



XIII INTERNATIONAL
SYMPOSIUM ON LITTORINID
BIOLOGY AND EVOLUTION

XIII ISOLBE, 24-27th July 2023, Vila do Conde

Abstract book

Dear XIII ISOLBE 2023 participant,

We are pleased to welcome you to the 13th edition of ISOLBE - the International Symposium on Littorinid Biology and Evolution, hosted at CIBIO – Research Centre in Biodiversity and Genetic Resources, University of Porto, Portugal.

During the four days of conference, we will discuss recent scientific findings of evolutionary significance within a highly polymorphic group of organisms with remarkable variation in life history traits.

Future promising avenues of research using littorinids as models to understand evolutionary process driving biodiversity, together with state-of-the-art tools to address them will be discussed. Altogether, the XIII ISOLBE edition aims to contribute to reinforce the

recognition of Littorinid species as key models to understand the forces influencing evolution.

The informal and inspiring scientific atmosphere of ISOLBE will enable researchers and students from different disciplines but with a common interest in Littorinids to present their work in a series of talks, as well as opportunities for group discussions/brainstorms on participants-selected topics of interest.

We hope that you learn and enjoy your time in this corner of Europe!

The Organizing Committee

Organizing Committee

Alfonso Balmori de la Puente

Isabel Martínez Monreal

João Carvalho

Juan Galindo

Maria Magalhães

Pedro Andrade

Pierre Barry

Ricardo Jorge Lopes

Rui Faria

Scientific Committee

Anja Westram, Institute of Science and Technology,
Austria and Nord University, Norway

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Emilio Rolán-Alvarez, Universidade de Vigo, Spain

Gray Williams, University of Hong Kong, China

Kerstin Johannesson, University of Gothenburg, Sweden

Natalia Mikhailova, Institute of Cytology Russian
Academy of Sciences, Russia

Roger K. Butlin, University of Sheffield, United Kingdom

Rui Faria, CIBIO-BIOPOLIS, Universidade do Porto,
Portugal

SUNDAY, 23rd JULY, 2023

18:00-20:00 Welcome
reception

MONDAY, 24th JULY, 2023

08:30 Registration
09:00 Opening Ceremony
09:30 Plenary talk
10:30 Coffee break
11:00 4 Regular talks
12:00 Extended talk
12:30 Lunch
14:00 5 Regular talks
15:15 Coffee break
15:30 4 Regular talks
16:30 Walking city tour

TUESDAY, 25th JULY, 2023

09:00 Plenary talk
10:00 2 Regular talks
10:30 Coffee break
11:00 6 Regular talks
12:30 Lunch
14:00 Visit to "Galeria da
Biodiversidade" – Porto

WEDNESDAY, 26th JULY, 2023

09:00 5 Regular talks
10:15 Coffee break
10:15 Extended talk
11:45 Regular talks
12:15 Lunch
14:00 Extended talk
14:30 1 Regular Talk
14:45 General Discussion
15:15 Coffee Break
15:30 Discussion Groups
17:30 Conclusions

THURSDAY, 27th JULY, 2023

09:00 Plenary talk
10:00 Regular talks
10:15 Coffee break
11:00 6 Regular talks
12:15 Lunch
13:45 Plenary talk
14:30 General Discussion
15:15 Coffee Break
15:30 Walking visit to the shore
20:00 Conference dinner

***Timetable in LOCAL TIME (Portuguese/UK time zone)**

MONDAY 24th

Chair | Rui Faria

09:30 *Plenary talk .1* Kerstin Johannesson - **Marine snails at the intersection of ecology and genomics**

10:30 Coffee break

11:00 *Talk .1* Roger Butlin - **Selection in *Littorina saxatilis* hybrid zones**

11:15 *Talk .2* James Reeve - **Snail-scape genomics: assessing diversity and the role of inversions in Swedish *Littorina saxatilis***

11:30 *Talk .3* Ana Peris - **Genomic architecture of parallel phenotypic divergence in the marine snail *Littorina saxatilis* along shore levels and latitudinal locations**

11:45 *Talk .4* Marco Gerdol – **First evidence of gene presence/absence variation in *Littorina***

12:00 *Extended Talk .5* Amin Ghane - **Sex-linked genomic variation in various populations of *Littorina saxatilis***

12:30 Lunch

Chair | Roger Butlin

14:00 *Talk .6* Alan le Moan - **A strong barrier to gene flow between ecotypes focused on multiple chromosomal inversions**

14:15 *Talk .7* Marina Rafajlović - **What generates bimodal hybrid zones?**

14:30 *Talk .8* Aurélien De Jode - **Parallel divergence but different levels of reproductive isolation in a marine snail**

14:45 *Talk .9* Alfonso Balmori - **What can SNPs tell us about Iberian populations of *L. saxatilis*?**

15:00 *Talk .10* Juan Galindo - **Gene flow between Galician ecotypes: Ecology versus history**

15:15 Coffee Break

Chair | Juan Galindo

15:30 *Talk .11* Emilio Rolán-Alvarez - **Negative assortative mating in *Littorina* (*Neritrema*) species: Field and laboratory insights into the evolutionary maintenance of a shell color polymorphism**

15:45 *Talk .12* Juan Gefaell - **A temporally stable and spatially recurrent color cline in *L. saxatilis* from four coastal inlets of NW Spain**

16:00 *Talk .13* Ramón Vigo - **Unravelling the mechanisms responsible for a shell color cline in *Littorina saxatilis***

16:15 *Talk .14* Rui Faria – **The Stone Raft: using population genomics to understand the evolutionary processes shaping *Littorina* diversity in Iberian Peninsula**

TUESDAY 25th

Chair | Sean Stankowski

09:00 *Plenary talk .2* Pierre-Alexandre Gagnaire - **Integrating recombination information into population genomics: why and how?**

10:00 *Talk .15* João Carvalho - **Differentiating scenarios of ecotype formation with pool-sequencing genomic data and Approximate Bayesian Computation**

10:15 *Talk .16* Pierre Barry - **The role of inversions in adaptation and speciation in *Littorina* multiple evolutionary timescales**

10:30 Coffee break

Chair | Anja M. Westram

11:00 *Talk .17* Diego Garcia - **Fast and Furious snails: the surprising tale of local adaptation in record time**

11:15 *Talk .18* April Blakeslee - **Phenotypic and genetic characterization of two prominent ecotypes in Northwest Atlantic populations of *Littorina saxatilis***

11:30 *Talk .19* Sarah E. Kingston - **What's in a snail? Divergence between moderate (wave-protected) and wave-exposed *Littorina saxatilis* populations in the Gulf of Maine, Western North Atlantic**

11:45 *Talk .20* Peter R. Teske - **Genomic distinctness of the southern African littorinid snails *Afrolittorina knysnaensis* and *A. africana***

12:00 *Talk .21* Li-Sha Hu - **Phylogeographic structure of periwinkle *Littorina brevicula* along costal China**

12:15 *Talk .22* Yun-Wei Dong - **Genomic sequencing of Littorinidae snails in China**

12:30 Lunch

Wednesday 26th

Chair | Elizabeth Boulding

- 09:00 *Talk .23* Sean Stankowski - **Selection on many loci drove the origin and spread of a key innovation**
- 09:15 *Talk .24* Anja M. Westram - **Do snails prefer their home habitat? A mark-recapture experiment across different shore levels**
- 09:30 *Talk .25* Ruth Turunen - **Mate choice and reproductive barriers in two cryptic intertidal gastropod species *Littorina arcana* and *L. saxatilis***
- 09:45 *Talk .26* Luisa Kumpitsch - **The role of eusperm in reproductive isolation and sperm competition in *Littorina* snails**
- 10:00 *Talk .27* Laurent Seront - **Littorinid behavioral ecology revisited: deciphering the known knowns and unknowns from the known and unknown unknowns**
- 10:15 Coffee break

Chair | Gray Williams

- 10:45 *Extended Talk .28* Elizabeth G. Boulding - **Effects of a planktotrophic larval stage on the intertidal recruitment of four periwinkles (*Littorina*: Gastropoda) during extreme El Niño events: Evaluation of four hypotheses**
- 11:15 *Talk .29* Christopher McQuaid - **Thermal tolerances of Littorinid snails from temperate and subtropical regions of South Africa**
- 11:30 *Talk .30* Sarah L.Y. LAU - **Less is more: metabolic depression in tropical high shore littorinids**
- 11:45 *Talk .31* Bingqian Han - **Phenotypic evidence for local adaptation to heat stress in *Littorina saxatilis* across small scales**
- 12:00 *Talk .32* Laurent Seront - **The shade of death: littorinids habitat choice as a key determinant to face heat/cold waves**
- 12:15 Lunch

Chair | Emilio Rolán-Alvarez

14:00 *Extended Talk .33* Luis Pereira - **Quantification of cardiac performance in mollusks under realistic simulations of intertidal thermal conditions**

