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Greenhouse Gastropods of the Hortus botanicus Leiden (Distribution) and other experiences in the Botanical Garden

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## Table of contents

1. Introduction
1.1 Hortus botanicus Leiden a 450 years old institution ..... p. 1
1.2 Collections ..... p. 2
1.3 The Naturalis Biodiversity Center ..... p. 3
2. Research Project: Greenhouse gastropods
2.1 Introduction ..... p. 4
2.2 Creation of the sampling patches ..... p. 6
2.3 Sampling methods ..... p. 15
2.4 Results ..... p. 19
2.5 Discussion ..... p. 25
2.6 Output of the research project ..... p. 30
3. Activities in the Botanical garden
3.1 Management ..... p. 30
3.2 Horticulture ..... p. 32
3.3 Conclusions ..... p. 35
4. Acknowledgments ..... p. 35
5. References ..... p. 36
6. Appendix ..... p. 38

## Riassunto

L'orto botanico di Leiden e il Naturalis Biodiversity Center sono due istituzioni che da tempo collaborano per promuovere la ricerca e la divulgazione del sapere scientifico. Il primo, fondato nel 1590, festeggia quest'anno il suo $425^{\circ}$ anno dalla fondazione promuovendo un gran numero di iniziative per portare il pubblico a conoscere le affascinanti e rare collezioni che ospita e, più in generale, le meraviglie della botanica. All'interno delle serre dell'orto sono presenti 6000 orchidee, molte delle quali originarie del Sud Est Asiatico. Questi esemplari, in parte raccolti in natura con lo scopo di portarli a fiorire in serra, rappresentano un punto di riferimento per tutti gli orti botanici e nuove specie vengono periodicamente descritte. Floridi sono gli scambi di vegetali con i diversi orti botanici. Il secondo, con basi più moderne, è sorto grazie alla collaborazione dei precedenti musei di storia naturale di Leiden e Amsterdam che hanno unito le loro collezioni, portando l'attuale museo al terzo posto tra i musei del mondo per quantità di campioni conservati nella collezione. Del museo fa parte anche l'erbario nazionale. Dotato sia di una parte espositiva che di una di pura ricerca, rappresenta una solida e attiva istituzione nel campo delle Scienze Naturali.

Il mio tirocinio è consistito in un lavoro di ricerca all'interno delle serre dell'Hortus con lo scopo di creare una collezione dei Gasteropodi ivi presenti, lavorando sinergicamente anche con il museo di storia naturale. I gasteropodi possono avere effetti negativi sulla crescita delle piante all'interno delle serre, danneggiandole e modificando l'equilibrio dell'ambiente. Le serre, inoltre, vengono mantenute nel modo più naturale possibile evitando, ad esempio, l'utilizzo di pesticidi. Questi ambienti caldi e umidi si configurano come luoghi ideali per l'instaurarsi di popolazioni di gasteropodi. Questi richiamano un grande interesse scientifico in quanto comprendono spesso oltre a specie endemiche anche specie aliene introdotte con gli scambi di piante tra orti botanici oppure tramite le piante raccolte in natura durante le spedizioni di ricerca. Spesso succede che specie nuove siano descritte grazie a esemplari raccolti nelle serre. Le specie di nuova introduzione possono rappresentare un pericolo per l'equilibrio della fauna e flora locali nell'eventualità che riescano ad uscire dalle serre. Le parti relative alla identificazione, conservazione e check-list sono trattate
nell'elaborato di Mattia Trivellato, con cui ho lavorato durante il tirocinio. In questo elaborato, invece, vengono presentate la creazione dei siti di campionamento, le modalità di raccolta e la distribuzione delle specie raccolte.

Per prima cosa sono stati definiti i siti di campionamento. Le diverse serre sono state interpretate come ambienti diversi e inizialmente separate tra loro nominandole con lettere in ordine alfabetico dalla A alla Z (Fig.1). Ogni serra successivamente è stata a sua volta suddivisa in singoli siti di campionamento per avere una risoluzione maggiore della presenza e distribuzione delle specie di gasteropodi (Fig.2,3,4,5). Per ogni sito sono stati registrati diversi parametri ambientali con lo scopo di trovare una correlazione tra questi e la distribuzione delle specie all'interno delle serre (Tab.1,2,3,4,5).

Per ottimizzare il campionamento, in ogni sito sono state utilizzate diverse tecniche. Alcune di queste, infatti, potrebbero privilegiare la raccolta di alcuni gasteropodi con un determinato habitat trascurandone altri con uno stile di vita differente. Solo sommando i contributi di più modalità di raccolta è possibile ottenere un'idea chiara e dettagliata dalla presenza di gasteropodi nei siti di campionamento. A questo scopo sono stati utilizzati vasetti con insalata fresca, vasetti contenenti birra, la floatation technique e la ricerca a vista. Dei contenitori con insalata sono stati appesi sulla chioma degli alberi e alcuni di questi sono anche stati scossi con lo scopo di far cadere delle lumache ma nessun campione è stato raccolto con queste due tecniche. Le diverse metodologie di cattura sono state ripetute più giorni in diversi momenti della giornata. Grande attenzione è stata data ai vari microhabitat dove queste specie potrebbero vivere.

Complessivamente sono state raccolte 52 specie delle quali 29 terresti e 23 di acqua dolce. 41 sono state identificate fino al livello di specie (Trivellato, 2015). 25 specie sono endemiche e 19 aliene includendo 3 specie non identificate considerate di origine tropicale da diversi esperti. Di 8 specie non è stato possibile determinare l'origine (Trivellato, 2015). In tutte le serre (Fig.9) sono state raccolte specie endemiche mentre i gasteropodi di origine aliena sono stati trovati principalmente nelle serre $\mathrm{A}, \mathrm{O}, \mathrm{U}$ e Z . Nella serra A , inoltre, molte specie sono state raccolte solo come conchiglia. Le serre H, I, LM e N ospitano per lo più specie endemiche. Osservando i dati a maggiore risoluzione (Fig.8) sono stati
riconosciuti siti dove i gasteropodi sono assenti (F1, F4) o poco rappresentati (O4, O5) e quelli dove riscontriamo il maggio numero di specie (A15, F2, O7, O15, O16, O18, O25, U2, Z1, Z2). Sono state individuate le specie terrestri e acquatiche più frequenti nelle serre (Fig.6,7). Inoltre, come riportato in Tab.7, è stato possibile eseguire un confronto tra questa collezione e le precedenti effettuate nelle serre e conservate nel Naturalis.

Questo progetto di ricerca dimostra la presenza di una ricca malacofauna all'interno delle serre dell'Hortus botanicus. Sia specie endemiche che aliene sono state raccolte. Per evitare una distorsione dei dati le specie rinvenute solo a livello di conchiglia (13 spp.) non sono state considerate nella valutazione dei dati. Le serre presentano anche una grande differenziazione nella composizione delle specie. Infatti l'indice di Whittaker I (Tab.7) calcolato su tutti i siti di campionamento, equivale a 8,26, molto simile al valore di 8,6 raggiunto in un Km quadrato della foresta tropicale presso Sabah, Borneo Malesiano (Schilthuizen and Rutjes, 2001). Le serre in esame sono estremamente diversificate in quanto a specie ivi presenti, similmente a quanto viene registrato in una foresta tropicale. Analizzando i dati raccolti è stato possibile inoltre osservare quali siano gli ambienti più favorevoli per i gasteropodi in generale nelle serre. Lo stesso si è potuto fare per le specie endemiche, da un lato, e le aliene, dall'altro. È stato quindi possibile dimostrate come siano principalmente le specie endemiche a rappresentare un pericolo per le piante e gli ecosistemi di serra per la loro maggiore adattabilità ad ambienti diversi e la dieta fitofaga di alcune specie. Le specie aliene, invece, essendo più legate ad ambienti prettamente umidi ed nutrendosi principalmente di detrito, non rappresentano un pericolo diretto né per le serre, né per l'ambiente al di fuori delle serre poiché non riuscirebbero probabilmente a superare l'inverno. Le specie più interessanti sono trattate in dettaglio. E stato esposto, infine, come i gasteropodi possano contribuire positivamente al funzionamento dell'ecosistema serra.

L'elaborato, nel suo terzo capitolo, descrive brevemente le conoscenze acquisite sulla gestione di un orto botanico e sulle pratiche orticulturali operate dai giardinieri per coltivare le diverse specie vegetali.

## Introduction

### 1.1Hortus botanicus Leiden a 450 years old institution

Thinking of botanical gardens, the first ideas usually coming to mind are the colours of beautiful and rare flowers, the special plants hosted in the greenhouses, the old trees planted in the garden and the general landscape inspiring serenity, and joy. Anyway, botanical gardens are much more than simple exhibitions of plants. As the BGCI suggests, botanical gardens are institutions where "plant diversity is valued, secure and supporting all life" (https://www.bgci.org/). This purpose was implemented in the Hortus botanicus since it's foundation that dates back to the 1590 thanks to the "wonder for diversity of the natural world" (Uffelen, 2015) that was emerging in people of the $16^{\text {th }}$ century. Before becoming the first prefect of the garden, Carolus Clusius, travelled a lot around Europe writing the very first floras. He knew many languages and had many contacts with people interested in botany. When he settled in Leiden, he designed "not just a herb garden, but a Hortus botanicus" (Uffelen, 2015), the first in The Netherlands. In fact, he carried to the Hortus many tubers, bulbs and seeds he collected during his travels that where not known at that time in the Netherlands. He was the first who managed to cultivate successfully the Tulips (Jelles, 2014) that nowadays are both a symbol and a pride for the country and are well known all around the world. These plants, originally from Central Asia like Kazakhstan, were taken by the botanist from Frankfurt (1593). Since the really first time, the Hortus was designed as a place for professors and students to learn how to identify both plants for medical use and study new introduced plants like the potatoes and the tomatoes. It was organised as an open garden for all the visitors interested in botany. The plants were displaced partly systematically and partly in groups according to their origin (e.g. plants from Crete). Students were provided with a booklet having a map of the garden and had to fill in the names of the plants in it (Jelles, 2014). A modern reconstruction of Clusius' garden is present today in the Hortus. The importance of this reconstruction is that the same plants Clusius planted in $16^{\text {th }}$ century were placed in their original position nowadays. This has been possible thanks to the conservation of the original book (Index stirpium) where the Hortus plan was designed. The Hortus become bigger during time,
always improving the collections, making connections with other institutions, exchanging plants and becoming one of the most important botanical gardens in Europe.

### 1.2 Collections

The South East Asian Orchids are the main collection of the Hortus. There are more than 6000 individual orchids grown in the greenhouses, many of them from wild origin. This collection was created for scientific purpose. In fact, in order to define an orchid species in the traditional way, it's necessary to have all morphological characters available. Unfortunately, in nature "less than $15 \%$ of the species are flowering in any given month" (Schuiteman, 2010). In order to be able and identify new species, many plants were collected in the wild during the last 30 years with the intention to make them grow in flower under good climatic conditions in the greenhouses. Ed de Vogel and other botanists collected many plants for the Hortus together with colleagues and institutions from the countries of origin. Many species have been described and named thanks to this collection (Schuiteman, 2010).

