participants) or cash (please bring exact change!) are possible.

## **Emergency contact**

Yumi Nakadera ([y.nakadera@vu.nl](mailto:y.nakadera@vu.nl), +31 6 83488604) or 112 for urgent medical or police assistance!

### 3. Abstracts of oral presentations

#### **Protandric simultaneous hermaphroditism in *Lysmata ankeri* – reproductive behaviour, implication for aquaculture and evolutionary questions**

Cristiana Maciel<sup>a</sup>, Jhonatan Costa<sup>a</sup>, Fernando Abrunhosa<sup>a</sup>, Murilo Maciel<sup>a</sup>, Chiara Benvenuto<sup>b</sup>

<sup>a</sup> Instituto de Estudos Costeiros, Universidade Federal do Pará UFPA, Brazil

<sup>b</sup> School of Science Engineering & Environment, University of Salford, UK

*Lysmata ankeri* is a commercially important species in the aquarium trade, given its bright colourful aspect and its role as a cleaner shrimp (removing parasites from fish) and pest control agent. However, shrimp are collected from the field, with ethical and conservation implications. This species is a protandric simultaneous hermaphrodite (i.e., individuals change sex from male to hermaphrodite), making it a very interesting study system for sexual system evolution.

Experiments were conducted in LAQUA, the aquaculture lab at the Federal University of Pará, Brazil, to develop a protocol of larval management for sustainable aquaculture. Animals kept in groups in the lab showed high level of aggression when hermaphrodites moulted and became receptive. When individuals (male + hermaphrodite, or hermaphrodite + hermaphrodite) were individually coupled, the highest success rate was obtained when pairing two hermaphrodites, of which the largest would act consistently as female. Males were often cannibalized by their partners as soon as they moulted, unless they were recognised as mates (more rarely). In other species of the same genus, reciprocal exchange of eggs has been reported, but here instead fixed pairs with fixed roles appear to be the norm. The reproductive cycle has been fully defined but a few questions still remain to be answered. It is not clear if the male-acting individual restrict its growth or change its moult frequency. Moreover, to optimize the protocol, the research group is still working on the most efficient way to pair individuals. This applied approach can also be used to answer evolutionary questions on sex allocation and the evolution of this unusual sexual system (so far described only in the genus *Lysmata*).

## Heatwave impacts on flatworm reproduction: are they sex function-specific?

Steven A. Ramm<sup>1,2</sup>, Sarthak Grover<sup>1,3</sup>, Suhaas Sehgal<sup>1,3</sup>, Sonja Schindler<sup>1</sup>, Athina Giannakara<sup>1</sup>

<sup>1</sup> Department of Evolutionary Biology, Bielefeld University, Germany

<sup>2</sup> UMR 6553 ECOBIO, Université de Rennes, France

<sup>3</sup> Indian Institute of Science Education and Research (IISER) Mohali, India

How animals cope with systematically changing and more variable and extreme environmental conditions is a key concern under climate change. A major question is how climate change will impact fertility, and the knock-on consequences this will have for population viability and biodiversity, as well as whether male and female reproduction is similarly impacted. Simultaneously hermaphroditic animals may be especially useful models to help resolve this debate, because they allow for impacts on gametogenesis to be assessed as a pure comparison of spermatogenesis vs. oogenesis in the same individual, unconfounded by additional factors that might influence these processes in separate-sexed taxa such as sexual dimorphism and sex-specific life histories and behaviour. We therefore performed a manipulative experiment subjecting individuals of the simultaneously hermaphroditic flatworm *Macrostomum lignano* to heatwaves of varying frequency and duration. We report results from sex-specific assays of their resulting fertility at two timepoints following the heatwaves, to assess both their immediate reproductive impact and the scope for recovery.



## **Thermoresistance in the hermaphroditic Cuban painted snail *Polymita muscarum* (Gastropoda: Cepolidae): implications in its conservation**

Mario Juan Gordillo-Pérez<sup>1</sup>, Martijn Heleven<sup>1</sup>, Bernardo Reyes-Tur<sup>2</sup>, Karen Smeets<sup>1</sup>, Thierry Backeljau<sup>3</sup>, Natalie Beenaerts<sup>1</sup>

1. Centre for Environmental Sciences, Hasselt University, Belgium
2. Universidad de Oriente, Cuba
3. Royal Belgian Institute of Natural Sciences, Belgium