14:30 *Talk .34* Aurélien De Jode - **Assembling the new reference genome of *Littorina saxatilis***

14:45 General Discussion: Developing Littorinid online resources

15:15 Coffee break

15:30 Group Discussions

17:30 Conclusions

Thursday 27th

Chair | Kerstin Johannesson

09:00 *Plenary Talk .3* Jonathan Henry - ***Crepidula* slipper snails: Key spiralian model organisms for biological research**

10:00 *Talk .35* Erica H. Leder - **Ecotype-specific gene expression related to shell formation in *Littorina saxatilis***

10:15 Coffee break

10:45 *Talk .36* Jorune Sakalauskaite - ***Littorina* shell ColourBiomes: molecular archives for biomineralization and archaeology**

11:00 *Talk .37* Caren Shin - **Shell shapes over space and time in Pacific *Littorina***

11:15 *Talk .38* Arina Maltseva - **Consequences of trematode invasion in *Littorina saxatilis* snails: transcriptomic, proteomic and metabolomic insights**

11:30 *Talk .39* Elizabeta Gafarova - **Conservative or variable: the composition of microbimoes associated with different Atlantic periwinkles**

11:45 *Talk .40* Dmitrii Kuriachii - **A study of temporal, geographic, and interspecific variability of the microbiome in a model of the related species *Littorina fabalis* and *L. obtusata***

12:00 *Talk .41* Solène Henry - **Foaming at the mouth: direct and indirect exposure to seafoam affect Littorinids change metabolic rate and behaviour**

12:15 Lunch

Chair | John Graham

13:45 *Plenary Talk .42* Gray Williams - **Trail following and aggregation formation: an informal ecological review of ISOLBE meetings**

14:30 General Discussion: Future prospects in Littorinid research

20:00 Conference dinner



Marine snails at the intersection of ecology and genomics

Kerstin Johannesson (1)

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Marine snails of the genus *Littorina* are emerging models for experimental evolutionary biology, and one major focus is to understand the evolutionary mechanisms involved in ecotype formation and in the establishment of reproductive isolation. I will review some early and some more recent studies that have laid the foundation for approaching more general questions, such as, how species evolve. Based on these findings, I will synthesis a "snail-view" on ecotype formation and speciation and compare it with the classical speciation continuum hypothesis - one that assumes speciation occurs in a stepwise manner along a one-dimensional trajectory. I will also superficially refer to work in other organisms to compare snail patterns with those of other type of organisms. Finally, I will speculate about future research questions that I think studies of snails can generate important contributions, and what challenges that need to be solved before such progress can be made.



Selection in *Littorina saxatilis* hybrid zones

Roger Butlin (1,2); Kerstin Johannesson (2); Anja Westram (3,4); The Littorina team

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Where *Littorina saxatilis* ecotypes meet at sharp environmental transitions, there are strong clines in allele frequencies for many genes and also for arrangement frequencies of chromosomal inversions. These clines have been interpreted as a balance between gene flow and selection due to crab predation and wave action. Here, we present a test of this interpretation based on the prediction that clines will change in form seasonally, because wave action is stronger in the winter and crab predation is stronger in the summer.

Snail-scape genomics: assessing diversity and the role of inversions in Swedish *Littorina saxatilis*

James Reeve (1); Kerstin Johannesson (1); Per Bergström (1); Eva Koch (2); Roger K. Butlin (1,3)

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Littorina saxatilis is a diverse species occupying many rocky shorelines throughout the North Atlantic. Strong clines in allele frequencies and phenotypes separate ecotypes across several sites in Sweden. However, these ecotypes are just a small part of the diversity in this species, which may only represent the extreme ends of a spectrum of diversity. Furthermore, it is not clear how inversions contribute to adaptation outside of these ecotypes. To answer some of these questions, we investigated every habitat and phenotype of *L. saxatilis* in a local region of Sweden by sampling from 200 randomly selected sites. Snails were photographed to measure shell shapes before being dissected for DNA sequencing. Samples were genotyped using 351 SNPs, which included markers of ecotype divergence, inversions, and the genetic background. Environment, phenotype, and genotype were analysed using Bayesian regression models to see i) how the environment explains phenotypes, ii) how inversions explain phenotypes, iii) and how inversion frequencies vary with environment. PCA showed that snails occupy a continuous phenotypic space, suggesting that ecotypes represent extreme parts of a continuum. We will describe the influence of both inversion frequencies and environmental variation on this continuous phenotypic variation.



Genomic architecture of parallel phenotypic divergence in the marine snail *Littorina saxatilis* along shore levels and latitudinal locations

Ana-Maria Peris Tamayo (1); Juan Galindo (2); Kerstin Johannesson (3); Roger Butlin (3,4); Rui Faria (4,5,6); Joost Raeymaekers (1); Anja Marie Westram (1,7)

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Understanding the ecological and physical factors driving intraspecific divergence and the origin of species is the “holy grail” of evolutionary biology. Parallel ecological divergence can occur in heterogeneous environments, showing different patterns at the genotype level under parallel phenotypic evolution. In this study, we focused on the genomic basis of temperature adaptation in the marine snail *Littorina saxatilis* in response to low-shore and high-shore from different latitudinal locations to understand the spatial distribution of adaptive genetic variation. We tested whether the same set of loci underlie shore level temperature adaptation in different geographical locations. We used pooled whole-genome resequencing for determining the allele frequency variation among locations. We found that while some genomic regions showed location-specific patterns, others are likely to contribute to adaptive divergence across locations. We also found overlap between these regions and candidate chromosomal inversions. Chromosomal inversions can increase differentiation across large stretches of the genome and control divergent phenotypes, which can be associated with local adaptation in the presence of gene flow, favouring rapid parallel adaptation to heterogeneous environments. Chromosomal inversions can play an important role in adaptation and speciation as has been seen in other polymorphic species.



First evidence of gene presence/absence variation in *Littorina*: can a single reference genome adequately represent intraspecific genetic diversity?

Marco Sollitto (1,2); Rui Faria (3); Marco Gerdol (1)

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Although molluscan genomes are relatively large, complex and highly heterozygous, little attention has been placed so far on the factors underlying these extreme levels of intraspecific genetic diversity and on the implications of these factors on phenotypic diversity and local adaptation. Recent studies have revealed that bivalve mollusks have a peculiar genomic architecture characterized by widespread hemizygoty. The association of protein-coding genes with genomic regions subjected to structural variation results in gene presence/absence variation (PAV), whereby genes can be either characterized as core or dispensable depending on their presence in all or just a fraction of individuals. Nevertheless, so far this phenomenon has only been marginally studied in gastropods. Here, we preliminarily investigated the occurrence of gene PAV in *Littorina fabalis*, based on the assembly and annotation of a draft genome assembly and on the analysis of whole-genome resequencing data from 12 individuals belonging to two distinct ecotypes (“large” and “dwarf”), and confirmed the presence/absence patterns predicted in silico through PCR analyses. Although not as prevalent as in bivalves, we show that gene PAV affects a significant fraction of protein coding genes in this species (i.e. more than 1,400, about 4% of the total). This phenomenon might have important phenotypic implications, providing a significant contribution to intraspecific genetic variation and local adaptation in this species, in addition to small-scale variants. These observations lie the ground for further in-depth analyses in other congeneric species, which might exploit the availability of high-quality chromosome scale assemblies and a higher number of samples.

Sex-linked genomic variation in various populations of *Littorina saxatilis*

Amin Ghane (1); Nicholas H. Barton (1); James Reeve (2); Kerstin Johannesson (2); Roger Butlin (2,3); Anja Westram (1,4)

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The identification of sex-determination systems in species with young homomorphic sex chromosomes is difficult due to low chromosomal differentiation. To better understand the evolutionary forces involved, we investigated the role of ecology in sex chromosome evolution. *Littorina saxatilis*, a species with two ecotypes (“Crab” adapted to crab predation and “Wave” adapted to wave exposure) and many contact zones, offers an excellent study system. The Crab ecotype has been found to have a female-heterogametic sex-determination system, but the sex-determination system in the Wave ecotype remains unclear. To address this, we collected samples from multiple intertidal habitats in Sweden and performed whole genome sequencing on 82 individuals (48 Crab and 34 Wave). Through a combination of variant-based analyses including fixation index (FST), genome-wide association study (GWAS), SNP-density, and a reference-free k-mer approach, we identified genomic regions associated with sex in each ecotype. Our findings confirm that chromosome 12 is sex-associated in the Crab ecotype and that sex determination might be driven by more than one locus in the Wave ecotype. Sexually antagonistic selection and local adaptation may explain the variation in the sex-determination system and sex-specific differentiation patterns.