The greenhouses are also hosting many other plants of scientific interest like the genus Nepenthes. The plants of this genus represent a wonderful example of adaptation to extreme habitats. In fact, due to the extremely low quantity of nutrients in soil where they live, they are adapted to up-take nitrogen from insects, using pitchers. Recently new identification techniques were studied on this group (Beveridge et al. 2013). Other plant genera generally listed as "carnivorous" are also present.

Another important collection, present in the Winter garden, consists of Cycads. The oldest plants arrived in the Hortus in 1851 (http://www.hortusleiden.nl/) and all the plants are planted in containers to be able and move them outside during summertime. These slow growing plants are threatened in nature.

One of the symbols of the Hortus is Amorphophallus titanium (Becc.) Becc.. This species presents a tuber under the soil surface. It produces one single big leaf that
lasts at least one year feeding the tuber. After that the old leaf decays the tuber needs a resting period and after that a new one is produced. After having stored enough nutrients the plant starts flowering producing the famous inflorescence which emits the horrible smell that lasts just some days. Many other Araceae are present in the Hortus. It is impressive to think that at least 120 species of plants (many of these members of this family) present in the greenhouses are known to be extinct in the wild as an effect of deforestation and are still present only in this and other botanical gardens.

Another famous plant is Victoria amazonica (Poepp.) J.C. Sowerby, the biggest Water Lily in the world. The flower appears at the end of summer and is quite small compared to the size of the leaves. Many other Nymphaeaceae are present in the greenhouses as well as other tropical plants.

Other collections are found in the Clusius garden, mentioned before, and the Systematic garden, where all the plants are planted in beds according to the more recent phylogenetical classification known as APG3.

The von Siebold memorial garden was designed as a Japanese garden by Japanese architects. It was created in order to remember Philipp Franz Balthasar von Siebold (1796-1866), a German doctor who collected, shipped and donated many plants to the botanical garden. Some of these original plants like Zelkova serrata (Thunb.) Makino are still present today.

Recently, a Chinese herb garden has been opened to the public and a collection of Dutch threatened flora was displaced in four beds in the garden. A collection of 400 fern spp. is also present and is mainly meant for scientific purposes.

### 1.3 The Naturalis Biodiversity Center

The Naturalis Biodiversity Center is an active and vital institution whose aim is to "describe, understand and explore biodiversity for human wellbeing and the future of our planet" (http://www.naturalis.nl/). In order to do so the museum is organised in three main parts. The collection is one of the biggest in the world, hosting 37 million objects. The main parts of the collection are the Naturalis
geology collection, the Amsterdam Zoological Museum and Naturalis zoology collections. The Dutch National Herbarium host the botanical collection. This impressive amount of natural objects will be expanded in future. This collection is curated and preserved for scientific purposes and focuses on the Europe. SouthEast Asia, tropical America and Africa are also well represented. This material has been of great importance in our research as it will be explained in (Trivellato, 2015). Recently, all objects have been digitalised and are easily accessible. This sector of the museum is closely linked with the part of the museum dedicated to do research. Here many closely collaborating scientists are present. The main important activities are focused on taxonomy, but morphology, phylogenetic, biogeography and global change biology are also important elements. Education is another great objective of this institution and many opportunities are often present for students at study any level. The museum has also an expositional part where people of any age can observe and be inspired by the astonishing objects displayed. This Museum is closely related to the Hortus botanicus doing collaborations in research and divulgation activities.

## Research Project: Greenhouse gastropods

### 2.1 Introduction

Greenhouse gastropods have recently become an interesting topic to focus on. These animals, even though may be really small (from 4 cm like Helix pomatia (Linnaeus, 1758) to some mm like the family Valloniidae) can have on one hand, a negative impact on the environment where they live, damaging plants if they become too abundant. The most dangerous group for plants are the slugs that most of the times are phythophagous damaging leaves and roots. Snails can be dangerous as well depending on their diet and size: in fact, small detritivores snails living in the litter will be less dangerous than big snails living directly on the plants and feeding on them. Moreover, gastropods may also transmit diseases through wounds in the plants. Commonly, in order to prevent these animals to damage cultivating plants in vegetable production greenhouses, periodically pesticide like metaldehyde (http://www.omafra.gov.on.ca) are used to remove the
populations. On the other hand, greenhouse gastropods may be proved to be of great scientific interest. Both endemic and alien species are commonly present in botanical garden greenhouses. Even though gastropods do not have a great dispersal potential themselves, they can be really easily moved from one place to another simply in moving the soil, the plants or the water where these species are present. The small size, the good protection the shell provides and the close relation with plants are the most important elements that make this group so indirectly and I would say accidentally introduced in the greenhouses or even in nature itself. Botanical gardens play an important role in the introduction of alien species both thanks to the exchange of plants between other institutions and carrying plants collected during research expeditions. Quarantine procedures are usually taken but seem sometimes not successful to prevent the dispersal of these species. You may just need to have a single egg to produce a new population if the slug is self-fertile. Once a gastropod arrives in a greenhouse, these warm and humid places provide an habitat where snails may potentially live and create stable populations. In studying them it's possible to understand their distribution both giving interesting data and allowing us to better understand their potential threat for the plants. New species, sometimes never found in nature, can also be detected. It is the case of Gulella io (Verdcourt, 1974) whose type was collected and named in greenhouses in the Czech Republic (Animalbase.org). Finally, as previously argued, having a reduced mobility these animals are not able to move in case the environmental conditions changes and can be considered as good bioindicators both for their natural environment and for the different environments present in a Greenhouse. Greenhouses are little "islands of biodiversity" that may host not only exotic plants but also various animal taxa with populations that sometimes are relict and genetically isolated. This small and "simplified" controlled environment gives us the opportunity to understand many dynamics that can be extended to all greenhouses and natural environments around the world. Some species, if the environment outside doesn't allow it, can't spread out of the greenhouses. On the contrary, some alien species may be invasive for the environment outside the greenhouses producing negative alterations. It is also possible for some endemic species to become invasive inside the greenhouses. In fact, what we consider to be an endemic species in Europe, may be seen as alien
in other countries like in Hawaii (R.H. Cowie, 1997). Analyzing the endemic populations in the greenhouse may make us aware of the potential threat they represents if they were introduced in a natural environment with similar climatic conditions. Finally, if necessary, it may be more easy to control the population knowing which of the present species represent the real threat for plants and where they are present. These are the reasons why a check-list of the species present in the greenhouses of the Hortus botanicus Leiden was created. All aspects related to the check-list, the identification, conservation of the material will be exposed and discussed in the report of Mattia Trivellato (Trivellato, 2015) whom I worked with. All information involving the creation of the sampling patches, the sampling and the data about distribution and abundance of the species will be presented in my report.

### 2.2 Creation of the sampling patches

In order to create an inventory of snails before starting the collection the places where to look for snails were defined. The map of the greenhouses was preliminary studied both to have a first idea of the metrical dimension of the places under study and to start thinking how to separate the sampling sites. According to the kind of greenhouse, attention was payed to the different climatic conditions where gastropods may live. In fact, different living conditions for such little moving animals may imply completely different habitats. All the different greenhouses were separated, considering them such as different localities and were named in alphabetical order from A to Z as it is shown below (Fig.1).


Fig
.1: Greenhouses named in alphabetical order from A to Z .

## -Greenhouse A

The tropical greenhouse, named A , is the biggest greenhouse present in the Hortus botanicus (Fig.2). All the plants inside are planted directly in the soil, allowing them to grow as if they were in nature. The temperature varies between $18-30^{\circ} \mathrm{C}$ and the humidity percentage is constantly around $70-80 \%$ thanks to an automatic vaporizer system. The soil is very poor and sandy: white sand is used in the low layer and the surface is composed of organic matter. This because in a real jungle the soil is also very poor except for the very top layer (Van Vugt pers. com.) The dead leaves and animals living in the greenhouses such as insects, frogs and, of course, snails provide a continuous renewal of this layer. In this greenhouse the plants are watered both directly on the soil twice a week and from the top daily. There is another part of the greenhouse at the first floor that is not shown on the map and was not studied because only a few pots are present there and it was assumed that the most of the species would have lived in the rest of the greenhouse. There are different paths for visitors to pass through the greenhouse that were considered as the starting point to separate the capture sites. These areas were divided in smaller patches trying to create as much as possible equidimensional sites. The environmental data of each single sampling site are described in Tab.1. In order to provide ecological data the tables were created
giving data that could have best described the environment focusing on the parameters possibly influencing the snails presence. Some data, like the amount of hours spent on searching snails (Rowson et al., 2010) were added to give a clearer idea of the spent on each site using each sampling technique that will be discussed later on.