Terrestrial molluscs are considered very susceptible to climate change. The limited vagility or low dispersal capability of these animals could be a problem for dispersion and for colonizing new habitats with favourable environmental conditions. The genus *Polymita* include six species of hermaphroditic tree snail inhabiting the eastern Cuba characterized for the presence of an accessory copulatory organ “dart apparatus” associated to a pair of gland and a remarkable polymorphism of the shells with vibrant colours. All the species are considered in a critical status of conservation taking in account the diminishing of suitable habitats areas due to climate change. We studied the short term effect of the air temperature on *P. muscarum* in laboratory conditions evaluating histological and molecular traits. The digestive gland and foot are structures involved in the thermoregulation of the species showing an increasing in the index calcium/digestive cells and tubule sizes and hypertrophy and hyperplasia in the foot mucocytes. Heat shock protein (Hsp 70) was upregulated when animals are treated with low and median temperatures, not exceeding 40° C. In individuals treated with 43° C is already observed a down regulation in the chaperon expression and tissue necrosis. Values dispersal even in the control group suggest an allostatic metabolism of thermoregulation in the species. In general, *P. muscarum* is less resistant to the warming than other tropical species in Mediterranean areas with a lethal temperature of 44° C after 5 hours of exposition. The predicted models for temperature increasing in Cuba already suggested an extinction scenario for the genus. The results are discussed taking in to account data related to mating duration and shell color luminance in *Polymita*.

## **Intraspecific trait variation in the land snail *Rumina decollata***

Lara Cifola<sup>1</sup>, Julia Pizá<sup>1</sup>, Nicolás Bonel<sup>1</sup>

<sup>1</sup> Genética y Ecología Evolutiva, CERZOS, CONICET-UNS, Camino La Carrindanga Km 7, Bahía Blanca, Argentina

Gastropods make up a large part of the animal biodiversity of terrestrial ecosystems. *Rumina decollata* is a hermaphrodite land snail native to the Mediterranean area with a current extensive distribution in Asia and America mainly due to anthropogenic introductions. In Argentina, it is currently inhabiting from Patagonia to the north of our country where it is established in regions with extreme climatic characteristics (aridity and wide range of temperatures). Environmental conditions exert a strong selection pressure on land snails, significantly modifying life history traits and, consequently, their ability to colonize new habitats. We seek to understand how *R. decollata* manages to establish itself in different ecosystems. The first step was to explore phenotypic variation and its association with environmental rearing conditions, reproductive strategies, and population origin. We compared fecundity rate, hatching time, juvenile survival, and individual growth between 1) wild-caught and first-generation lab-reared snails, 2) selfing and outcrossing individuals, and 3) wild-caught individuals from native and non-native populations. Preliminary results indicated contrasting trait variability within each group. The next step is to obtain more generations of snails raised in common garden laboratory conditions to estimate heritability and to perform quantitative genetic analyses. Understanding what processes are driving trait variation is essential to determine the potential of this cosmopolitan species to increase its distribution ranges.

## Distribution and origin of introduced populations of the land snail *Rumina decollata* (Linnaeus 1758) in Argentina

Guerrero Spagnuoli, J.<sup>1,\*</sup>, Dop, N.S.<sup>1</sup>, Pizá, J.<sup>1</sup>

<sup>1</sup> *Genética y Ecología Evolutiva, CERZOS, CONICET-UNS, Camino La Carrindanga Km 7, Bahía Blanca, Argentina*

The land snail *Rumina decollata* is native to the Mediterranean region but has a worldwide distribution due to accidental or voluntary introductions in several countries of Asia, Africa and America. Its biological characteristics (facultative self-fertilization, omnivory, and xeroresistance) favoured the establishment and colonization in new environments. In Argentina, it was reported in 1988 in Buenos Aires and had expanded its distribution since then. It is considered an invasive species and crop pest in several countries. Previous works postulated that introduced populations belong to the same phylogenetic group called “Clade A” and associated it with the dark-colored morph (being the native populations composed of both, dark and light morphs). The aim of our study was to update the distribution of *R. decollata* in Argentina and to investigate the origins of Argentine populations through a comparative molecular and morphological analysis with those of the native range of distribution. We performed a Citizen Science project; we posted a survey on social media to get information on distribution, habitat, food preferences, and damage caused. As a result, we got 700 responses revealing that this species inhabits urban and peri-urban areas from a broad area of Argentina (16 provinces from Misiones to Patagonia and from the pre-cordillera to the Atlantic coast). We also received living snails from 20 populations in 11 Argentine provinces for genetic analyses. We extracted total genomic DNA and amplified and sequence the mitochondrial Cytochrome oxidase I gene. Subsequently, a maximum likelihood phylogenetic tree and a haplotype network were constructed. We determined that there is a predominant haplotype included within the Clade A, identical to the one found in populations from Spain and Portugal. Only one of the studied populations presented differences in the haplotype sequence. Besides, the studied individuals presented variable coloration, so we could not detect a clear coloration pattern and rejected the hypothesis of the relationship of the invasive populations with the dark morph. Our data confirm that *Rumina decollata* has spread fast in Argentina during the last thirty years and it is likely that it colonizes natural environments in a near future. Studying life history traits and the environmental and biological factors influencing them is crucial to determine its dispersal capacity.