A strong barrier to gene flow between ecotypes focused on multiple chromosomal inversions

Alan Le Moan (1,2); Marina Rafajlovic (2); Sean Stankowski (3); Lee Chin Choo (4); Olga Ortega-Martinez (1); Rui Faria (5); Roger Butlin (2,4); Kerstin Johannesson (2)

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Reproductive barriers can cluster in the genome notably due to the presence of chromosomal rearrangements, but we still lack general insight into whether and how such chromosomal rearrangements results in the completion of speciation. Marine snails of the genus *Littorina* have been developed as a model system to study this type of question. Here, I will present some of my work on the evolution of a large and a dwarf ecotype of *L. fabalis*. These ecotypes are repeatedly segregating across wave exposure gradients on west Europe coastlines. By analysing whole genome polymorphism data, I found 14 putative inversions reaching near differential fixation along two transects crossing hybrid zones between the two ecotypes. These inversions covered around 20% of the genome but carried 93% of the SNPs that showed clinal variation along the transect, i.e. SNPs that are directly or indirectly affected by a barrier to gene exchange. The inversions were found in strong linkage disequilibrium and the hybrid zones showed clear bimodality, suggesting that coupling between inversions generate to strong reproductive barriers. However, these barriers had little effect on the collinear part of the genome (80% of the genome) where extensive gene flow was detected. I will discuss the evolutionary origins of these hybrid zones and provide a first comparative genomics insight into ecotypic divergence by comparing my work on *L. fabalis* to the previous work made on *L. saxatilis* ecotypes.

What generates bimodal hybrid zones?

Marina Rafajlović (1,2); Alan Le Moan (2,3) Kerstin Johannesson (2,4);
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Hybrid zones are geographical areas wherein genetically and phenotypically distinct parental populations interbreed and form hybrids. Hybrid zones are often characterized by the spatial shape and width of the underlying allelic and phenotypic clines. An additional classification can be made based on the shape of (local) distributions of individuals' genotypes (i.e., hybrid index) and phenotypes. The two extremes of such a classification are referred to as unimodal hybrid zones, wherein intermediate genotypes/phenotypes predominate, and bimodal hybrid zones, wherein intermediate genotypes are very rare or nonexistent. Intermediates between the two extremes also exist, and they are referred to as flat hybrid zones. According to the classic theory, hybrid zones are expected to be unimodal. Thus, classic theory is unable to explain the existence of bimodal hybrid zones. Notably, classic theory of hybrid zones rests on a number of simplifying model assumptions, including random mating, adaptive loci being arranged on collinear chromosomes, absence of genetic incompatibilities, absence of locally divergent selection (i.e., local patchiness). In this study, we use computer simulations to assess how these model assumptions, both individually and in combination, influence the characteristics of hybrid zones, and especially their modality. We use our results to deduce which mechanisms contributed to generate bimodal hybrid zones in the marine snail *Littorina fabalis* but not in *L. saxatilis* on the west coast of Sweden.



Parallel divergence but different levels of reproductive isolation in a marine snail

Aurélien De Jode (1,2,3); Francesca Raffini (4,5,6); Rui Faria (7,8); Kerstin Johannesson (1); Juan Galindo (9); Emilio Rolán-Alvarez (9); Roger Butlin (1,4)

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Speciation typically occurs at time frame too long to be observed. One way to work around that issue, is to study pairs of populations at different stages of the speciation continuum. Population pairs showing different levels of divergence within clades constitute a particularly interesting case as levels of reproductive isolation are not confounded with other differences among taxa. In *L. saxatilis*, the repeated occurrence of ecotypes adapted to either crab predation or wave action found on shores from Portugal to Norway therefore constitute a good case to study speciation. In Sweden, ecotypes occur at the same shore levels and the differentiation between ecotypes is relatively weak. In Spain, ecotypes are found at different shore levels with the wave ecotypes living very low, almost always submerged, and the crab ecotype found up to levels rarely under water. We sampled transects in Spain and Sweden going from crab to wave habitats. Using low coverage whole genome sequencing we characterized and compared the genomic patterns of differentiation in Spain and Sweden. We also characterized the phenotypical differentiation between the Spanish ecotypes using shell features, growth parameters and behavior. In Spain, individuals clustered in 2 distinct genetic groups and genetic differentiation between those (F_{st} of 0.11) was higher between Swedish ecotypes (F_{st} = 0.06). Though no F1 hybrids could be identified, our analyses suggest that some level of gene flow still occurs. Genomic inversions were detected in both localities and differentiation in inverted regions was more pronounced in Spain. The Spanish genetic clusters are phenotypically highly differentiated, though a few individuals present discordance between their genetic background and phenotype characteristics. Overall, Spanish populations represent a later stage of the speciation continuum and future studies will aim to determine assess factors contributing to those higher levels of reproductive isolation.



What can SNPs tell us about Iberian populations of *L. saxatilis*?

Alfonso Balmori-de la Puente (1); Aurélien De Jode (2); James Reeve (2); Francesca Raffini (3); Pierre Barry (1); Juan Galindo (4); Anja M. Westram (5); Kerstin Johannesson (2); Roger Butlin (3); Rui Faria (1,3)

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The rough periwinkle (*Littorina saxatilis*) shows a high phenotypic variability (e.g., 'wave' and 'crab' ecotypes), in part resulting from parallel divergence associated to similar environmental pressures in the intertidal across multiple locations in European Atlantic shores. Populations in the Iberian Peninsula, in particular, present distinct genetic and phenotypic diversity, which may be key for adapting to varying environmental conditions and diversify at the southernmost range of the species. Recent studies based on whole-genome sequencing (WGS) and phenotypic data revealed i) stronger reproduction isolation between Iberian ecotypes (when compared with Sweden), consistent with late stages of speciation; and that ii) many of the 20 chromosomal inversions detected de novo in the northernmost range are also present in the Iberian Peninsula. However, this is largely based on the analysis of a single location. Thus, we still need to extract the full potential from the genomic information gathered across Iberian *L. saxatilis* populations to reconstruct the history of the genetic variation underlying ecotype divergence and speciation in this (and other) areas of the species distribution range. In order to reach this goal, a SNP genotyping assay consisting of ~200 SNPs divided in three different categories (i- diagnostic (or nearly) between ecotypes; ii) linked to inversions; iii) randomly selected from collinear regions) was designed based on WGS data from a single Spanish site. The analysis of for SNP set across samples collected across three transects from low (Wave) to high (Crab) shore in Iberia Peninsula revealed: i) strong but variable degree of reproductive isolation across transects; ii) individuals with phenotypic and genetic ancestry consistent with ongoing hybridization (despite rare); iii) differentiation between populations within the same ecotype (although lower than between ecotypes); and iv) the top SNPs involved divergence between ecotypes are often located within inversions, while the top SNPs contributing for phylogeographic differentiation tend to be found in collinear regions. Finally, I will describe our current efforts to extend this array to incorporate more informative SNPs about the various evolutionary processes we aim to study in Iberian populations of this species.



Gene flow between Galician ecotypes: Ecology versus history

Inés González-Castellano (1); Ana Peris (2); Armando Caballero (1), Emilio Rolán-Alvarez (1), Juan Galindo (1)

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The Crab and Wave ecotypes from Galicia (NW Iberian Peninsula) present high levels of phenotypic and genetic divergence associated to different microhabitats within shore, and only tens of meters apart. These ecotypes overlap at the mid-shore but the width of the hybrid zone and the ecological/environmental conditions at the mid-shore have been shown to vary between localities. Therefore, local microhabitat conditions seem to play an important role in the level of gene flow between the ecotypes, and consequently the recombination of their genomes. Here we study Crab and Wave ecotypes across 14 localities from the Rias Baixas (South of Galicia) using two approaches: ddRAD and PoolSeq. The obtained SNPs were used to determine the level of introgression between ecotypes at those localities and also detect genomic regions of high divergence (outliers). The results show differential levels of introgression at different localities, this introgression can be correlated with different ecological/environmental characteristics of the localities rather than their geographical position. The detection of outliers is greatly improved by the increment of independent sampling sites rather than the number of individuals within locality. As expected, some of these outliers have been associated to previously described chromosomal inversions. Finally, even though the high levels of genetic divergence previously found pointed to a potential secondary contact of the ecotypes, the current results point to a potential role of the local conditions in the divergence, even if it was a case of secondary contact.