Fig.2: Sampling patches in greenhouse A

| Patch | Size | Env | Ground conditions | S. R. G. | Litter | Vegetation | P.-h.: Eye+FI_S.T._B. T. | Notes |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\mathrm{m}^{\wedge} 2$ |  |  |  |  |  | hours_days_days |  |
| A1 | 11,5 | T | Soil alternatively dry and humid | No | Abundant | F, Ch | 1_6_2 | Ficus sp. |
| A2 | 11,2 | T | Soil ways humid | No | Present only in small areas | F, Ch, S | 1_6_2 | Little pond |
| A3 | 12,9 | T | Soil ways humid | Yes | Abundant | F, Ch, S | 1_6_2 | Little pond |
| A4 | 11,5 | T | Soil partly dry and partly alternatively dry and humid | Yes | Present only in small areas | F, Ch, S | 1_6_2 |  |
| A5 | 13,8 | T | Soil alternatively dry and humid | Yes | Abundant | F, Ch, E | 2_6_2 |  |
| A6 | 14,0 | T | Soil alternatively dry and humid | Yes | Abundant | F, Ch, E, M | 2_6_2 |  |
| A7 | 6,0 | T | Soil always humid | No | Abundant | F, Ch, E | 0,5_6_2 | Dead trunk on the ground |
| A8 | 6,4 | T | Soil always humid | No | Abundant | F, Ch, E | 0,5_6_2 |  |
| A9 | 11,3 | T | Soil alternatively dry and humid | No | Absent | F, Ch | 1_6_2 | Dead trunk on the ground, Banan tree |
| A10 | 8,4 | T | Soil alternatively dry and humid | No | Present only in small areas | F, Ch, E | 1_6_2 |  |
| A11 | 4,8 | T | Soil alternatively dry and humid | No | Present only in small areas | F, Ch, E | 0,5_6_2 |  |
| A12 | 11,6 | T | Soil alternatively dry and humid | No | Abundant | F, Ch, E, S | 1_6_2 |  |
| A13 | 9,0 | T | Soil alternatively dry and humid | No | Abundant | F, Ch, E, S | 1_6_2 |  |
| A14 | 9,9 | T | Soil alternatively dry and humid | No | Abundant | F, Ch, E, S | 1_6_2 | Dead trunk on the ground, really abundant vegetation |
| A15 | 15,5 | T | Soil always humid | No | Abundant | F, Ch, E, S | 2_6_2 | Really abundant vegetation |
| A16 | 10,2 | T | Soil alternatively dry and humid | No | Abundant | F, Ch, E | 1_6_2 | Dead trunk on the ground |
| A17 | 9,4 | T | Soil alternatively dry and humid | No | Absent | F, Ch, E | 1_6_2 |  |
| A18 | 11,5 | T | Soil always humid | No | Absent | F, Ch, E | 1_6_2 | Little pond |
| A19 | 11,6 | T | Soil alternatively dry and humid | No | Absent | F, Ch, E, M | 1_6_2 | Dead trunk on the ground |
| A20 | 9,3 | T | Soil alternatively dry and humid | No | Absent | F, Ch, M | 1_6_2 | Dead trunk on the ground |
| A21 | 5,6 | T | Soil alternatively dry and humid | No | Present only in small areas | F, E | 0,5_6_2 |  |
| A22 | 3,8 | T | Soil alternatively dry and humid | No | Present only in small areas | F, E | 0,5_6_2 |  |
| A23 | 3,1 | T | Soil alternatively dry and humid | No | Present only in small areas | F, Ch, E | 0,5_6_2 |  |
| A24 | 3,0 | T | Soil alternatively dry and humid | No | Present only in small areas | F, Ch, E | 0,5_6_2 |  |
| A25 | 4,0 | T | Soil alternatively dry and humid | No | Absent | F, Ch, E | 0,5_6_2 |  |
| A26 | 4,2 | T | Soil alternatively dry and humid | No | Absent | F, E | 0,5_6_2 |  |
| A27 | 6,8 | T | Soil alternatively dry and humid | Yes | Absent | F, E | 0,5_6_2 |  |
| A28 | 2,5 | T | Soil always humid | Yes | Abundant | F, E | 0,5_6_2 |  |

Tab.1: Environmental data of the patches in greenhouse A. Env. Indicates the kind of environment (Terrestrial, Freshwater or both of them), S.R.G. says whether the solar radiation reaches directly the ground or not. The vegetation is described using the biological forms (F:Phanerophyte, Ch:Chamaephyte, E: Hemicryptophyte) and it is said if Selaginellae (S) and mosses (M) are present. The next column P.h. (Person-hours)
indicates how many hours were spent overall on searching snails in each plot by naked eye and using the floatation technique(Eye +Fl .), how many days the "salad trap" (S.T.) was used and the quantity fot the "beer trap" (B.T.).

## -Greenhouses F, H, I, M, N

The greenhouse F (Fig.3) is a hallway with four different areas in which plants are planted directly on the soil that has the same composition of the soil present in greenhouse A . The temperature goes from a minimum of $18^{\circ} \mathrm{C}$ to a maximum of $30^{\circ} \mathrm{C}$ and the humidity percentage is quite variable: it's really high when the plants are watered twice a week and is lower when it's evaporated. No vaporizer system is present here. These four areas were divided in four sampling sites. No snails were present on the ground on the walkway. The greenhouses H, I, LM and N are the nurseries of the Hortus botanicus. In greenhouse H (Fig.3) resting dry seasonal plants, sick orchids and some succulent plants are present. Both the temperature and the humidity are variable. The patch H 1 is composed of three different tables having plants placed in pots on pebbles that commonly are moved among these three areas. The soil is variable from pot to pot. The place H 2 is a small pond where aquatic plants are stored. On the bottom of the pool silt and little stones are present. Greenhouse I (Fig.3) is similar to the previously described greenhouse in all its aspects but here on the tables it is possible to find plants that need to be potted in a bigger pot and new arrived tropical plants. Many different kinds of soil are present in the pots. The greenhouses L and M (Fig.3) were considered as a single greenhouse (LM) because after a first look no dispersal barriers were present and the environment was very similar. The temperature and the humidity are very variable and the plants are placed in pots on tables with pebbles. Here are placed the new arrived plants that will be displaced outside the greenhouse. The soil condition is very variable. The N greenhouse (Fig.3) is similar in all its aspects to the LM but it hosts less plants and it is generally dryer hosting succulent plants on sandy soil. The environmental data of each single sampling site are described in Tab.2.


Fig.3: Sampling patches in greenhouses F, H, I, M, N

| Patch | Size | Env. | Ground conditions | S. R. G. | Litter | Vegetation | P.-h.: Eye+FI_S.T. | Notes |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\mathrm{m}^{\wedge} 2$ |  |  |  |  |  | hours_days |  |
| H1 | 4,4 | T | Tables with pots and pebble stones | Yes | Absent | Ch, E, M | 1_0 | Plant coming from outside and malate plants |
| H2 | 0,6 | A | Aqatic plants, wet surface of the pool | No | / | I | 0,5_0 | Stream present |
| 1 | 4,6 | T | Tables with pots and pebble stones | No | Absent | Ch | 1_0 | Plant coming from outside and ill plants |
| F1 | 0,5 | T | Soil lternatively dry and humid | No | Absent | Ch | 0,25_1 |  |
| F2 | 5,4 | T | Soil lternatively dry and humid | No | Present only in small areas | Ch, E | 0,5_1 | Really abundant vegetation |
| F3 | 5,4 | T | Soil lternatively dry and humid | No | Present only in small areas | Ch, E | 0,5_1 | Really abundant vegetation |
| F4 | 0,5 | T | Soil lternatively dry and humid | No | Absent | Ch | 0,25_1 |  |
| LM | 8,2 | T | Tables with pots and pebble stones | No | Absent | Ch, E, M | 1,5_0 | Plants coming from outside the greenhouses and new arrivals |
| N | 3,2 | T | Tables with pots and pebble stones | No | Absent | Ch, E | 1_0 | Plants coming from outside the greenhouses and new arrivals |

Tab.2: Environmental data of the sampling patches in greenhouses H, I, F, LM and N.
Env. Indicates the kind of environment (Terrestrial, Freshwater or both of them), S.R.G. says whether the solar radiation reaches directly the ground or not. The vegetation is described using the biological forms (F:Phanerophyte, Ch:Chamaephyte, E:

Hemicryptophyte, I:Hydrophytes) and it is said if Selaginellae (S) and mosses (M) are present. The next column P.h. (Person-hours) indicates how many hours were spent overall on searching snails in each plot by naked eye and using the floatation technique(Eye+Fl.), and how many days the "salad trap" (S.T.).

## -Greenhouse $\mathbf{O}$

This is the greenhouse (Fig.4) with the highest variability in environments present in the Hortus botanicus. Both terrestrial and aquatic plants are present alternatively all around and plants are planted directly on the soil. On the bottom
of each pool silt is present. The soil of the terrestrial beds are made with universal soil except for O 4 and O 5 that present a sandy soil with small pebbles. Some pots are also present on the corner of the pools. Moreover, it is also the warmest greenhouse. The temperature varies from a minimum of $20^{\circ} \mathrm{C}$ when the temperature outside are cold and there is no sunlight to a maximum of $35^{\circ} \mathrm{C}$ when outside is warmer and the sun is shining. Anyway, the standard temperature is between 23 and $25^{\circ} \mathrm{C}$ (Van Vugt, pers. com.) The humidity is always high (80-90 $\%)$ thanks to the evaporation of the water from the pools. The sampling patches were created looking at the characteristic of each single bed. For example, if there were similar areas close each other with Terrestrial humid soil, they were placed in the same sampling site. If one was terrestrial and the next one aquatic they were considered as different sampling sites. Most of the aquatic sampling sites also had some pots with terrestrial plants in it that were considered as part of the same sampling site. Finally, some aquatic sites have the ground level half under the water level and half emerging. These were considered to be the same sampling site. The big pool was divided in two to be able to better analyse it. The environmental data of each single sampling site are described in Tab.3.


Fig.4: Sampling patches in greenhouse O

| Patch | Size | Env. | Ground conditions | S. R. G. | Litter | Vegetation | P.-h.: Eye+FI_S.T. | Notes |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\mathrm{m}^{\wedge} 2$ |  |  |  |  |  | hours_days |  |
| 01 | 1,8 | T | Soil alternatively dry and humid | No | Absent | Ch | 0,5_3 |  |
| 02 | 2,6 | A, T | aquatic plants, wet corners and bottom of the pool, pots | No | Absent | He | 0,75_0 | Stream absent |
| 03 | 1,1 | A, T | aquatic plants, wet corners and bottom of the pool, pots | Yes | Absent | 1 | 0,75_0 | Stream absent |
| 04 | 1,1 | T | Soil alternatively dry and humid | Yes | Absent | Ch, E | 0,25_3 |  |
| 05 | 1,1 | T | Always dry soil | Yes | Absent | Ch, E | 0,25_3 |  |
| 06 | 1,5 | T | Always dry soil | No | Absent | Ch, E | 0,5_3 |  |
| 07 | 4,0 | A, T | aquatic plants, wet corners and bottom of the pool, pots | Yes | Absent | E, I, He | 1_3 | Stream absent |
| 08 | 1,2 | A, T | aquatic plants, wet corners and bottom of the pool, pots | No | Absent | I | 0,75_0 | Stream absent |
| 09 | 1,1 | T | Soil alternatively dry and humid | Yes | Absent | Ch, E | 0,5_3 |  |
| 010 | 0,4 | T | Soil alternatively dry and humid | No | Absent | Ch, E | 0,25_3 |  |
| 011 | 0,4 | T | Soil alternatively dry and humid | No | Absent | Ch, E | 0,25_3 |  |
| 012 | 2,3 | T | Soil alternatively dry and humid | No | Absent | Ch, E | 0,5_3 |  |
| 013 | 1,1 | A, T | aquatic plants, wet corners and bottom of the pool, pots | Yes | Absent | He | 0,75_0 | Stream absent |
| 014 | 1,8 | T | Soil alternatively dry and humid | No | Absent | Ch, S | 0,5_3 |  |
| 015 | 2,3 | A, T | aquatic plants, wet corners and bottom of the pool, pots | Yes | Absent | 1 | 0,75_0 | Stream absent |
| 016 | 1,4 | A, T | aquatic plants, wet corners and bottom of the pool, pots | Yes | Absent | He, I | 0,75_0 | Stream absent |
| 017 | 1,1 | T | Soil alternatively dry and humid | Yes | Absent | Ch, E | 0,5_3 |  |
| 018 | 1,1 | T | Soil alternatively dry and humid | No | Absent | Ch, E | 0,5_3 |  |
| 019 | 1,5 | T | Soil alternatively dry and humid | No | Absent | E | 0,5_3 |  |
| 020 | 2,9 | T | Soil alternatively dry and humid | Yes | Absent | Ch, E | 0,5_3 |  |
| 021 | 1,1 | A, T | aquatic plants, wet corners and bottom of the pool, pots | No | Absent | 1 | 0,75_0 | Stream absent |
| 022 | 2,7 | T | Soil alternatively dry and humid | No | Absent | Ch, E | 0,5_3 |  |
| 023 | 1,2 | T | Soil alternatively dry and humid | Yes | Absent | Ch | 0,5_3 |  |
| 024 | 1,6 | T | Soil alternatively dry and humid | Yes | Present only in small areas | F, E | 0,5_3 |  |
| 025 | 4,4 | A, T | aquatic plants, wet corners and bottom of the pool, pots | Yes | Absent | F, Ch, E, I | 0,5_3 | Stream absent |
| 026 | 4,4 | T | Soil alternatively dry and humid | No | Present only in small areas | F, E | 0,5_3 |  |
| 027 | 4,4 | T | Soil alternatively dry and humid | No | Absent | F, E | 0,5_3 |  |
| 028 | 27,8 | A, T | aquatic plants, wet corners and bottom of the pool, pots | Yes | Absent | F, Ch, E, I | 2_3 | Stream present |
| 029 | 27,8 | A, T | aquatic plants, wet corners and bottom of the pool, pots | Yes | Absent | F, Ch, E, I | 2_3 | Stream present |