## The diversity of mating behaviours in *Lehmannia* slugs

John M.C. Hutchinson

Senckenberg Museum of Natural History Görlitz, Germany

*Lehmannia* is a genus of terrestrial slugs in which ejaculate is swapped reciprocally between everted penes. I will show videos of the mating behaviour of five species of *Lehmannia* s.s. and make comparisons with species of the closely related genus *Ambigolimax*. In all five species, courtship consists of one individual following the other closely, the leader then doubles back, they both nibble the partner's right flank as they circle around each other, and then soon each protrudes part of the penis. Once the partially protruded penes touch each other, the rest of each penis everts rapidly. In four of the species, the penes curve round and deposit sperm on the basal part of the partner's penis. In the fifth species, *L. carpatica*, the penis is a long tube, which spectacularly wraps around the partner's penis to form a double helix as it everts further, with the ejaculate swapped between the tips. In *L. rupicola* and *L. macroflagellata*, additionally a long penial flagellum everts over the partner's body; at least in *L. macroflagellata* this deposits a secretion, as in *Deroceras* slugs. The flagellum in *L. marginata* appears too short reliably to reach the partner. Whereas in four of the species ejaculates are swapped and penes retracted within 5–40 s of the start of the rapid eversion, in *L. szigethae* the everted penes may remain in contact for over 53 min. Uniquely in *L. macroflagellata*, after penis retraction the partners remain in contact, circling around each other in a dance continuing for hours.

## Does the penial gland secretion influence egg production in *Deroceras* slugs?

Heike Reise, Esther Carlitz, John M.C. Hutchinson

Senckenberg Museum of Natural History Görlitz, Am Museum 1, 02826 Görlitz, Germany

Mating in the terrestrial slug genus *Deroceras* involves mutual sperm exchange between the partners' everted and entwined penes. Given the opportunities, individuals mate repeatedly, and they remate long before they run out of allosperm. Penis morphology varies remarkably within the genus, but most of the 100+ species have a more or less elaborate digitiform penial gland, which is everted during copulation, transferring a secretion onto the partner's external body surface. In the common pest species *D. invadens*, this happens well after sperm exchange, implying that the function of the secretion might be to manipulate the partner to the donor's advantage. In a series of experiments we have investigated several hypotheses about what trait could be affected. Here, we present preliminary results from experiments investigating a potential influence of the gland secretion on egg laying. Counter to our expectations, individuals that had received the gland secretion laid lighter eggs than the control group, but we did not find a significant difference in overall egg numbers, clutch size, or time to the start of egg laying. We are currently replicating the experiment and adding a further control group.

## As search for sexual cues in hermaphrodites

Elise Jeanne, Estelle Le Tessier, Franco Robles-Guerrero, Maria-Cristina Lorenzi

Experimental and Comparative Ethology Laboratory (LEEC) UR 4443, University Sorbonne Paris North