Negative assortative mating in *Littorina* (Neritrema) species: Field and laboratory insights into the evolutionary maintenance of a shell color polymorphism

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Shell color polymorphism is a widespread phenomenon among marine gastropods, especially within the genus *Littorina*. Among the various mechanisms that have been put forward to account for the evolutionary maintenance of (shell) color polymorphism is negative frequency-dependent selection (NFDS), where the least frequent morphs have higher fitness than their counterparts at higher frequencies. One indirect way of determining whether NFDS is acting in a population is through a mate-choice-driven negative assortative mating (NAM), where individuals mate with those individuals with a different color than their own. It has been shown that both NFDS and NAM may be responsible for the maintenance of shell color polymorphism in the intertidal snail *Littorina fabalis*, but why this is the case and whether such a mechanism also holds for other species within the genus *Littorina* is not yet well known. Here, we present field and laboratory data verifying the existence of NAM as a proxy of NFDS in the snail *Littorina saxatilis* and *Littorina obtusata*, close relatives of *L. fabalis*. While field data confirms the existence of NAM in these three *Littorina* species, thereby suggesting a possible ancestral behavioral mechanism driving such mating pattern (Gefaell et al. 2021), laboratory data on *Littorina saxatilis* only provides an incomplete validation of the observed mate choice. We discuss the possible causes of this potential mismatch between field and laboratory data.



A temporally stable and spatially recurrent color cline in *L. saxatilis* from four coastal inlets of NW Spain

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L. saxatilis from the Rías Baixas (NW Spain) shows a remarkable shell color polymorphism that follows a clinal pattern, with fawn-like individuals in the inner localities of the rías, high diversity of colors in the intermediate localities, and lined snails in the more wave exposed localities, usually coinciding with the outer part of the rías. In the Ría de Vigo, this clinal pattern in shell color has been recently shown to have kept relatively stable over the last 40 years (Gefaell et al. 2023). In addition, preliminary data from our group suggest that it also shows spatial replication not only within (i.e., north and south latitudes) but also between rías, as at least the rías of Pontevedra and Muros show a similar pattern of color distribution. Despite this, unlike other kinds of genetic polymorphism in the Galician system of *L. saxatilis*, clinal shell color polymorphism has been much less studied in the past. In this talk, we aim to present the abovementioned results on the stability and spatial replication of clinal color polymorphism in these species, together with a discussion of potential predictor ecological variables and some preliminary experimental results on its evolutionary maintenance mechanisms. On the whole, these results point to a very interesting case of parallel evolution of a color cline that opens the door to future genomic analyses intended at revealing its molecular underpinnings.



Unravelling the mechanisms responsible for a shell color cline in *Littorina saxatilis*

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Marine snails of the genus *Littorina* are highly polymorphic for shell color. It has been recently verified that a color cline of *L. saxatilis* in the Ria de Vigo (NW Spain) remains relatively stable after at least half a century with a dominance of lighter colors in the inner, protected localities and darker and lineated morphs in the outer and wave-exposed areas, showing the intermediate localities a high diversity of colors. Here we present preliminary data in order to test two possible explanations for the appearance and maintenance of this cline: differences in shell heating by solar radiation at low tide and visual predation by crabs. For the first one, laboratory data show that darker morphs are more heat-sensitive, probably due to the absorption of a wider range of visible light, in line with the color cline: darker shells in the outer and exposed zones, where insolation is relatively less important. In the case of predation, field data from a pilot tethering experiment suggests differences in shell damage for some colors, but do not fully validate a crypsis hypothesis, where those specimens with an apparently cryptic color and pattern with the substrate are less vulnerable to predation. We discuss these preliminary results and the possibility of extending the study with mortality, stress and in situ temperature measurements as well as the continuation of tethering experiments in other seasons of the year.



The Stone Raft: Understanding the evolutionary processes shaping *Littorina* diversity in Iberian Peninsula

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The Iberian Peninsula comprised one of the most important glacial refugia of European biodiversity, with populations from many different species in the region harboring unique genetic variation. This is the case of periwinkles, one of the most informative models for studying adaptation and speciation, where unique phenotypic and genetic diversity previously described for several species representing different degrees of reproductive isolation across the speciation continuum, including *Littorina saxatilis* and *L. fabalis*. Here, I will start by summarizing ongoing work on *Littorina* species across Iberia, with a particular focus on the role chromosomal inversions (previously detected in the in these taxa). This will be followed by a description of the findings from a study aiming to characterize the genetic basis of colour polymorphism in these same species using whole genome pool-sequencing. The meaning of the different associations between inversions and shell colour across species as well as their evolutionary implications in terms of adaptation and speciation will be discussed.

Integrating recombination information into population genomics: why and how?

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Recombination is a fundamental mechanism by which new allelic combinations arise within populations, generating evolutionary novelty. Analysing recombination signals in molecular data can provide valuable information about eco-evolutionary processes. In particular, recombination can inform key questions that require the integration of processes operating at different timescales, a common problem in research at the interface of ecology and evolution. I will present examples showing how accounting for recombination can improve molecular estimates of divergence times in the presence of gene flow, and how the history of gene flow can be inferred and used to assess reproductive isolation. I will then illustrate how we can learn about recent demography and contemporary evolution from genomic segments that are identical-by-descent. These different layers of information, relating to different aspects of genetic ancestry, are all embedded in a genome-wide collection of genealogical trees that link individuals to their shared genetic ancestors. A complete description of such genetic relationships, in the form of multiple correlated trees along the genome, is provided by the Ancestral Recombination Graph (ARG). The ARG contains all available information on past recombination events, demographic and selective processes for a given set of recombining genomes. It is therefore an ideal framework for inferring the evolutionary history of a species. Recent development of approaches to reconstruct the ARG from genomic sequences offers promising new perspectives for inferring demographic and selective processes over evolutionary and ecological timescales.



Differentiating scenarios of ecotype formation with pool-sequencing genomic data and approximate Bayesian computation

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Understanding the interplay of selection and gene flow during the early stages of divergence is a key question in evolutionary biology. The formation of ecotypes, driven by adaptation to different ecological niches, offers a chance to study how divergent selection drives local adaptation despite gene flow. A fundamental question is whether ecotype formation occurred independently in multiple locations with gene flow (parallel origin), or whether it occurred once followed by secondary contact (single origin). Here, we developed an Approximate Bayesian Computation (ABC) method to reconstruct demographic history tailored to Pool-sequencing data. Pool-sequencing is a cost-effective method for sequencing multiple populations but introduces errors in allele frequencies that can bias downstream analyses. We model explicitly those sources of error (i.e., unequal individual contribution, variation in depth of coverage and sequencing errors), such that parameter estimation and model comparison are not affected by them. By conducting a simulation study and applying our method to populations of two ecotypes of the marine gastropod *Littorina saxatilis*, we found that our ABC method can effectively distinguish between scenarios of ecotype formation and estimate relevant parameters using standard Pool-sequencing datasets. For *Littorina saxatilis*, the most likely scenario of ecotype formation depends on the geographical scale. For Swedish populations, a single origin is the most likely scenario. However, when comparing populations from Sweden and Spain, the parallel scenario has the highest support. Furthermore, there is evidence indicating gene flow between distinct ecotypes coexisting in the same location. We conclude that our method can be used to identify the most likely scenario of ecotype formation, enabling the development of suitable null models for species with ecotypes in multiple locations and enhancing our understanding of the adaptive process.



The role of inversions in adaptation and speciation in *Littorina* multiple evolutionary timescales

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Inversions are particular genomic rearrangements where the order of genes are reversed. Growing numbers of empirical and theoretical studies are showing that they are prone to accumulate reproductive isolation barriers between nascent species by reducing recombination between the two rearranged parts and keeping in high linkage disequilibrium genes of local adaptation and/or intrinsic genetic incompatibilities. However, there is still lack of knowledge about the appearance of inversions and the underlying processes of barriers formation within it during speciation. Here, we are assessing the role of multiple inversions, compared to the collinear part of the genome, in shaping the genomic landscape of differentiation and divergence in multiple hybrid zones of two ecotypes of *L. saxatilis* along the Iberian coast. In parallel, we are conducting a whole-genomic multispecies approach of 8 species of the *Littorina* genus to detect polymorphic and fixed inversions between species and to infer their evolutionary history. These two approaches at different timescales will unravel the mechanisms of barriers accumulation in inversions and particularly assess whether they are kept as polymorphism during long evolutionary times or appeared independently within each lineage and eventually shared afterwards by introgression.



Fast and Furious snails: the surprising tale of local adaptation in record time

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Human-introduced populations offer great opportunities to study the genetic basis of evolution in the short term. We present the results of a 30-year local adaptation experiment. Our study organism, the snail *Littorina saxatilis*, shows divergent ecotypes, locally adapted to wave-exposed vs. crab-predation habitats across European shores. Genomic and phenotypic differentiation between ecotypes is well-studied; however, there is no direct evidence that selection caused these differences. In 1992, we collected ~700 Crab ecotype snails and relocated them to an uninhabited, crab-free islet exposed to strong waves. Thirty years after introduction, the population on the islet has established itself and undergone significant morphological changes that differ from the parental population. For instance, the individuals have become relatively smaller, with thinner and smoother shells, and have acquired greater color and patterning diversity. These changes are all characteristic of the Wave ecotype. To investigate the genetic basis of this local adaptation, we used genetic markers in both collinear regions and inversions. Samples were collected from the parental Crab ecotype, the islet, and a nearby Wave ecotype population in 1992, 2005, 2018, and 2021. Consistent with the phenotypic observations, the population genetics analysis indicates that the islet population has diverged from the Crab ecotype over time. The signals of divergence are stronger in genomic regions that are known to differentiate Crab and Wave ecotypes in other locations. Candidate-selected regions have shifted in frequency to values comparable to the Wave ecotype, providing strong evidence for selection on the Wave-adapted allele. Furthermore, the greatest amount of genetic and phenotypic divergence occurred within the initial decade following the introduction. Our findings demonstrate that adaptive evolution is not necessarily a long-term process, but it can occur in a few decades.