Tab.3: Environmental data of the sampling patches in greenhouse O. Env. Indicates the kind of environment (Terrestrial, Freshwater or both of them), S.R.G. says whether the solar radiation reaches directly the ground or not. The vegetation is described using the
biological forms (F:Phanerophyte, Ch:Chamaephyte, E Hemicryptophyte,
I:Hydrophytes, He:Elophytes) and it is said if Selaginellae (S) and mosses (M) are present. The next column P.h. (Person-hours) indicates how many hours were spent overall on searching snails in each plot by naked eye and using the floatation technique(Eye+Fl.), and how many days the "salad trap" (S.T.).

## -Greenhouses $\mathbf{U}, \mathbf{V}, \mathbf{Z}$

The greenhouse U (Fig.5) is one of the two greenhouses hosting the collection of South East Asian Orchids that were collected during research expeditions by the botanist E. Tevogel in Indonesia between 1991 and 1997. Other taxa of plants are also present like e.g. Nepenthes. The plants are placed in small pots on tables with pebble stones to make the water flow away easily after the watering. The soil is mostly composed of pine cortex but cork, arboreal fern cortex and common soil are also used. The temperature reaches a minimum of $13^{\circ} \mathrm{C}$ during the night to a maximum of $25^{\circ} \mathrm{C}$ during the day. The optimum for this greenhouse is between 14 and $23^{\circ} \mathrm{C}$ (Van Vugt, pers. com.). The humidity percentage is constant at $70-80 \%$ thanks to an automatic vaporizer system. The patches U1 and U3 consist in two
different tables each because the plants present there are very similar and commonly are exchanged from a table to the near one. U2 is one big table and U4 and U5 are the places where pots are hanged on pipes. The greenhouse V (Fig.5) is another hallway where plants are placed in big pots on the corner of the room. The temperature and the humidity are variable during the day and the soil is sandy in the dry plants and richer in organic material in the big pots. The greenhouse Z (Fig.5) is the second of the greenhouses hosting South East Asian Orchids and other plant groups. The soil is mostly composed of pine cortex but cork, arboreal fern cortex and common soil are also used. The temperature is higher than the other greenhouse and varies from a minimum of $20^{\circ} \mathrm{C}$ to a maximum of $30^{\circ} \mathrm{C}$ (Van Vugt, pers. com.). The humidity is set at $70-80 \%$ using an automatic vaporizer system. The sampling sites are organised symmetrically with greenhouse U .


Fig.5: Sampling patches in greenhouses U, V, Z

| Patch | Size | Env. | Ground conditions | S. R. G. | Litter | Vegetation | P.-h.: Eye+Fl_S.T. | Notes |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\mathrm{m}^{\wedge} 2$ |  |  |  |  |  | hours_days |  |
| U1 | 4,9 | T | Tables with pots and pebble stones | Yes | Absent | E, M | 2_4 | Orchids |
| U2 | 3,1 | T | Tables with pots and pebble stones | Yes | Absent | E, M | 4_4 | Orchids |
| U3 | 12,5 | T | Tables with pots and pebble stones | Yes | Absent | Ch, E, M | 2 -4 | Orchids |
| U4 | 5,0 | T | Pots hunged on pipes | Yes | Absent | E, M | 3_4 | Orchids |
| U5 | 3,3 | T | Pots hunged on pipes | Yes | Absent | E, M | 3_4 | Orchids |
| V | 11,3 | T | Pots and corners of the hallway | Yes | Absent | Ch, E | 1_0 |  |
| Z1 | 4,9 | T | Tables with pots and pebble stones | Yes | Absent | Ch, E, M | 2_4 | Orchids |
| Z2 | 3,1 | T | Tables with pots and pebble stones | Yes | Absent | E, M | 4_4 | Orchids |
| Z3 | 12,5 | T | Tables with pots and pebble stones | Yes | Absent | E, M | 2 -4 | Orchids |
| Z4 | 5,0 | T | Pots hunged on pipes | Yes | Absent | E, M | 3_4 | Orchids |
| Z5 | 3,3 | T | Pots hunged on pipes | Yes | Absent | E, M | 3_4 | Orchids |

Tab.4: Environmental data of the sampling patches in greenhouses U, V, Z. Env. Indicates the kind of environment (Terrestrial, Freshwater or both of them), S.R.G. says whether the solar radiation reaches directly the ground or not. The vegetation is described using the biological forms (Ch:Chamaephyte, E: Hemicryptophyte) and it is said if mosses ( M ) are present. The next column P.h. (Person-hours) indicates how many hours were spent overall on searching snails in each plot by naked eye and using the floatation technique(Eye+Fl.), and how many days the "salad trap" (S.T.).

## -Aquariums A_Aq_Dx and Small_Aq

These are two of the aquariums present in the Botanical garden. The A_Aq_Dx is the left aquarium present in greenhouse A. It is a big aquarium hosting many tropical fishes and plants. Many algae and dead trunks are present. The Small_Aq is a smaller aquarium present in the offices of the staff for ornamental purpose. No trunks are present there.

| Patch | Size | Env. | Ground conditions | S. R. G. | Litter | Vegetation | P.-h.: Eye+FI_S.T. | Notes |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | L |  |  |  |  |  | hours_days |  |
| A_Aq_Dx | Approx. 500 L | A | Pebbles | No | $/$ | I | N. D._0 | Stream present |
| Small_Aq | Approx. 30 L | A | Pebbles | No | $/$ | I | N. D._0 | Stream present |

Tab.5: Environmental data of the sampling patches in A_Aq_Dx and Small_Aq. Env. Indicates the kind of environment (Terrestrial, Freshwater or both of them), S.R.G. says whether the solar radiation reaches directly the ground or not. The vegetation is described using the biological forms (I: Hydrophytes) and it is said if mosses (M) are present. The next column P.h. (Person-hours) indicates how many hours were spent overall on
searching snails in each plot by naked eye and using the floatation technique(Eye+Fl.), and how many days the "salad trap" (S.T.).

### 2.3 Sampling methods

In order to maximise the quantity of captured snails it is important to be focused on the biology of the gastropods. These animals are usually more active when the environment around is more humid (Schilthuizen pers. com.), with low solar intensity and high temperature. This means that usually snails are more dynamic during the night and at early morning when the humidity is still high thanks to the dew. Moreover, not all the snails use to eat leaves on plants moving on the soil. Many snails species are detritivores and live in the soil or in the litter eating dead leaves. Some other are also carnivorous like Clea helena (Philippi, 1847). Many different capture techniques were used to look for gastropods. In this way all the microhabitats present in the greenhouses were analysed to collect the most of the species present in the greenhouses.

In the Hortus the greenhouses are divided in three types: the High tropical greenhouse where plants are directly planted in the soil, the greenhouses with the orchid's collection planted in pots placed on tables or hanged on pipes and a last greenhouse, the Victoria amazonica's with lots of pools for Hydrophytes. The way of collecting snails was adapted for each kind of environment to optimise the collection which will be explained beneath.

In the High tropical greenhouse (Greenhouse A), it was noticed that most of the plants have hard leaves both on trees and in the litter. These may have been difficult to eat for small snails. For this reason, in order to have a first idea of the species present in the different areas of the greenhouse, saucers with fresh salad coming from the vegetable garden of the Hortus botanicus were displayed in the middle of each patch. Hypothetically, snails would be attracted by more fresh, wet and soft leafs, than the most commonly hard leaves present in this greenhouse, reducing the manipulation time and increasing their fitness. After leaving these so called "salad traps" for one day snails were collected from the salad, the saucers, the soil around the saucer and the soil under it that remains more humid. This
technique resulted really useful to capture slugs (Deroceras leave (O.F.Müller, 1774), Lehmannia valentiana (Férussac, 1823)) and the most common species such as Subulina octona (Bruguière, 1789), Zonitoides arboreus (Say, 1816) and many others.

Some salad was also placed in pots hanged on plants of the canopy (Tab.6) to try and attract some arboreal species. Twelve pots were placed on different plants, trying to extend the arrangement in different areas of the canopy with different leaves hardness.

| Species | Family | $N^{\circ}$ Pots |
| :--- | :--- | ---: |
| Alsomitra macrocarpa (Blume) M. Roem. | Cucurbitaceae | 1 |
| Alsomitra sarcophylla (Wall.) M. Roem. | Cucurbitaceae | 1 |
| Bucida buceras L. | Combretaceae | 1 |
| Cinnamomum burmannii (Nees \& T. Nees) Blume | Lauraceae | 1 |
| Musa x paradisiaca L. | Musaceae | 1 |
| Strongylodon macrobotrys A. Gray | Fabaceae | 2 |
| Tectona grandis L. f. | Lamiaceae | 1 |
| Terminalia catappa L. | Combretaceae | 1 |
| Thunbergia mysorensis (Wight) T. Anderson | Acanthaceae | 3 |

Tab.6: Plant species where the hung salad traps were placed. The names of the plants have been taken from the labels and checked with http://www.theplantlist.org/.

Unfortunately using this technique no snails were collected. Even though no traces of snails feeding was present on the plants, some snails as it will be explained later were found on trees' leaves. We found this technique useless to collect snails. Anyway, some caterpillars present on the Banan were attracted and found on our "hanged salad trap".

Another method is to observe the area and find snails with the naked eye (Schilthuizen, 2011). Snails and slugs were looked for in each possible microenvironment present in the research area like the soil, moist places, leaves, dead trunks, and trees. This search was repeated three times: the beginning, it was done $\mathrm{Cm}^{2}$ per $\mathrm{Cm}^{2}$ collecting every snail avoiding missing some species difficult to distinguish at a first look. Than it was done other times only looking for new snails' species. The most of the samples were collected on the humid soil nearby
the dead leaves, in the litter, under the dead trunks and in little holes in the soil. Just five samples were collected on the plants: two Juveniles probably of Helicidae on a Ficus sp. in A1 and two other on small plants in A15. These snails were not identifiable and are not mentioned in this work. One Undetermined sp. 2 (Charopidae) was found on a Banana's leaf.