Hermaphrodites adjust their reproductive resources in response to the variations of local mating group size, as predicted by Charnov's theory. However, little is known about how they assess the number of conspecifics or their prevalent sex allocation. We addressed this question in the simultaneously hermaphroditic worms *Ophryotrocha diadema*, which adjust their sex allocation based on mating opportunities; in large groups, they invest relatively more resources in male traits, whereas, when isolated or paired, they invest relatively more in female traits. These worms perceive mating opportunities via water-borne chemical cues, but it is unclear what information they use to adjust their sex allocation. To test whether there is a difference in the chemical cues produced by male-biased vs female-biased worms, we exposed pairs of sexually mature worms or isolated larvae to either control water or homospecific conditioned waters where mature conspecifics were kept either in isolation (so that they biased their sex allocation towards the female function) or in groups (male-biased sex allocation). We measured the investment of experimental worms in the male (i.e., motility, larval length of the protandrous phase) and the female (i.e., production of eggs or propensity to lay) functions. Surprisingly, mature worms allocated their reproductive resources significantly differently depending on whether they were exposed to "male" or "female" cues, but not consistently among replicates. As for the isolated larvae, there was no difference in their development depending on the cues they were exposed to. However, when they were sexually mature and paired with mature partners, they were more prone to lay first if they were exposed to female-biased cues during early development. Overall, although the differences between worms exposed to prevailing "male" vs "female" cues were often subtle and not always consistent among replicates, they provide a chance to discuss what factors to take into account when investigating sexual cues/signals in hermaphrodites and serve as an initial trial to identify other relevant factors for future experiments.

## Identifying seminal fluid proteins via transcriptomics in a simultaneously hermaphroditic snail species

Yumi Nakadera, Miao Chen, Joris M. Koene

Ecology and evolution, A-LIFE, Vrije Universiteit Amsterdam

Seminal fluid proteins (SFPs) play significant roles in reproduction, and their genes are often observed to evolve rapidly. The current status of SFP research is not able to fully resolve the patterns of SFP evolution, as the prevalent model systems are almost exclusively separate-sexed species. We consider the great pond snail *Lymnaea stagnalis* as a fruitful model system to investigate SFP evolution, since they are simultaneous hermaphrodites and their reproductive biology is well documented. To establish a hermaphroditic model system that allows for in-depth SFP studies, we screened transcriptomes of *L. stagnalis* for SFPs. First, in order to obtain a male accessory gland-specific transcriptome we compared the transcriptome of a male and female reproductive gland: the prostate, which produces the majority of SFPs, and albumen gland, which produces the material the eggs are provisioned with. Second, we examined differential expression in prostate gland transcriptomes of snails reared in different social conditions – isolation, pairs and quintets. Based on a previous study, we predict that SFP genes are up-regulated in paired and grouped individuals, since these snails have mating and thus transferred SFPs to their partners. Using this approach, we found candidate SFP genes highly expressed in prostate glands as well as SFPs that are differentially expressed between isolated and paired and/or grouped treatments. We will discuss the criteria for candidate SFP genes and their implications.

#### 4. Abstracts of poster presentations

##### **Dart apparatus use and transfer of its products from accessory glandular structures: potential delivery behaviours and in vitro effects in the Cuban painted snail**

Bernardo Reyes-Tur<sup>1</sup>, Joris M. Koene<sup>2</sup>, Mario J. Gordillo-Pérez<sup>3</sup>, Doyle Montoya-Tamayo<sup>1</sup> & Ianna Y. Benavides-Oro<sup>1</sup>

<sup>1</sup>Department of Biology & Geography, Faculty of Natural & Exact Sciences, Universidad de Oriente, Santiago de Cuba (Cuba)

<sup>2</sup>Ecology & Evolution, Amsterdam Institute for Life and Environment (A-LIFE), Faculty of Science, Vrije Universiteit, Amsterdam (The Netherlands)

<sup>3</sup>Centre for Environmental Sciences, Hasselt University (Belgium)

Hermaphroditic snails display a remarkable range of complex reproductive morphologies and behaviours, many of which are related to the transfer of accessory-gland products (ACPs). Such socially transferred materials generally affect the sperm recipient by enhancing the sperm donor's fertilization success. When this is in disagreement with the recipient's reproductive optimum, a conflict of interest ensues. The love dart of snails is arguably one of the best examples of how such conflict can escalate evolutionarily. This sharp, calcareous device is stabbed through the partner's body wall to inject ACPs that affect the recipient's physiology. Here, we redescribe how such darts are used in hermaphroditic Cuban painted snails, *Polymita picta*; previous reports only described repeated wiping, rubbing and stabbing but did not include analysis of dart apparatus interactions (*i.e.* 'dart wrestling'). In addition, we describe the functional morphology of the dart apparatus based on results of *in vitro* effects of ACPs on the reproductive system. The dart apparatus consists of three accessory glandular structures (*i.e.* bilobed gland, atrial sac and pedunculated gland). The low frequency of stabbing, as well as the presence of cylindrical epithelium, a ribbed surface alongside abundant keratinized sharp-ended structures, and mucus in the everted atrial sac, suggest a key role for wiping and rubbing for transfer of ACPs via the body wall of the partner. Physiologically, atrial sac and bilobed gland extracts induce a fast reaction with a longer-lasting shortening effect on the bursa tract. However, the pedunculated gland extract produces a briefer-lasting and small shortening effect on the bursa tract and a delayed bursa elongation. Our findings suggest that the peculiar mating interactions and morphology of these snails offer a unique potential for avoiding the ACPs' effects via 'dart wrestling'. With the upcoming genome we hope to elucidate the identity of some of the ACPs and contribute to conserving this amazing species.