Phenotypic and genetic characterization of two prominent ecotypes in Northwest Atlantic populations of *Littorina saxatilis*

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Littorina saxatilis is an abundant and ecologically-important intertidal snail in the North Atlantic. It is also widely-studied for its multiple “ecotypes” (=phenotypically distinct forms resulting from local adaptation), which have been identified and characterized in numerous North Atlantic populations. The evolution of these distinct ecotypes, sometimes found just meters apart, is believed the result of the snail’s direct-developing (brooding) reproductive strategy, along with habitat heterogeneity arising from multiple abiotic and biotic forces (e.g., wave action, predators) and distinct habitat types (e.g., rock cobble, crevices, barnacle-covered boulders). Though ecotypic formation has been extensively researched in the Northeast Atlantic (NEA), little is yet known in the Northwest Atlantic (NWA). To address this knowledge gap, we sampled *L. saxatilis* from 19 sites ranging from Nova Scotia to Long Island during summers 2012-2013. We took multiple phenotypic measures and analyzed genomic data to characterize two ecotypes (“typical” and “barnacle”) from two respective habitat types (small rock/cobble vs large barnacle-covered boulders/jetties) found at our sites. Phenotypic data revealed significant differences between ecotypes in individual measures of shell length, weight, brood pouch size, and embryo count, but not embryo size across our sites. A PCA examining multiple phenotypic measures found distinct differences in both ecotype and geographic region (north/south of Cape Cod); however, morphometric analyses of shell shape did not differ between ecotypes. We also revealed particular genomic regions showing location-specific patterns of local adaptation, whereas other regions likely play an important role in facilitating adaptive divergence across locations. Curiously, chromosomal inversions, which are strongly associated with ecotype divergence in NEA, did not show clear patterns in NWA samples, even though many were polymorphic. Altogether, our study represents the first effort to characterize ecotype differences of *L. saxatilis* in NWA and can contribute to future work examining parallelism of ecotypic formation across the North Atlantic.



What's in a snail? Divergence between moderate (wave-protected) and wave-exposed *Littorina saxatilis* populations in the Gulf of Maine, Western North Atlantic

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Analyses of the architecture of divergence among Eastern North Atlantic ecotypes of the charismatic intertidal snail, *Littorina saxatilis*, have offered valuable insight into the processes of parallel evolution and reproductive isolation. While there is tremendous morphological variation in the species in the Western North Atlantic, less is known about the extent and structure of any underlying genomic basis of this observed variation. Here, we characterized *L. saxatilis* snails from wave-protected and wave-exposed sites in the Gulf of Maine to assess both morphological and genomic divergence between moderate (wave-protected) and exposed (wave-exposed) ecotypes. We reveal that while moderate (wave-protected) and wave-exposed ecotypes of the Western North Atlantic *L. saxatilis* populations also exhibit divergence patterns consistent with parallel evolution, geographic differentiation and isolation by distance are marked in the Gulf of Maine. Additionally, contrasts along a tidal height gradient elucidate many genomic regions under diversifying selection among populations geographically separated by mere meters. Lineage-based models also reveal a complex combination of ecotype and geographic divergence.



Genomic distinctness of the southern African littorinid snails *Afrolittorina knysnaensis* and *A. africana*

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The supratidal zone of southern African rocky shores is dominated by small littorinid snails that were originally described as *Littorina knysnaensis* and *L. africana*. Collectively, these display a change in shell colour from west to east, with the western populations having dark brown shells, and eastern ones light grey shells. This difference was previously attributed to a morphological cline in a single species, but currently, the two are considered to be distinct species, *Afrolittorina knysnaensis* (brown) and *A. africana* (grey). We used partial genomic data from snails collected in Namibia, South Africa and Mozambique to confirm that the species status of each is not merely a result of phenotypic plasticity.

Phylogeographic structure of periwinkle *Littorina brevicula* along costal China

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In the context of global warming, species are predicted to shift their ranges in response to climate change, impacting peripheral populations at trailing and leading edges. The periwinkle *Littorina brevicula*, a widely distributed intertidal species along the northwest Pacific coast, was studied to investigate the effects of global warming and human activities on its population genetics. The genetic structure of 5 natural and 5 artificial rocky populations was analyzed and a genome-wide association study (GWAS) revealed two major haplotype groups (A and B). Subei Biogeographical Barrier (~33°–34°N, SBB) on the central coast of Jiangsu province, separated these groups with A group distribution in the north and B group in the south. The Yancheng population represents the northern edge of group B. Populations at the southern distribution limit in Xiamen and the northern distribution limit of group B in Yancheng have been observed to disappeared during the summer, likely due to high temperatures in Xiamen and frequently heat waves in Yancheng. This study highlights *L. brevicula* as a suitable model organism for further research on the adaptation mechanisms of intertidal species in the face of global warming and human activities.

Genomic sequencing of Littorinidae snails in China

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Rocky intertidal Littorinids inhabit at the upper intertidal zone from temperate to tropical Pacific regions face harsh heat environments. So far, many studies based on these species have demonstrated behavioral, physiological, and evolutionary adaptation to high temperatures. Despite mounting studies on these intertidal snails, the genomic resources for these species remain limited. Corresponding to this background, we first got over the difficulties that the pure DNA for sequencing from small-size periwinkles that were hard to be derived, utilized the updated PacBio highly accurate long sequencing (HiFi) sequencing technologies and finally constructed high-quality genomes for some Littorinids. By utilizing these genomes, more useful information will be found, greatly developing our understanding of the physiology and ecology of Littorinidae species. For example, we can conduct phylogenetic and comparative genomic studies to identify the evolution innovations related to the high heat resistance or utilize these genomes as references for genome-wide association analyses to compare the genetic structures among different populations and figure out specific molecular pathways correlated with thermal adaptation.



Selection on many loci drove the origin and spread of a key innovation

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Key innovations are fundamental to biological diversification, but their genetic architecture is poorly understood. A recent transition from egg-laying to live-bearing in *Littorina* snails provides the opportunity to study the architecture of an innovation that has evolved repeatedly in animals. Samples do not cluster by reproductive mode in a genome-wide phylogeny, but local genealogical analysis revealed numerous genomic regions where all live-bearers carry the same core haplotype. Associated regions show evidence for live-bearer-specific positive selection, and are enriched for genes that are differentially expressed between egg-laying and live-bearing reproductive systems. Ages of selective sweeps suggest live-bearing alleles accumulated gradually, involving selection at different times in the past. Our results suggest that innovation can have a polygenic basis, and that novel functions can evolve gradually, rather than in a single step.



Do snails prefer their home habitat? A mark-recapture experiment across different shore levels

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Species with limited dispersal abilities can adapt to the environment on small scales. As a result, different species, as well as different ecotypes within the same species, may show distinct, non-random distributions even when sampling small areas. However, it is often unclear whether these distributions are maintained solely by selection or partly driven by active habitat preferences. *Littorina* snails are an ideal system to test for active habitat preference because of their suitability for field experiments and demonstrated microscale habitat separation both within and between species. *Littorina arcana* typically dominates the higher part of rocky shores, which is associated with higher temperatures and higher desiccation risk, and is relatively rare on the low shore. *Littorina saxatilis* has two distinct ecotypes, one inhabiting the high and one inhabiting the low shore. In this study, we tested for habitat preferences in this system on the Norwegian coast near Bodø. We ran a mark-recapture experiment for which we marked snails from the high and low shore, placed subsets in their native habitat as well as in the opposite habitat, and recorded movement over several days. Afterwards we dissected the snails for species identification. We found strong evidence for habitat preferences; in particular, high-shore snails placed on the low shore showed clear upshore movement. We will discuss this and further results, as well as implications for local adaptation within species and reproductive isolation between species.