Another capturing technique consisted of pouring some beer in the same saucers previously used as "salad traps" to try and attract different species of snails. These so called "beer traps" are traditionally used in gardens to kill gastropods. This capture method is very useful because the animal dies after reaching the trap, whereas using the other capture techniques it is possible to miss some snails just because you didn't look for it in the right moment when it was there. Using the beer it's possible to collect all gastropods that reached the trap from the moment it was placed to the time it was checked. Mostly slugs were collected.

Another common way to collect snails is the so called "floatation technique" (Cameron and Pokryszko, 2005). In each patch 3 pieces of soil in different parts were taken using a small shovel. Approximately quadrats of $20 \times 20 \mathrm{Cm}$ were dug each time removing at least $1 \mathrm{~m}^{3}$ of soil. This material was placed in a bowl that was filled in with water. When the soil is wet it precipitates on the base of the bowl whereas the snails come up thanks to air bubbles inside the shell. This technique resulted really useful to collect empty shells deposited in the soil like Kaliella microconus (Mousson, 1865) and some empty shells of aquatic snails like Radix labiata (Rossmässler, 1835). Really small living snails present in the soil were also collected like Hawaiia minuscula (Binney, 1841) and Vallonia enniensis (Gredler, 1854).

Finally, another technique was tried. Some different 5-6 m high trees were shaken to try and make snails present on leaves falling on the ground (Schilthuizen and Rutjes, 2001). A cover was previously placed under the tree to see more easily what was falling down. No samples were collected using this technique. Probably either there weren't snails on the trees, as the other analysis suggested, or they were really well hidden.

In the hallways (greenhouses F and V ) the same techniques were used. In the patches F2 and F3 many Cornu aspersum (O.F.Müller, 1774) were collected on the leaves various plants with big leaves. All Discus rotundatus (O.F.Müller, 1774) in this greenhouse were found on the glasses eating little algae. In the V greenhouse most of the samples were collected in or near or under the big pots present there.

In the greenhouses with the collection of orchids (Greenhouses $\mathrm{U}, \mathrm{Z}$ ) snails were looked in each single pot, on the plants, soil, on the outside and in the saucer which is the most humid place and on the soil where the pots are placed on. Some snails were also collected in the pebble stones on the tables were pots are displaced and on the ground were little ferns are present. Most of the snails were collected in the saucers and on the mosses growing on the pots of the orchids. All the snails collected on the pots were really small like the Charopidae and Lauria cilindracea ( Da Costa, 1778). On the pebble stone mostly empty shells were found and on the ground mostly Zonitoides arboreus and Oxychilus sp. were present. Salad traps and the floatation technique were used as well. This time, in order to do the flotation, pebbles on the tables were taken after a first check by sight.

The same techniques were used in the nurseries (Greenhouses H1, I, LM, N). In the terrestrial patch of greenhouse H the snails were collected only on the table close to the little aquarium and on the ground. In the greenhouses LM and N all the samples were collected on or under the pots.

The environment of the Victoria amazonica greenhouse ( O ) is much more complex for the collecting of snails. Sometimes the water is turbid and you don't manage to observe snails under water. The best way to proceed is to look for snails starting from the top going down, first on the emerging plants, than on the corners of the pools, on both sides of floating leafs and on the plants living under the water level. Some snails are also living on or in the mud. Some species are easy to find because they are quite big like Melanoides tuberculata (O.F.Müller, 1774) and Helisoma anceps (Menke, 1830). One can just touch and feel them between the soil particles. Some soil was also taken and cleaned with a filter removing the black silt to be able and see the white small dead snails. Floating
pots were put on the water of the big pools (O25, O28, O29). It resulted useful to collect small snails living on floating leafs because it is more easy to see them on the dark pot. Finally, salad traps were used on the places with terrestrial plants.

In the little aquarium in the greenhouse $\mathrm{H}(\mathrm{H} 2)$ all the samples were collected by sight. The samples of the other aquariums (A_Aq_Dx and Small Aq) were collected during the cleaning procedures of these aquariums by the gardeners. The snails of A_Aq_Dx were removed sucking up the pebbles present in the aquarium with a tube. Not all the species were collected in this capture site due to the risk of damaging the expositional site. The snails collected in Small_Aq were collected changing the water of the aquarium, cleaning the glasses and the plants.

During the collection no snails were found on toxic plants or in completely dry environments. In all the greenhouses snails were searched in different moments of the day, from the sunrise to the sunset, both before and after the plants and the soil were watered by the gardeners. This resulted in the collection of all snails with different activity periods. Using all these techniques for each spot of each greenhouse under study, we noticed that it's by sight that you collect the most of the snails but only summarising all different techniques, it's possible to collect the biggest number of species occupying different habitats and microhabitats.

### 2.4 Results

In total 52 species were collected of which 29 terrestrial and 23 freshwater. 41 were identified at the species level (Trivellato, 2015). 25 species are endemic and 19 alien including 3 of the unidentified species (Unidentified sp. 3 (Charopidae), Unidentified sp. 4 (Charopidae), Unidentified sp. 5 (Charopidae)) that were considered to be alien from many consulted experts (De Winter T., Vermeulen J., Stanisich J., Köhler F., Cameron R.A.D., Horsák M.) probably of South East Asian origin. We can't say for sure whether Paralaoma sp., Pristiloma sp, Littorina sp., Planorbarius sp., Undetermined sp. 2 (Lymneidae), Undetermined sp. 8 (Zonitidae), Unidentified sp.9, Unidentified sp. 10 are endemic or alien (Trivellato, 2015).

Generally speaking (Fig.9) in all the greenhouses under study endemic gastropods were collected whereas most of the alien species were found in greenhouse A and O. Moreover, in A g. many species where only collected as empty shells and the diversity of species for such a big greenhouse is relatively low compared to much smaller greenhouses like U and Z that appear to have the same diversity of species. The O g . is the one hosting the biggest diversity in alive gastropods species. Most of the not identified species, all of them of tropical origin, were collected in greenhouses U and Z . Greenhouses $\mathrm{H}, \mathrm{I}, \mathrm{LM}$ and N are hosting most of all endemic terrestrial species except for the freshwater snails present in Hg . Having a look with a deeper resolution at the species collected in the sampling sites (Fig.8), as already suggested by the previous Fig.9, in most of the patches it's possible to find endemic gastropods. There are just two sites (F1, F4) where no gastropods were collected and some other like O 4 and O 5 hosting just one species. In A g. most of the empty shells are concentrated, in patches A7, A8, A9, A10 whereas alien species are present in almost all patches. In greenhouse O , great differences in diversity of species among the sampling sites was observed. U and Z have a comparable quantity of species. The sites with greater diversity of species in all the greenhouses are A15, F2, O7, O15, O16, O18, O25, U2, Z1, Z2.

The greenhouses U and Z are hosting medially the biggest number of the species per plot (Tab.7). Whittaker's index I "calculated as the overall species richness divided by the mean number of species per plot"(Schilthuizen and Rutjes 2001), is 10,13 considering all the sampling sites in all greenhouses and varies between different greenhouses. These data have been later analysed considering only the alive gastropods. By doing so, greenhouses $\mathrm{A}, \mathrm{H}$ and U appear similar for the mean of species found per plot. I calculated in all sampling sites is 8,26 . This parameter becomes comparable in $\mathrm{A}(3,13)$ and $\mathrm{F}(3,27)$ like in $\mathrm{U}(1,90)$ and Z $(1,97)$ whereas it is much higher in $\mathrm{O}(5,36)$.

The most frequent terrestrial species in the greenhouses is Deroceras leave (Fig.6). Subulina octona, Zonitoides aroreus and Zonitoides nitidus (O.F.Müller, 1774) are also quite common. All the other terrestrial species are less frequent. The more frequently collected species among the pools (Fig.7) are Physella acuta
(Draparnaud, 1805), Melanoides tuberculata and Pseudosuccinea columella (Say, 1817). Some other common species are Radix labiata and Bithinia tentaculata (Linnaeus, 1758). Ten freshwater species were only found as empty shells.

Having a look to the previous collections in the greenhouses of the Hortus present in the Naturalis Biodiversity Center (Tab.8), we immediately observe the great difference in species collected between the collection carried on this year and the previous ones. For sure in the past only few species were collected in the greenhouses and the research was not done in deep resolution. It is only possible to know when some species were collected but we don't know if many of the not recorded species in the past were really not present. The first reliable collection was done in 1995 by T. De Winter. Two alien species (Trivellato, 2015), Subulina octona and Helisoma duryi (Wetherby, 1879), have been recorded in the past and still are present alive today. 12 endemic species were recorded in the past and are still present there. 13 spp . that were collected in the past are not present in our collection. 38 species, of whom 13 only as empty shells were recorded in the greenhouses for the first time in 2015.

All data that were used to create Fig.6, Fig.7, Fig.8, Fig. 9 and Tab. 7 are present in the Appendix.


Fig.6: Frequency expressed in percentage; the terrestrial species collected both as alive specimen (blue bar) and empty shell (red bar) in the terrestrial sampling sites are indicated.