## Does sex affect long-term memory formation? - A case study in a simultaneous hermaphroditic snail species

Yumi Nakadera<sup>1</sup>, Beatriz Álvarez Díaz<sup>2</sup>, Joris M. Koene<sup>1</sup>

1. Ecology and Evolution, A-LIFE, Vrije Universiteit Amsterdam, the Netherlands

2. Department of Psychology, Universidad de Oviedo, Spain

Mating has been shown to enhance long-term memory formation in female *Drosophila melanogaster*, via Seminal Fluid Proteins (SFPs). However, it remains to be tested whether this effect is universal and sex-specific. Here, we tested if mating enhances long-term memory formation in a pond snail species, *Lymnaea stagnalis*. This simultaneous hermaphrodite is a well-established model species for operant conditioning of air-breathing behaviour. In this experiment, we let the snails copulate, and subsequently operant-conditioned the male- and female-mated individuals to test for any difference in long-term memory formation. Compared to the non-copulating control snails, we did not detect any difference in long-term memory formation in male- or female-mated snails. These findings indicate that the effect of mating, probably mediated by SFPs, on memory formation is not universal, and inspires further investigation to reveal the relationship between copulation and memory formation.

## **Ginger snail project – revealing the inheritance mode of shell colour polymorphism in *Lymnaea stagnalis***

Yumi Nakadera<sup>1</sup>, Matthijs Ledder<sup>1</sup>, Alexandra, Staikou<sup>2</sup>, Joris M. Koene<sup>1</sup>

1. Ecology and Evolution, A-LIFE, Vrije Universiteit Amsterdam, the Netherlands
2. Department of Zoology, School of Biology, Aristotle University of Thessaloniki

Snail shell colour polymorphism is a classic study system for evolutionary genetics. However, the genetic mechanism of determining shell morphs remains to be fully elucidated. Here we propose a new model system to reveal the genomics of shell polymorphism. Recently, our research group discovered a unique shell colour polymorphism in the great pond snail *Lymnaea stagnalis*. Part of the snails from a Greek population show a distinct red shell colour (nicknamed as ginger snail), while the shell colour of this species is usually beige. In order to evaluate if this shell colour polymorphism is useful for genomic investigation, we compared the inheritance mode of this shell colour as well as several life history traits (e.g., reproduction, behaviour) between ginger and wild type snails that were all offspring of the Greek parent population. The life history traits of ginger snails are very similar to wild types, and the mode of inheritance of the ginger phenotype seems to fit simple, single locus Mendelian inheritance. Therefore, we believe that this new variant of *L. stagnalis* provides a promising opportunity for further exploration of the genetic basis of shell polymorphism.

## Hermaphrodite anatomy: a *Lymnaea* anatomy lesson for the future

Yumi Nakadera, Wouter van der Vegt, Elloha Tajzai, Sander van Iersel<sup>1</sup>, Tim Visser<sup>1</sup>, Mieke Roth<sup>2</sup> & Joris M. Koene

Amsterdam Institute for Life and Environment (A-LIFE), Section Ecology & Evolution, Faculty of Science, Vrije Universiteit Amsterdam, The Netherlands

<sup>1</sup> <https://www.jesterandwylde.com>

<sup>2</sup> <https://www.miekeroth.com>

Anatomy lessons are an essential part of life science education and via this intensive form of teaching, students have learned, for centuries, to interpret and understand how humans, animals and plants function. Such interactive education is relevant for a wide range of disciplines and levels, not only for dissecting organisms but also for species identification, biodiversity research and general three-dimensional (3D) understanding of morphology, function and structure. However, hands-on lab teaching is under pressure due to declining teacher availability, growing student numbers and pandemics. That is why we are developing a sustainable and innovative digital solution to relieve this pressure. We intentionally focus on modern, student-oriented educational techniques, using the most contemporary video and animation practices. With the pilot project (SLAB) we have transformed a first classic biological dissection practical - using our favourite hermaphrodite *Lymnaea stagnalis* - into an instructional video. Students can complete this independently in its entirety, or flexibly by working on subsections via their online learning environment. To follow this up, we now aim to enhance this educational innovation by implementing the latest 3D illustration, animation, interaction and printing techniques. We also invite experts on other species to join in this effort so that we create a unique, uniform and high-quality teaching collection that will benefit students, teachers and scientists. We expect this modernisation and digitisation to further enrich and deepen learning experiences, and thereby support the understanding of the material more effectively.