Mate choice and reproductive barriers in two cryptic intertidal gastropod species *Littorina arcana* and *Littorina saxatilis*

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In recent years more and more marine "species" have turned out to be complexes of multiple cryptic species that are sometimes closely related. This has raised new interesting questions about reproductive barriers and mate choice in the marine environment: Which barriers allow (cryptic) marine species to remain separate when they occur in sympatry? To study ongoing speciation and mate choice in marine habitats, we analysed mating behaviour in the closely related and coexisting cryptic intertidal gastropods *Littorina saxatilis* and *Littorina arcana* in the field in the UK and Norway. What makes *L. arcana* and *L. saxatilis* a good model system to study speciation is that previous work indicates very low unidirectional gene flow, suggesting that there is an incomplete reproductive barrier, yet no hybrids have been found in the field. We will present results on the degree of assortative mating and discuss whether this is driven by mate choice, microhabitat differences leading to reduced encounter rates, or both. In the course of this study we also analysed the morphology of the two species in Northern Norway. Preliminary results suggest shape differences between *Littorina arcana* and *Littorina saxatilis* on the local scale, indicating that the species are in fact not fully cryptic. These results suggest that at least locally, the two species may to some extent be distinguishable based on external characteristics.



The role of eusperm in reproductive isolation and sperm competition in *Littorina* snails

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Reproductive isolation is a key mechanism for maintaining species and preventing hybridization. This either functions through prezygotic barriers, such as different mating behaviours or gamete incompatibility, or postzygotic barriers, such as sterile offspring. Sperm length is a trait that has been suggested to contribute to prezygotic reproductive isolation in birds and freshwater fish. In species with female sperm storage organs, like *Littorina* snails, sperm length might be under selection to fit into the female storage organs, which has been shown for passerine birds and fruit flies. Moreover, in species with sperm competition, i.e. multiple matings, sperm length might be under selection pressure to outcompete the sperm from competitor males. This suggests that high intensities of sperm competition would lead to low variation in sperm length – selection for an optimal size. To better understand speciation in *Littorina* snails, we examined the variation of eusperm length within and among several species of *Littorina* including two different ecotypes of *L. saxatilis* and *L. fabalis* and locations in Sweden, Spain, and the United Kingdom. To examine sperm competition, we analysed the variation in their sperm length and quantified matings of different *Littorina* species and ecotypes in the laboratory and the wild.



Littorinid behavioral ecology revisited: deciphering the known knowns and unknowns from the known and unknown unknowns

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As most moving organisms, littorinids fundamentally search for food and mates, while avoiding predators in spatially and temporally complex, structured environment. Specifically, littorinids exhibit a vast behavioural repertoire: (i) they do not move following straight lines, but along pathways that can be more or less convoluted, (ii) they alternate periods of activity with periods of relative stasis, (iii) when they move their speed often fluctuates erratically, which is incompatible with a description based on mean speed, and (iv) they exhibit a range of taxes that contribute to their navigation in various landscapes. In this context, the present work will first describe the existing models available to describe the complexity of movement behaviour before addressing their respective strength and limitations, in particular in relation to the scales at which the organisms are considered. I subsequently described how the explicit consideration of both the geometric and stochastic components of littorinid movements can be used to assess their fundamental and realized behavioural niches in the absence of extrinsic stimulus and as a response to abiotic and biotic stressors, and more generally to quantify response strength across stimuli and species. This aspect will be illustrated using examples based on the response of different species of littorinids to a range of both naturally occurring chemical cues (e.g. injured conspecifics, predators, conspecifics of the opposite sex, domoic acid) and a range of anthropogenic contaminants such as plastic leachates, hydrocarbon, caffeine and antidepressant. These results will be discussed in the general context of the biological, ecological and evolutionary advantages the observed behavioral flexibility and adaptability provides to littorinids.



Effects of a planktotrophic larval stage on the intertidal recruitment of four periwinkles (*Littorina*: Gastropoda) during extreme El Niño events: Evaluation of four hypotheses

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Life history traits that simultaneously affect dispersal ability could have large effects on the responses of species to global climate change. Yet long-term population studies of marine species that differ in life-history traits are rare. I compared the recruitment of two intertidal gastropod (*Littorinidae*) species with direct benthic development (DD) with that of two species with a planktotrophic larval stage (PD) by monitoring their count anomalies and their size-distribution at two wave-exposed sites near Bamfield Marine Sciences Centre (Vancouver Island, Canada), between 1993-2021. Two extreme El Niño-Southern Oscillation (ENSO) events that occurred during this study period (1997/1998 and 2015/2016), were followed by large increases in the PD species that were synchronous at both sites. I observed that the peak abundances of planktotrophic and direct-developing species were separated in time. Four single-factor hypotheses that could explain this negative correlation were statistically evaluated. Three could be rejected: 1) Interspecific competition, 2) Differential responses to warmer sea surface and air temperature anomalies, 3) Differential predation by the lined shore crab that temporarily invades after major El Niño events. The fourth hypothesis - that increased recruitment of the PD species resulting from rapid transport of their larvae from California by El Niño-augmented winter Davidson current - was supported. Multivariate Autoregressive State-Space (MARSS) models for Winter and Summer at each site estimated positive slopes for the seawater temperature anomaly during the preceding February (SST-Feb) with respect to the juvenile count anomaly of the PD species. In contrast the MARSS SST-Feb covariate slopes for the DD species count anomaly were either significantly negative or not significantly different from zero. My long-term study using *Littorina* spp. shows that the mechanisms behind population responses to climate change can be complex and can differ for species that vary in dispersal potential.



Thermal tolerances of Littorinid snails from temperate and subtropical regions of South Africa

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Environmental temperature affects ectotherm performance and fitness by altering body temperature because physiological performance increases with temperature up to a sublethal optimum. Optimum values are not fixed, but depend on species, individual and the history of the individual. We used four species of South African littorinids in laboratory experiments to explore the influence of species identity, size and thermal history on their thermal tolerances. The South African coast encompasses conditions that range from subtropical to cool temperate, with our species distributed across this gradient. We found that heat coma temperatures (HCT) were lower than lethal (LT50) temperatures for all species, but that HCT was greater for juvenile individuals while LT50 was greater for adults. Thermal tolerance was higher for adults of all species, which showed the same ranking by both metrics: *Echinolittorina natalensis* > *Littoraria glabrata* (both subtropical) > *Africana africana* (subtropical/warm temperate) > *A. knysnaensis* (warm/cool temperate). Intraspecific comparisons for the two *Africana* species from different biogeographic regions showed non-significant differences, with individuals from the warmer region showing slightly higher tolerances. When the four species were subject to acclimation temperatures ranging from 20-35°C for 14 days, the only significant differences in HCT were between field fresh individuals and all other treatments. Comparing LT50 values for different seasons showed non-significant acclimatization with higher values for summer for all four species. Overall, thermal tolerance reflects the biogeography of these species and appears fixed for species occurring across different regions with only moderate seasonal acclimatization. The fact that the effect of size differed between our two metrics of tolerance and that field fresh individuals showed lower HCT values than laboratory treated individuals indicates the need to account for the effects of the metric used to measure thermal tolerance and of the laboratory environment when interpreting the results of lab work.



Less is more: metabolic depression in tropical high shore littorinids

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Tropical shores are among the most thermally dynamic and extreme habitats, with surface temperature often exceeding 50°C. To withstand such severe thermal stress, organisms will have to invest in energetically costly thermal defense mechanisms such as heat shock responses. Such an energy demand could, however, be challenging to meet in high shore species, given the generally constrained activity hence limited energy acquisition. As a result, exhibiting metabolic depression to conserve energy during aestivation appears to be a key strategy for organisms to inhabit the thermally harsh, yet energy-limited tropical high shores. To investigate how metabolic depression may benefit survival, cardiac thermal performances of a widespread tropical high shore littorinid, *Echinolittorina malaccana*, were examined. Six populations experiencing different climatic conditions (from aseasonal to seasonal subtropical) were examined, either immediately (field fresh) or after acclimating to hot or cool regimes in common garden experiments to assess how metabolic depression varies across these populations, and whether such variation is caused by phenotypic plasticity or genetic variation. Results showed that the variation in thermal tolerance of field fresh snails coincided with the thermal but not the latitudinal gradient of the populations, indicating that local conditions (e.g. tidal cycle and wave exposure) overrode latitudinal, climatic effects in driving the thermal responses of these snails. Upon acclimation, the cardiac thermal performance of snails from all populations converged to a similar pattern where hot-acclimated snails showed an overall more depressed metabolism and slightly enhanced thermal tolerance as compared to cool-acclimated snails. Such convergence highlights phenotypic plasticity in the metabolic depression trait and suggests that recent thermal history could be key to invoke energy balance strategies in thermally dynamic and extreme habitats.