Fig.7: Frequency expressed in percentage, The freshwater species collected both as alive specimen (blue bar) and empty shell (red bar) in the aquatic sampling sites are indicated. Species that were collected only as empty shells in terrestrial sampling sites have $0 \%$ of frequency.

| N Nocies/Plot |
| :--- |


| IdentificationVerb | Uncertain | 1891 | 1909 | 1911 | 1935 | 1938 | 1942 | 1964 | 1995 | 2002 | 2015 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Aegopinella nitidula (Draparnaud, 1805) |  |  |  |  |  |  |  |  |  |  |  |
| Archiphysa parkeri (Currier, 1881) |  |  |  |  |  |  |  |  |  |  |  |
| Australorbis nigricans (Spix, 1827) |  |  |  |  |  |  |  |  |  |  |  |
| Balea biplicata (Montagu, 1803) |  |  |  |  |  |  |  |  |  |  |  |
| Bithynia tentaculata (Linnaeus, 1758) |  |  |  |  |  |  |  |  |  |  |  |
| Candidula intersecta (Poiret, 1801) |  |  |  |  |  |  |  |  |  |  |  |
| Carychium tridentatum (Risso, 1826) |  |  |  |  |  |  |  |  |  |  |  |
| Catinella cf. arenaria (Bouchard-Chantereaux, 1837) |  |  |  |  |  |  |  |  |  |  |  |
| Cecilioides acicula (O.F.Müller, 1774) |  |  |  |  |  |  |  |  |  |  |  |
| Cepaea nemoralis (Linnaeus, 1758) |  |  |  |  |  |  |  |  |  |  |  |
| Clea helena (Philippi, 1847) |  |  |  |  |  |  |  |  |  |  |  |
| Cochlicopa lubrica (O.F.Müller, 1774) |  |  |  |  |  |  |  |  |  |  |  |
| Cornu aspersum (O.F.Müller, 1774) |  |  |  |  |  |  |  |  |  |  |  |
| Deroceras laeve (O.F.Müller, 1774) | x |  |  |  |  |  |  |  |  |  |  |
| Discus rotundatus (O.F.Müller, 1774) |  |  |  |  |  |  |  |  |  |  |  |
| Euconulus fulvus (O.F.Müller, 1774) |  |  |  |  |  |  |  |  |  |  |  |
| Gyraulus albus (O.F.Müller, 1774) |  |  |  |  |  |  |  |  |  |  |  |
| Gyraulus cf. parvus (Say, 1817) |  |  |  |  |  |  |  |  |  |  |  |
| Gyraulus laevis (Alder, 1838) |  |  |  |  |  |  |  |  |  |  |  |
| Hawaiia minuscula (Binney, 1841) |  |  |  |  |  |  |  |  |  |  |  |
| Helisoma anceps (Menke, 1830) |  |  |  |  |  |  |  |  |  |  |  |
| Helisoma duryi (Wetherby, 1879) |  |  |  |  |  |  |  |  |  |  |  |
| Kaliella microconus (Mousson, 1865) |  |  |  |  |  |  |  |  |  |  |  |
| Lamellaxis cf. gracilis (Hutton, 1834) |  |  |  |  |  |  |  |  |  |  |  |
| Lamellaxis clavulinus (Potiez \& Michaud, 1838) | x |  |  |  |  |  |  |  |  |  |  |
| Lauria cylindracea (Da Costa, 1778) |  |  |  |  |  |  |  |  |  |  |  |
| Lehmannia valentiana (Férussac, 1823) | x |  |  |  |  |  |  |  |  |  |  |
| Lithoglyphus naticoides (Pfeiffer, 1828) |  |  |  |  |  |  |  |  |  |  |  |
| Littorina sp. |  |  |  |  |  |  |  |  |  |  |  |
| Lymnaea stagnalis (Linnaeus, 1758) |  |  |  |  |  |  |  |  |  |  |  |
| Melanoides tuberculata (O.F.Müller, 1774) | x |  |  |  |  |  |  |  |  |  |  |
| Neritina natalensis (Reeve, 1855) |  |  |  |  |  |  |  |  |  |  |  |
| Oxychilus cellarius (O.F.Müller, 1774) | x |  |  |  |  |  |  |  |  |  |  |
| Oxychilus draparnaudi (H.Beck, 1837) |  |  |  |  |  |  |  |  |  |  |  |
| Oxyloma dunkeri (L,Pfeiffer, 1865) |  |  |  |  |  |  |  |  |  |  |  |
| Paralaoma sp. |  |  |  |  |  |  |  |  |  |  |  |
| Physella acuta (Draparnaud, 1805) |  |  |  |  |  |  |  |  |  |  |  |
| Planorbarius sp. |  |  |  |  |  |  |  |  |  |  |  |
| Pomacea bridgesii (Reeve, 1856) |  |  |  |  |  |  |  |  |  |  |  |
| Pristiloma sp. |  |  |  |  |  |  |  |  |  |  |  |
| Pseudosuccinea columella (Say, 1817) | x |  |  |  |  |  |  |  |  |  |  |
| Punctum cf. pygmaeum (Draparnaud, 1801) |  |  |  |  |  |  |  |  |  |  |  |
| Radix labiata (Rossmässler, 1835) |  |  |  |  |  |  |  |  |  |  |  |
| Radix ovata (Draparnaud, 1805) |  |  |  |  |  |  |  |  |  |  |  |
| Semisalsa stagnorum (Gmelin, 1791) |  |  |  |  |  |  |  |  |  |  |  |
| Stagnicola palustris (O.F. Müller, 1774) |  |  |  |  |  |  |  |  |  |  |  |
| Subulina octona (Bruguière, 1789) |  |  |  |  |  |  |  |  |  |  |  |
| Subulina striatella (Rang, 1831) |  |  |  |  |  |  |  |  |  |  |  |
| Succinea sp. |  |  |  |  |  |  |  |  |  |  |  |
| Trichia hispida (Linnaeus, 1758) |  |  |  |  |  |  |  |  |  |  |  |
| Undetermined sp. 2 (Lymneidae) |  |  |  |  |  |  |  |  |  |  |  |
| Undetermined sp. 3 (Charopidae) |  |  |  |  |  |  |  |  |  |  |  |
| Undetermined sp. 4 (Charopidae) |  |  |  |  |  |  |  |  |  |  |  |
| Undetermined sp. 5 (Charopidae) |  |  |  |  |  |  |  |  |  |  |  |
| Undetermined sp. 9 |  |  |  |  |  |  |  |  |  |  |  |
| Undetermined sp. 10 |  |  |  |  |  |  |  |  |  |  |  |
| Vallonia enniensis (Gredler, 1856) |  |  |  |  |  |  |  |  |  |  |  |
| Vallonia excentrica (Sterki, 1893) |  |  |  |  |  |  |  |  |  |  |  |
| Vallonia pulchella (O.F.Müller, 1774) |  |  |  |  |  |  |  |  |  |  |  |
| Valvata piscinalis (O.F.Müller, 1774) |  |  |  |  |  |  |  |  |  |  |  |
| Vitrea cristallina (O.F.Müller, 1774) |  |  |  |  |  |  |  |  |  |  |  |
| Viviparus contectus (Millet, 1813) |  |  |  |  |  |  |  |  |  |  |  |
| Zonitoides arboreus (Say, 1816) | x |  |  |  |  |  |  |  |  |  |  |
| Zonitoides cf. nitidus (O.F.Müller, 1774) |  |  |  |  |  |  |  |  |  |  |  |
| Zonitoides nitidus (O.F. Müller, 1774) |  |  |  |  |  |  |  |  |  |  |  |
| Zonitoides sp. |  |  |  |  |  |  |  |  |  |  |  |
| Tot | 7 | 1 | 1 | 2 | 1 | 1 | 6 | 1 | 21 | 1 | 52 |

Tab.8: Years in which each species was collected in the greenhouses of the Hortus according to the Naturalis' collection.

### 2.5 Discussion

This research project shows the presence of an extremely rich and diverse malacofauna hosted in the greenhouses of the Hortus botanicus composed of 52 species (Trivellato, 2015), both endemic and alien in a comparable quantity of species suggesting that both alien and endemic species can similarly live and be collected in the greenhouses. Anyway, it is important to notice that 13 of the collected species were only found as empty shells and only 39 were alive. This means that overall we collected 13 alien species alive, 6 alien species dead, 19 endemic species alive, 6 as empty shell, 7 alive species we don't know the origin and one other as empty shell.

In order to have a clearer idea of the distribution of the species in the greenhouses, all data were elaborated separating the alive species from the empty shells. Unfortunately, the presence of empty shells can't be easily interpreted. In some cases, like for patches A7, A8, A9 and A10, we know that the abundance of empty shells is related to the activities of cleaning the aquariums present in greenhouse A. The shells, in fact, are of freshwater species. In case of terrestrial species we can't say if the species arrived in the greenhouses alive and then the population got "extinct" or come directly dead and never lived in the greenhouses. A salty water species, Littorina sp., was also collected as empty shell on the soil of greenhouse A but we don't know how it arrived there. To avoid data distortion, empty shells were not considered in the discussion about the distribution of the species. Moreover, the data may have been influenced by the creation of the sampling patches, their size, shape (Brummer et al., 1994, Cameron and Pokryszko, 2005) and the collecting techniques used.

Anyway, the greenhouses show not only a great diversity in species (39 alive species), but also a high differentiation in snails composition between all the greenhouses. In fact, Whittaker's index $I$ calculated for the alive species in all sampled plots (Tab. 7) is 8.26 , really similar to the 8.6 reached in a square Km of the tropical forest in Sabah, Malaysian Borneo (Schilthuizen and Rutjes, 2001). The group of greenhouses where the research was carried on are a surprisingly diversified in gastropods composition similarly to what can be registered in a tropical natural forest. The differentiation in snail composition in each single
greenhouse, instead, is quite small and do not show a significant difference between the sampling sites. Anyway, as expected, greenhouse O has a good value of $I$ index $(5,36)$. This is related to the great diversity of microhabitats present in this greenhouse that, in fact, hosts the biggest number of species. These data suggest a direct relation between the environmental conditions (Temperature, humidity, kind of soil) of the greenhouses and the community of gastropods present there. As an example, greenhouses U and V have quite the same kind of plants, same humidity percentage in the air, same kind of soil used for plants and same size of sampling patches. The only differing parameter is the temperature, lower in $\mathrm{U}\left(13-25^{\circ} \mathrm{C}\right)$ and higher in $\mathrm{Z}\left(20-30^{\circ} \mathrm{C}\right)$. As a result, only these greenhouses have 6 species in common but 5 species present in $U$ were not recorded in Z . On the contrary 9 species collected in Z are not present in U .

Generally speaking, comparing our environmental data with the abundance of species present in each sampling site, it was possible to observe that terrestrial gastropods living in the greenhouses are much more abundant in the plots where the vegetation is really dense and organised in different layers with Phanerophyte, Chamaephyte and Hemicryptophyte according to Raunkiaer's life form system making the environment more stable and humid. It is not important the flora present on the site but the vegetation covering the ground, where most of the samples were collected. Other important parameters are the presence of decaying material (most for alien species) like litter and dead trunks, the absence of solar radiation on the ground and the high humidity percentage in the air. A dry substrate, with poor vegetation cover, no litter, direct sunlight drying out the surface and the presence of toxic plants make the environment unhospitable for gastropods. All these factors contribute together to the presence and distribution of the terrestrial species in the greenhouses. It was not possible to understand the environmental condition making freshwater species more abundant due to the small number of sampling sites available (14).