## **Snail Sex and the City: On the Effects of Urbanization on Morphological Traits Involved in Sexual Selection in the Grove Snail (*Cepaea nemoralis*)**

Brendan den Duijn<sup>1</sup>, Geert Stevens<sup>1</sup>, Menno Schilthuis<sup>1,2</sup>

1. Institute of Biology Leiden, Leiden University, Leiden, the Netherlands
2. Naturalis Biodiversity Center, Leiden, the Netherlands

Urbanization is ever increasing in our world, with this comes a change in both biotic and abiotic factors. The effects of these changes have been somewhat studied in the context of natural selection, however they have hardly even been studied in context to sexual selection. In this study we studied the effect of urbanization on morphological traits of the male reproductive system in *Cepaea nemoralis*. For this, the urban and non-urban populations of Amsterdam and Leiden were collected and parts of the reproductive systems (mainly the male parts) were measured and compared. Most traits did not show any difference between the populations, however there were some interesting results that sparked interest and more work is currently being performed to better gain an insight on how urbanization might influence sexual selection.

## 5. List of participants

First name	Last name	Affiliation	Email address
Chiara	Benvenuto	University of Salford	c.benvenuto@salford.ac.uk
Vera	Bökenhans	Instituto de Biología de Organismos Marinos-IBIOMAR, LARBIM - CCT CENPAT (CONICET)	verebok@gmail.com
Nico	Bonel	Genetica y Ecología Evolutiva, CERZOS, CONICET-UNS	nicobonel@gmail.com
Lara	Cifola	Genetica y Ecología Evolutiva, CERZOS, CONICET - UNS	laracifola99@gmail.com
Lynda	Delph	Indiana University	ldelph@indiana.edu
Brendan	den Duijn	Leiden University/Naturalis	brendandenduijn@hotmail.com
Athina	Giannakara	Bielefeld University	athina.giannakara@uni-bielefeld.de
Mario Juan	Gordillo Pérez	Centre of Environmental Sciences, Hasselt University	mariojg755@gmail.com
Sarthak	Grover	Indian Institute of Science Education and Research, Mohali	Sarthakgrover@gmail.com
Julian	Guerrero Spagnuoli		juliangfull@gmail.com
John	Hutchinson	Senckenberg Museum of Natural History Görlitz	majmch@googlemail.com
Elise	Jeanne	LEEC	eli4.jeanne@gmail.com
Joris	Koene	Vrije Universiteit Amsterdam, the Netherlands	joris.koene@vu.nl
Curtis	Lively	Indiana University	clively@indiana.edu
Cristina	Lorenzi	LEEC-Laboratoire d'Ethologie Expérimentale et Comparée, Université Sorbonne Paris Nord	lorenzi@univ-paris13.fr
Cristiana	Maciel	Universidade Federal do Pará	macielufpa@gmail.com
Murilo	Maciel	Professor Universidade Federal do Pará	cmtmaciel@ufpa.br
Yumi	Nakadera	Vrije Universiteit Amsterdam, the Netherlands	y.nakadera@vu.nl
Cindy	Norton	St. Catherine University	cgnorton@stkate.edu
Julia	Piza	Group Genetic and Evolutionary Ecology- Cerzos (Conicet). Argentina	julipiza@gmail.com
Pooja	Radhakrishnan	University Sorbonne Paris Nord	pooja.rk93@gmail.com
Steve	Ramm	Université de Rennes, France	steven.ramm@univ-rennes.fr
Heike	Reise	Senckenberg Museum of Natural History Görlitz	heike.reise@senckenberg.de
Takumi	Saito	Masaryk University, Brno, Czech Republic	saito.zef@gmail.com
Santhosh	Santhosh	Department of Environmental Sciences, University of Basel.	santhosh.s@unibas.ch
Alexandra	Staikou	Department of Zoology, School of Biology, Aristotle University of Thessaloniki	astaikou@bio.auth.gr