Phenotypic evidence for local adaptation to heat stress in *Littorina saxatilis* across small scales

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Understanding the physiological differences between organisms that inhabit different temperature environments is crucial for gaining insights into their survival and adaptation strategies. In particular, the rocky intertidal zone is at high risk of being impacted by ocean warming, making it an important model system for studying thermal adaptation. The unique biodiversity of this ecosystem has adapted to localized spatial changes and rapid fluctuations in temperature, providing a valuable opportunity to investigate the physiological responses of different species to varying thermal conditions. In this study, we compared two physiological traits, heart rate change and drop-down time, in high and low shore populations of *Littorina saxatilis*, which are adapted to distinct ambient thermal conditions due to tides. Although no significant difference during resting, the heart rate of high shore snails under thermal stress reaches higher maximum values shortly after exposure than that of low shore snails. In addition, high shore snails showed more stable heart rate after hours of exposure. Surprisingly, the drop-down time of high shore snails was significantly shorter than that of low shore snails, probably reflecting different escaping strategies under strong summer sunlight rather than capacity of heat tolerance. Our findings provide insights into the physiological mechanisms that underlie the differential thermal responses of these snails, and have important implications for understanding how marine organisms may cope with changing temperatures in the future.



The shade of death: behavioral flexibility as a key determinant in littorinids

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Heat/cold wave frequency, duration and intensity are expected to increase as climate change progresses. Intertidal invertebrates in general, and littorinids in particular, are vulnerable to thermal stress because they have minimal ability to behaviorally thermoregulate and/or rely on the presence and availability of thermally benign microhabitats and their ability to locate and occupy them. Understanding what factors influence the mortality of littorinids and how heatwaves and cold waves might impact their ability to use thermally benign habitats is hence critical. In this context, we illustrate on the basis of a heatwave and a cold wave that both occurred along the French coasts of the eastern English Channel between January 2021 and July 2022 how two common species of littorinids, *Littorina littorea* and *L. saxatilis*, take advantage of both abiotic and biogenic habitats to survive. Under severe cold wave conditions, the survival of three localized populations of *L. saxatilis* was directly driven by the availability of thermally benign microhabitats that offered thermal conditions up to 4°C warmer than in their absence. In the most extreme instance where thermally benign microhabitats were absent, one population of *L. saxatilis* went extinct. Under several heatwave conditions, *L. littorea* were able to select thermally benign microhabitats either in the form of (i) localized small patches of the common mussels *Mytilus edulis* with decreased temperature due to endolithic infestation or within large mussel beds or (ii) the shaded area offered by the shell of dead *M. edulis*. The relevance of these results will be discussed in the general context of the increasing thermal stress expected in our era of thermal change.



Quantification of cardiac performance in mollusks under realistic simulations of intertidal thermal conditions

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Intertidal systems are among the most thermally complex environments on Earth. They are inhabited by marine organisms that must withstand terrestrial conditions during low tide, and whose responses are therefore regarded as early indicators of climate change. Despite recent progress in our understanding of their physiological response to thermal stress, major limitations persist, severely restricting our ability to forecast the true ecological consequences of climate change. Current methodologies are neither good at simulating realistic conditions nor at measuring sub-lethal thermal stress. In this talk we will describe a methodological pipeline whose main focus is to bring realism to laboratorial thermal stress trials. It starts with the collection of long series of temperature data at the scale of the studied organisms using autonomous loggers in the field. Then, it uses a self-contained, experimental system that simulates the intertidal environment to replicate those realistic thermal profiles in the lab. It's like a PCR machine for the intertidal, featuring automatic control of tides, light, water temperature and infra-red heating. It can be remotely monitored and experimental conditions changed in real time through a dedicated webpage. Finally, heat stress can be measured in a non-invasive way by means of an infra-red heart frequency logger which can record the cardiac frequency of up to ten animals simultaneously. It has been extensively validated in mollusks such as limpets, mussels, periwinkles, or oysters. Since the device relies on non-invasive technology, it is harmless and can be used on live animals, providing on-the-fly data on their physiological performance. This pipeline can be used to study current conditions or to experiment with “what-if” scenarios involving virtual translocation of species or predicted future environmental conditions.



Assembling the new reference genome of *Littorina saxatilis*

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The intertidal gastropod, *Littorina saxatilis* is model system to study speciation and local adaptation. The repeated occurrence of crab and wave ecotypes found on the shores from Portugal to Norway showing different levels of divergence makes *L. saxatilis* particularly suited to study different stages of the speciation continuum in the same species. Moreover, the presence of several large genomic inversions with important contribution to the divergence between the ecotypes raises questions about the role of those inversions in the formation of ecotypes. The genome of *L. saxatilis* is 1.35Gb and composed of 17 chromosomes. The existing reference genome was assembled using Illumina data, is highly fragmented (N50 of 44kb) and quite incomplete with a BUSCO completeness of 80% on Metazoan dataset. A linkage map of one full sibling crab family allowed to place 587 Mbp of the genome in 17 linkage groups. Nevertheless, the fragmented nature of the existing reference genome of *L. saxatilis* is limiting our ability to fully understand the interplay between divergent selection and gene flow in the repeated formation of ecotypes. I will present the new reference genome of *L. saxatilis*. A total of 135X PacBio CLR reads were assembled with CANU and haplotigs were removed using the `purge_dups` pipeline. The obtained contigs were scaffolded using Hi-C reads and the `yahs` scaffolder. Finally, the assembly was manually curated using Pretext and the genome curation suite. The newly obtained reference genome is highly contiguous with a N50 of 67 Mb and 90% of the assembly total length placed in 17 super scaffolds. It is also highly complete with a BUSCO completeness of 96% of the Metazoa dataset. This new reference will allow a better characterization of the genomic regions implicated in ecotype formation and the relative contribution of the inversions compared to other parts of the genome.



***Crepidula* slipper snails: Key spiralian model organisms for biological research**

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Marine gastropod molluscs in the genus *Crepidula* have been used as subjects for developmental studies for well over 100 years. There are around 120 extant members of the Calyptraeidae, which include over 60 described species of *Crepidula* living in marine habitats around the world. Two species in particular, *C. fornicata* and *C. atrasolea* have emerged as molluscan/spiralian models for cell, molecular and developmental studies. These animals exhibit many key advantages that make them well suited for this purpose. They are accessible, readily cultured and have naked eggs and embryos that can be easily microinjected for lineage tracing and molecular functional manipulations. In this talk I will introduce these *Crepidula* model systems and describe developments in cell and molecular developmental biology, live imaging, transgenesis, genomic resources and automated aquaculture that make these animals excellent molluscan models for biological studies.



Ecotype-specific gene expression related to shell formation in *Littorina saxatilis*

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Parallel evolution arises when similar phenotypes evolve repeatedly and independently in response to local adaptation in a similar environment. The marine snail, *L. saxatilis*, has evolved ecotypes that are locally adapted to different shoreline microhabitats, and this has occurred repeatedly in populations in Sweden, the United Kingdom and Spain. The “crab” ecotype exists where there is less disturbance from wave action and where crab predation is high. These snails are larger, have a thick shell, and shy behavior. The “wave” ecotype exists in exposed areas where there is substantial wave action. These snails are smaller, have thinner shells, and bolder behavior than the crab ecotype. The mantle tissue is involved in shell formation and is a likely candidate for investigating shell-related differences in these ecotypes. In order to understand the genetic basis of shell differences between these forms, we sequenced mRNA from mantle tissue from both ecotypes from two distant populations, one from Sweden and one from Spain. Our findings comparing the ecotypes and populations, as well as basic identification of the genes transcribed in the mantle tissue will be presented.



***Littorina* shell ColourBiomes: molecular archives for biomineralization and archaeology**

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Molluscs adorn their shells with an incredible diversity of colours and patterns which were highly appreciated since ancient times. Shell biochromes encode important biological information - they may play a role in immunity, shell strengthening, protection from thermal stress and UV radiation. They are also extremely durable in time – pigments can be found in archaeological and palaeontological shells dating from several thousands to millions of years. What if shell biochromes were among the most promising ancient biomolecules? However, despite the great diversity of shell colours, the biochemical knowledge of shell coloration for many molluscs, including the colourful littorinids, is limited. Therefore, how biochromes are produced, mineralised and why they preserve so well, is poorly understood. I will present my current research project on *Littorina* shell biochromes. The project aims to investigate the biochemistry of *Littorina fabalis* shell pigments, which display colour polymorphism. These shells are also abundantly found in archaeological record - *Littorina sp.* were widely used by prehistoric populations as jewellery objects and the colours may have had a special meaning in transmitting social or cultural messages. To study colour associated shell biomolecules, I will employ so-called ‘ShellOmic’ techniques - transcriptomics and proteomics. Together with our team, we will aim to develop a CRISPR-Cas9 gene editing approach to verify the sequence involved in shell colouration. Gene editing is still challenging for non-model organisms and has only been successfully applied to a handful of molluscan species. I will discuss the feasibility of this approach to *L. fabalis*. If successful, this method could help to elucidate the nature and function of shell biochromes, as well as provide grounds to study a diversity of questions. Finally, in the future, this information will help to trace ancient shell colour sequences and study their preservation in time.