This research also proved the endemic species to be a real threat for the greenhouses and potentially for the tropical ecosystem itself. In fact, these species are distributed almost in all the sampling sites and seems to be very well adaptable to the tropical climatic conditions. They don't seem to be closely related to the
environmental parameters of temperature and humidity, nor of vegetation type and kind of soil. Moreover, some of these species, like the Helicidae and the slugs, are herbivorous creating great damages to the plants. It was in fact seen in greenhouse F that in many plants having wounds Helicidae were present. The widest distributed land slug is Deroceras leave, an endemic species for The Netherlands (Trivellato, 2015), was observed in two different forms: a dark one only present in greenhouse O and a grey form distributed in all the other greenhouses. Studying the genitalia of 20 collected samples it was possible to see that most of them had only the female reproductive system. This species are known to be self-fertile (De Winter pers. com.). We can assume that these are two different populations of the same species having two separated morphotypes. These two forms moreover occupy two different environments: the dark form prefers always humid and warm areas of the greenhouse O like the corners of the pools and the leaves of the emerging plants. The grey one prefers terrestrial environments with a variable percentage of humidity on the ground typical in the other greenhouses. Biogeographical barriers like the doors of the greenhouse O moreover separate these two populations. Plants are not usually moved from this place to the other greenhouses. It may be interesting to do DNA analysis to see if there's some isolation effect that in the future may result in a speciation. Another endemic dangerous species is Lehmannia valentiana, a less distributed slug often found feeding on orchids and other plants. Also two of the most abundant freshwater nails are endemic: Physella acuta and Pseudosuccinea columella. These species are known to be invasive but do not represent a direct threat for plants as they are feeding on detritus and algae (http://www.molluscs.at/, Zarco et al., 2011). These species of course were introduced with plants and soil because they can't tolerate the terrestrial environment and would dry very quickly. The introduction of species caused by human activities is also really easily observed having a look at the species present in the nursery greenhouses $\mathrm{H}, \mathrm{I}, \mathrm{LM}, \mathrm{N}$ : in these places where plants are moved inside the greenhouses and new arrived plants are placed, only endemic terrestrial species are present. Endemic species are well recorded in previous collections and it is possible to assume that since the foundation of the greenhouses these gastropods were present there. As said before, they are a real threat for the tropical greenhouses and probably for the tropical ecosystem itself
thanks to the easily way they are disperse, the good adaptability to many different climatic conditions and, for some species, the herbivorous diet.

The alien species also are present and well distributed in the greenhouses. These species comparing their distribution with the environmental data, are much more abundant and distributed in the greenhouses $\mathrm{A}, \mathrm{O}, \mathrm{U}$ and Z . What these greenhouses have in common is a constant humidity percentage provided by the automatic vaporizers. In the other greenhouses, we find really variable humidity percentage and very few tropical snails were collected. The most common freshwater alien species is Melanoides tuberculata (Fig.7) that, like the previous collected snails, does not feed on plants. What makes this species interesting is that, like for the case of Deroceras leave, two morphotypes in different plots were found: the "smoothed shell" one lives in O28 and O29, the central pool of Victoria amazonica greenhouse. A more ornamented shell type was collected in all the other sites. It leaves in the lime present in the pools eating detritus and do not feed directly on plants. Moreover, it's interesting to notice that the two of the not identified Charopidae, that were assumed to be of south east Asian origin, are only present in the greenhouses where south east Asian orchids are present. Just one of these species is also present in other greenhouses. Hypothetically, these snails were brought to the Hortus with the orchid of wild origin. Terrestrial alien species quite well distributed are Zonitoides arboreus and Subulina octona (Fig.6). These species do not feed on plants (Jurickova, 2006; Karlin, 1956) but on decaying plant material. Zonitoides arboreus is the only terrestrial alien species collected in previous collections (1995) (Tab.8) that is still present today. Another alien species collected in the past and found nowadays is Helisoma duryi, known to be present in the greenhouses since 1964. Alien species general do not seem to represent a direct threat for plants in the greenhouses both for their habitat closely related to the high humidity percentage, warm temperature and for their apparently poor presence record in the past. It is also possible to notice that, even though greenhouse A is much more bigger and was analysed with 28 sampling sites, the total amount of species is relatively low and similar to what was recorded in greenhouses U and Z , much smaller. This may be a delayed effect of the restoration of the greenhouses that was done in 2012 when the soil where snails lived was exposed to open air for two winters and probably, reducing the
diversity of alien species hosted in the greenhouse. It's possible to assume that most of the tropical species, once escaped from the greenhouses, would not survive the cold winters in The Netherlands. Further studies are anyway necessary because some of our collected species like Hawaiia minuscula are known to be present in temperate natural ecosystems (Lori and Cianfanelli, 2007).

Having a deeper look at the comparison between the more recent collection and the previous ones it is possible to see that many species collected in other collection are not present any more in the greenhouses under study and that many new collected species are present today. The greenhouses appear to be a place in which periodically, due to different activities, new species are introduced. Alien species, anyway, do not represent a real threat for the plants. These animals are mostly detritivores, feeding on decaying litter being collected on the soil and rarely on vegetation. Moreover, one single species usually is not widely distributed, except the mentioned ones, in all the greenhouses. They occupy just some parts of the environment and, sometimes, due to some events, like a restoration could represent, may not manage to survive. This is to say that, on the other hand, according to our data, most of the alien species present in the botanical garden, are not dangerous for plants. Anyway, like the endemic species, they can easily become invasive even though without any kind of apparent damage. Much more threat, instead, is represented by some endemic species as previously argued.

Finally, greenhouse gastropods in general, can also represent an unexpected value for the greenhouses. Feeding on the litter snails can improve the process of decomposition of the leaves, contributing to create new material useful for plant nutrition. Moreover, these animals, like some insects present in the greenhouses, may be a good prey for other animals hosted there like 8 frogs that were seen in the greenhouses. These frogs can be seen in some matter a natural way of controlling the growth of gastropod's population.

### 2.6 Output of our research project

Our collection, stored in 415 vials, was donated to the Natural Biodiversity Center with many related data (half of them are present in the appendix in Tab. $13,14,15,16,17$. The rest of the ecodata are present in "Trivellato, 2015" that will be useful for future analysis as a little reference collection of the Hortus's malacofauna of 2015. This research resulted also in a poster that was presented at the Molluscan forum 2015 at the Natural History Museum of London (19/12/2015). It had been a great occasion to meet Phd students and Professors that gave us important pieces of advice. Our work will also be submitted to a specialised journal. Finally, a poster will be created and placed in the greenhouses with the intention to show the visitors the presence and distribution of the greenhouse gastropods. By doing so visitors of the Hortus will be able to find and recognise the present gastropods.

## Activities in the Botanical garden

### 3.1 Management

The responsible function of all the personal, public and financial activities is the prefect of the botanical garden. He has a high representative function for the Hortus in meetings and events with various groups within the University and other partners. His roles are also related to teaching, supervise students, taking care of the administrative affairs and the financial management of the garden. Part of the budget is provided by the University. It is anyway necessary, working with the head of marketing and sales, to generate income to finance expenses like restorations, investments, the development of new projects. The tickets sold, the catering activities and the museum shop are a various sources of income. Than sponsors, funding, the association of the friends of the garden and funds for contributing for events help in financing the activities. Friends of the garden are people paying a membership to the association. By doing so they obtain one year free entrance. At the end of the year the association transfers a certain percentage of the income to the Hortus. The Hortus organises the "tree adoption" programme
via the "Friends". Anyone can adopt a tree for one year or longer giving a donation. The person having adopted the tree will have the opportunity to have the fruits produced by this plant or will be informed about any care operations done on the plant. These money will be used to pay specialist if some plant would need attention.

In order to invest in the best way the budget they have, a management team discusses the plans, programs and the strategic ways to develop the garden for the year. Inside the Hortus botanicus people work together. This helps to know each other better and makes the work easier. All the people working in the garden use to meet each other during the breaks to socialise, discuss problems and share ideas. This stimulates people in doing their daily work. Once a year daily excursions are organised inviting all the staff members to join an experience being colleagues-friends and making of all the group one single team. Visitors are also asked to give their opinion about the garden. This participatory approach is really useful to improve the quality of the Hortus. The people working in the garden of course look differently at the collections than a visitor may do.

It is also important to discuss and organise other activities and collaborations with other partners like museums and other gardens in Leiden. The Hortus is a member of the Dutch association of museums and the Dutch Botanical garden association. The 24 botanical gardens in The Netherlands work together and are all specialised in a different plant group or are focused on a different region of the world. Nowadays it is crucial to collaborate and pay much attention to University Botanical gardens. In The Netherlands, as an example, only three Botanical Gardens are still part of a University (Delft, Leiden, Utrecht). Five other of them were transformed to non university gardens, becoming more similar to parks. Once this happens teaching and research risks to be cut off the garden, loosing an important part of its strategic role in education and the scientific world. Anyway, the association has great plans for the future facing this problem enhancing public activities and making all gardens work closer each other.

### 3.2 Horticulture

Working together with gardeners many cultivation methods and problems in taking care of the plants were learned. The daily working experience done during the internship make us learn many practical ways to cultivate plants relating them to their biology.

## -Orchids

The most important substrate to make the orchids grow is the cortex of fern tree and Sphagnum moss. When some new orchids arrive in the greenhouses, it's fundamental to be informed on where the plant was collected to understand the plant's needs. If nothing is known about the plant's habitat, the best thing to do is to experiment various kind of substrate and climatic conditions, also using the personal experience, and see where the plant lives better. Orchids are watered every day using Osmose water to keep them grow well. In fact, the roots of these plants would be damaged by Calcium present in the normal drinkable water. Some plants prefer to have a little concentration of calcium that is provided by a specific kind of soil. Any orchid must become completely dry before it is watered again. The turnover of dry and wet substrate is vital for them. If an orchid is too dry, the best thing to do is to place the roots in water at least six hours. It will absorb all the necessary water and recover. In the greenhouses hosting orchids, moreover, a ventilation system is present. This reduces the risk of fungal infection on plants.

## -Victoria amazonica

The Victoria amazonica (Poepp.) J. C. Sowerby is a famous plant all around the world. In its natural areal, this perennial plant has almost a constant solar intensity and many sunlight hours all year long. At the end of the summer at our latitudes the decrease of the sunlight hours make the plant suffer producing small yellowish leaves. For this reason every year in winter the plant is composted and replaced planting seeds the next spring. This problems is much higher for tropical plants in botanical gardens at high latitudes. Many of them, in fact, may not survive with low solar intensity and seven hours light a day or less in winter time. This is the reason why sometimes it is necessary to put some lamps to add 5 hours light a day and make the plants survive but it's really expensive solution.

## -Tropical greenhouse

In tropical forests, as mentioned before, the soil is relatively poor and only the very first layers are rich in organic material. The same criteria was used for the soil in the Tropical greenhouse, generally sandy and poor in nutrients but rich in organic material on the surface. The plants are watered every day from the canopy to wash the leaves. The water must be given gently using a nebulised low intensity avoiding the leaves to be damaged. This operation takes at least one hour to water all plants well. By doing so the soil receives only a small part of the water given from the top. For this reason twice a week all the plants in the greenhouse are also watered from the ground paying attention to each individual plant needs. Cycas, for example, must be watered much less and Banana tree otherwise the apical bud may rot provoking the death of the plant. Sometimes disinfestations of plant's pests are necessary. The Hortus uses usually biological control methods. This year caterpillars were found on Banana's leaves and are now being reduced in number using particular tubes attracting insects.

For the perfect functioning of a greenhouse, many operations like the humidity regulation system, the heating and the opening of the windows are automatically controlled from a computer. This, on one hand is really helpful for people working in the garden and for sure, creates the best conditions for plant's grow. On the other hand it is a great threat. If a black-out appends, these delicate environment may be strongly damaged. During winter time no heating may make plants suffer and, more dangerously, in summertime the tropical house with closed windows and no vaporised water may reach extremely high temperatures, drying all the plants and the soil. The only way to avoid this risk is to have an emergency generator.