Shell shapes over space and time in Pacific *Littorina*

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Most gastropod species are traditionally recognized and identified by conchological characters, as the shell is most readily preserved. However, shell shape can be highly variable, particularly depending on the studied species. Using Pacific *Littorina* species from the west coast of North America (the sister species pair *L. plena* and *L. scutulata*; *L. keenae*), this study evaluates the consistency of shell shape with morphometric techniques. Shell shapes of these species are compared using modern specimens, Pleistocene fossils, and with an extinct species (\dagger *L. petricola*), to assess the variability of *Littorina* shell shape over space and time.



Consequences of trematode invasion in *Littorina saxatilis* snails: transcriptomic, proteomic and metabolomic insights

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The effects of the trematode parasites on the *Littorina* snails are complex and include changes in behaviour, morphology and physiology. Numerous studies describe the infection-induced shifts in particular metabolic pathways (such as carbohydrate and lipid metabolism) and deep structural changes such as hepatopancreas tissues replacement, castration and the shell remodelling. Our study aimed at characterisation of complex functional transformation in mollusks *Littorina saxatilis* infected by trematodes *Microphallus piriformes* / *M. pygmaeus* at the transcriptomic, proteomic and metabolomic levels. Snails were collected from the natural populations at three distant geographical locations: Chupa Bay (White Sea), Kola Bay and Dalnye Zelentsy (Barents Sea). The total RNA, metabolites and proteins were extracted from tissues not damaged by the parasites. The cDNA libraries were prepared for the Illumina NovaSeq sequencing. Then transcriptome assembly and gene expression analysis were performed. Proteomes were analysed with the LC-MS/MS system (Bruker Daltonics, Germany). The metabolomic analysis was based on the GC-MS/MS (LECO Corporation, USA) profiling. After the comparative transcriptomics analysis, 78 significantly differentially expressed genes (DEGs) with at least 4 times difference in expression between infected and control individuals were identified. These genes are involved in such processes as: catabolism, metabolism of fatty acids, proteolysis, cell death and morphogenesis. These results are consistent with ones of the metabolomic analysis which also revealed the shifts in catabolic processes due to parasitic infection; e.g. significant increase in lactate and decrease in malate abundance indicate the transition from aerobic to anaerobic cellular respiration in the infected individuals. Also, there were signs of more active catabolisation of the amino acids instead of glucose (presumably transported to the parasite) after the infection. Finally, the proteomic analysis in agreement with the other ones revealed changes in abundance of proteins involved in the amino acids metabolism, translation and proteolysis processes and cell signalling. Importantly, besides revealed metabolic shifts in the infected snails, there also were changes in the immune system of hosts. For example, several identified DEGs encode for immune effectors and regulators, such as NO-synthase, Dual oxidase, lectin-domain and CUB-domain containing proteins, TNF-related protein. Also worth noting is that few DEGs were annotated as transposable elements that potentially may cause serious rearrangements in the host genome structure and functioning.



Conservative or variable: the composition of microbiomes associated with different Atlantic periwinkles

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Symbiotic microorganisms provide remarkable contribution to the normal functioning of multicellular organisms. An organism and its microbiome function and evolve as an integrated system, holobiont. The composition of the associated microbiome is shaped by various factors, including the host's diet, immunity, or interactions between hosted bacteria. Considering closely related species, the spatial separation of their sympatric populations and host food preferences can also affect the spectra of engulfed microorganisms. This could have evolutionary significance as the species-specific microbiome composition may affect the host's adaptive potential. We assessed bacteria associated with a set of *Littorina* species inhabiting Northeast Atlantic shores. Three types of samples were taken from four sites on the coasts of the North and Norwegian seas: environmental biofilms, snails' mantle fragments, and hindgut. Microbial composition was analyzed using 16S-rDNA metabarcoding, followed by OTU identification in the SILVA database. This is the very first description of the microbiomes associated with *Littorina* from the Northeast Atlantic.



A study of temporal, geographic, and interspecific variability of the microbiome in a model of the related species *Littorina fabalis* and *L. obtusata*

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It is well known now, that microorganisms, associated with metazoans, can influence adaptive scope of their hosts. This poses an important question about the fate of associated microbiome during ecology-driven speciation. In this way, the characterization of microbiomes associated with recently diverged species, demonstrating ecological shift, may help to clarify this point. When associated microbiome is described, temporal and spatial variability should be taken into account. In our study, we analysed microbiomes associated with sister-species *Littorina fabalis* and *L. obtusata* collected from two remote regions (Barents Sea and White Sea); in the latter region, the samples were obtained in the summer and autumn seasons. The gut samples were obtained individually, samples of the mantle (pooled ones) and of environmental biofilms, taken from living (fucoids) and non-living (stones) substrates, were also used in the analysis. Bacterial composition was analyzed using 16S-rDNA metabarcoding, followed by the ASV identification in the SILVA database. Several principal conclusions can be done. [1] The environmental and body-surface microbiomes significantly differ from the gut microbiomes. Species richness and community evenness in the environmental samples were higher than in the gut-associated ones. [2] There were weak but significant differences between the gut-associated microbiota of the two species in the analysis. Interestingly, the number of bacterial lineages typically associated with *L. obtusata* was significantly higher than of those specific for *L. fabalis*. [3] Seasonal variability of the gut-associated microbiota was strong in both analysed species. In the autumn, the snail-associated bacterial communities significantly decreased in lineages richness; moreover, between-species differences in the microbiome composition were insignificant in this season. Possible interpretations of these phenomena will be presented in my talk.



Foaming at the mouth: direct and indirect exposure to seafoam affect Littorinids change metabolic rate and behaviour

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Foam formation is common in marine environments and its accumulation on coasts may have important ecological, social and economic implications. Although the presence and important role of foam deposits in the ecology of sandy coasts and estuaries is well established, their impact on the structure and function of rocky intertidal ecosystems remains under-documented. Through our research work, we studied how different foams, either young and aged, could influence the activity patterns of the periwinkle *Littorina littorea* under laboratory conditions. Our results showed that *Littorina littorea* actively avoided the foam and, in addition, never went through it. Furthermore, additional investigations showed that exposure to foam in situ resulted in a significantly higher metabolic rate in the gastropod *Littorina saxatilis*. Finally, behavioral experiments conducted in the laboratory revealed that this species is able to detect chemicals contained in the foam, and actively avoids areas covered by foam. Its reactivity is also increased in presence of foam. Our results suggest that foam accumulation on intertidal rocky shores may have important consequences for species relying on airborne chemosensory and tactile cues for movement, foraging, and mating. In particular, altered activity, whether inhibited or enhanced, combined with increased metabolic requirements, may affect species fitness and highlight potentially important ecological consequences in rocky intertidal ecosystems where the presence of long-lived foams is favored by strong hydrodynamics and high organic matter content.



Trail following and aggregation formation: an informal ecological review of ISOLBE meetings

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Being found almost all around the world in coastal habitats, littorinid snails have attracted the interest of natural historians and scientists. As David Reid commented, the amount of interest in this one family, the Littorinidae, is unusual and consequently has spawned a regular series of symposia. These meetings have been running since 1986 and have stretched across continents, attracting numerous followers to aggregate at various locations to share their findings; discuss recent advances in the field; and share their common interest in these highly abundant snails. Given this relatively long history, it is interesting to reflect on focal research areas and the changes in research directions over time. There are clear indications of convergent themes as a result of either recent advances in techniques, or discoveries which have attracted researchers and shaped the development of research into the evolution and ecology of littorinids. This paper presents an informal overview of the ISOLBE series; the contributions of various trail-blazing researchers; and subsequent developments in littorinid ecology and behaviour. Finally, the talk will reflect on potential future directions of these meetings to consider how they can further develop and promote research into these ubiquitous snails.



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Welcome reception



We will start the conference with a Welcome Reception in Vila do Conde, a charming coastal town north of Porto. You can register and enjoy some drinks in a relaxed atmosphere, at one of the most beautiful buildings, the Centro da Memória.

Venue

Centro da Memória
Vila do Conde

[Website](#)

Address

Largo de S. Sebastião
4480-706 Vila do Conde

Conference



The conference will be held in an informal but stimulating scientific atmosphere in beautiful room, providing an excellent opportunity for strong interaction and brainstorming between students and more experienced researchers.

Venue

Centro Municipal de Juventude
Vila do Conde

[Website](#)

Address

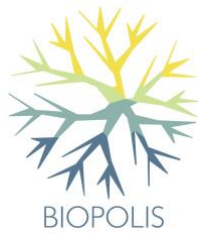
Avenida Júlio Graça
4480-672 Vila do Conde

Social events



We will do a city tour in the cozy town of Vila do Conde, visit a local beach and a modern gallery in Porto (Galeria da Biodiversidade) where the arts connect with biology and natural history and savour Portuguese cuisine.

Organization



Partners



Funding