## -Other greenhouses

A plant in a greenhouse, the more it is far from the glass, the less light it receives. This is the reason why plants that needs a lot of sunlight like Lithops should be placed really close to the glass to grow well. Most of the succulent plants needing direct and intense sunlight, in fact, are displaced in the nearer of the glasses in the greenhouses. For this kind of plants the soil composition consists in $1 / 3$ of sand,
$1 / 3$ of little pebbles and $1 / 3$ of universal soil. It is the best compromise to fulfil the plant's needs of a poor and dry soil, allowing drainage of the water.

Nepenthes, that are also abundant in the greenhouses, are watered two or three times a week. By doing so it's important not to fill in the pitchers directly with water. In fact, when it rains, plants living in nature receive very fey water inside their modified leaves. The rest is provided by the plant itself regulating the concentration of the solution to digest insects. The soil must be poorly fertilised because too many nutrients may be toxic.

## -The garden

Much work is also required by plants living outside the greenhouses. Gardeners and volunteers work together to take care of them. The soil present in the Hortus is most of all made of either clay or sand and the aquifer is just half a meter under the surface level. The combination of these two factors can make the soil really inhospitable for plants in certain occasions. In fact, the sand very easily makes water flow down making the soil drying faster than what it may be expected in a Oceanic climatic region. Moreover, roots can't be oxygenated at the aquifer level without any airier parenchyma. So many plants can only produce roots in a small layer of substrate that can easily dry out. For this reason in summertime water is daily pumped from the canal and irrigation systems are working many hours a day.

Many plants planted in the garden during the winter time are covered to manage and survive the night frosts. The more delicate plants are planted in pots and are placed in the Winter garden or in the Orangery in this period. Much care is also needed for ancient trees present in the Hortus like Ginkgo biloba L. planted in 1785 and Laburnum anagyroides Medik. dated from 1601. These plants are so delicate both for the risk of being attacked by infections and for the damages a strong storm may cause. Periodically specialised gardeners are called to take care of these plants and, in case of bad weather condition, the garden is closed for security reason.

### 3.3 Conclusions

During their study path students are told about a simplified world where all its single elements are put in different groups. This is a really helpful way to learn how scientists described nature and how to be able and talk with other people about its aspects. Anyway, once all these information has been understood, it is important to have a more open and interdisciplinary vision on nature. It's only in this way that it's possible to catch all present aspects from different points of view, avoiding as much as possible to obtain a partial and distorted idea of what you are looking at. This is what my experience told me. Working in a the Hortus botanicus and in Naturalis, I learned that interaction with other people is crucial not only in life, but also in scientific activities and in the working environment. Sharing ideas, working side to side with other people, knowing different points of view and being open minded are the key for a both personal and professional grout. Erasmus opportunities like the one was offered me are extremely important not only to better understand, in my case, gastropods or plants, but also are a great tool to make students more aware of the world around them, creating connections and doing experiences that will be remembered for the rest of their life.

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## 6. Appendix



Tab.9: It is shown where each species was collected, A-small-Aq being the names of the sampling sites. These data were used to create Fig. 9 and the colours are the same: blue (endemic species), green (alien species), red (not defined), violet (empty shells).

| Terrestrial snails | $A^{\prime \prime}$ | $B^{\prime \prime}$ | $C^{\prime \prime}$ | \% alive | \% dead |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Cepaea nemoralis (Linnaeus, 1758) | 3 | 0 | 3 | 0,04 | 0,00 |
| Cornu aspersum (O.F.Müller, 1774) | 6 | 0 | 6 | 0,08 | 0,00 |
| Deroceras laeve (O.F.Müller, 1774) | 50 | 0 | 50 | 0,66 | 0,00 |
| Discus rotundatus (O.F.Müller, 1774) | 11 | 0 | 11 | 0,14 | 0,00 |
| Euconulus fulvus (O.F.Müller, 1774) | 13 | 0 | 13 | 0,17 | 0,00 |
| Hawaiia minuscula (Binney, 1841) | 19 | 0 | 19 | 0,25 | 0,00 |
| Kaliella microconus (Mousson, 1865) | 1 | 1 | 0 | 0,00 | 0,01 |
| Lamellaxis cf. gracilis (Hutton, 1834) | 2 | 0 | 2 | 0,03 | 0,00 |
| Lamellaxis clavulinus (Potiez \& Michaud, 1838) | 23 | 0 | 23 | 0,30 | 0,00 |
| Lauria cylindracea (Da Costa, 1778) | 11 | 0 | 11 | 0,14 | 0,00 |
| Lehmannia valentiana (Férussac, 1823) | 18 | 0 | 18 | 0,24 | 0,00 |
| Oxychilus cellarius (O.F.Müller, 1774) | 3 | 1 | 2 | 0,03 | 0,01 |
| Oxychilus draparnaudi (H.Beck, 1837) | 3 | 1 | 2 | 0,03 | 0,01 |
| Paralaoma sp. | 3 | 0 | 3 | 0,04 | 0,00 |
| Pristiloma sp. | 1 | 0 | 1 | 0,01 | 0,00 |
| Punctum cf. pygmaeum (Draparnaud, 1801) | 1 | 1 | 0 | 0,00 | 0,01 |
| Subulina octona (Bruguière, 1789) | 30 | 0 | 30 | 0,39 | 0,00 |
| Subulina striatella (Rang, 1831) | 3 | 0 | 3 | 0,04 | 0,00 |
| Undetermined sp. 3 (Charopidae) | 10 | 0 | 10 | 0,13 | 0,00 |
| Undetermined sp. 4 (Charopidae) | 1 | 0 | 1 | 0,01 | 0,00 |
| Undetermined sp. 5 (Charopidae) | 1 | 0 | 1 | 0,01 | 0,00 |
| Undetermined sp. 8 (Zonitidae) | 12 | 0 | 12 | 0,16 | 0,00 |
| Undetermined sp. 9 | 2 | 0 | 2 | 0,03 | 0,00 |
| Undetermined sp. 10 | 1 | 0 | 1 | 0,01 | 0,00 |
| Vallonia enniensis (Gredler, 1856) | 1 | 1 | 0 | 0,00 | 0,01 |
| Vallonia pulchella (O.F.Müller, 1774) | 9 | 0 | 9 | 0,12 | 0,00 |
| Zonitoides arboreus (Say, 1816) | 32 | 0 | 32 | 0,42 | 0,00 |
| Zonitoides cf. nitidus (O.F.Müller, 1774) | 3 | 0 | 3 | 0,04 | 0,00 |
| Zonitoides nitidus (O.F.Müller, 1774) | 27 | 0 | 27 | 0,36 | 0,00 |

Tab.10: In this table it is shown for each species: in how many of the sites the terrestrial species were collected (A"), of whom were only found as empty shells ( $\mathrm{B}^{\prime \prime}$ ). C" represents the total number of sites where the species were collected alive ( A " -B "). \%alive indicates the frequency alive terrestrial snails were collected in the terrestrial sampling sites (C"/76). \%dead indicates how many times a terrestrial species was only collected in a terrestrial sampling site as empty shell ( $\mathrm{B}^{\prime \prime} / 76$ ). These data were used to create Fig. 6.

| Freshwater snails | $A^{\prime}$ | B' | $C^{\prime}$ | D' | \% alive | \% dead |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Archiphysa parkeri (Currier, 1881) | 1 | 1 | 0 | 1 | 0,00 | 0,00 |
| Balea biplicata (Montagu, 1803) | 2 | 0 | 2 | 0 | 0,14 | 0,00 |
| Bithynia tentaculata (Linnaeus, 1758) | 10 | 2 | 8 | 2 | 0,57 | 0,00 |
| Catinella cf. arenaria (Bouchard-Chantereaux, 1837) | 1 | 1 | 0 | 0 | 0,00 | 0,07 |
| Clea helena (Philippi, 1847) | 1 | 1 | 0 | 1 | 0,00 | 0,00 |
| Gyraulus cf. parvus (Say, 1817) | 4 | 0 | 4 | 0 | 0,29 | 0,00 |
| Gyraulus laevis (Alder, 1838) | 3 | 1 | 2 | 1 | 0,14 | 0,00 |
| Helisoma anceps (Menke, 1830) | 8 | 4 | 4 | 4 | 0,29 | 0,00 |
| Helisoma duryi (Wetherby, 1879) | 8 | 3 | 5 | 3 | 0,36 | 0,00 |
| Lithoglyphus naticoides (Pfeiffer, 1828) | 1 | 1 | 0 | 0 | 0,00 | 0,07 |
| Littorina sp. | 1 | 1 | 0 | 1 | 0,00 | 0,00 |
| Lymnaea stagnalis (Linnaeus, 1758) | 1 | 1 | 0 | 1 | 0,00 | 0,00 |
| Melanoides tuberculata (0.F.Müller, 1774) | 14 | 2 | 12 | 2 | 0,86 | 0,00 |
| Neritina natalensis (Reeve, 1855) | 1 | 1 | 0 | 0 | 0,00 | 0,07 |
| Oxyloma dunkeri (L. Pfiffer, 1865) | 1 | 1 | 0 | 1 | 0,00 | 0,00 |
| Physella acuta (Draparnaud, 1805) | 13 | 0 | 13 | 0 | 0,93 | 0,00 |
| Planorbarius sp. | 2 | 0 | 2 | 0 | 0,14 | 0,00 |
| Pomacea bridgesii (Reeve, 1856) | 1 | 1 | 0 | 1 | 0,00 | 0,00 |
| Pseudosuccinea columella (Say, 1817) | 12 | 0 | 12 | 0 | 0,86 | 0,00 |
| Radix labiata (Rossmässler, 1835) | 11 | 3 | 8 | 3 | 0,57 | 0,00 |
| Semisalsa stagnorum (Gmelin, 1791) | 2 | 0 | 2 | 0 | 0,14 | 0,00 |
| Undetermined sp. 2 (Lymnaeidae) | 6 | 0 | 6 | 0 | 0,43 | 0,00 |
| Valvata piscinalis (0.F.Müller, 1774) | 1 | 1 | 0 | 0 | 0,00 | 0,07 |

Tab.11: In this table for each species it is shown: in how many of the sites the aquatic species were collected ( $\mathrm{A}^{\prime}$ ), of whom were only found as empty shells ( $\mathrm{B}^{\prime}$ ). C' represents the total number of sites where the species were collected alive ( $\mathrm{A}^{\prime}-\mathrm{B}^{\prime}$ ). $\mathrm{D}^{\prime}$ indicate in how many terrestrial sites freshwater empty shell was collected. \%alive indicates the frequency alive freshwater snails were collected in the aquatic sampling sites ( $\mathrm{C}^{\prime} / 14$ ). \%dead indicates how many times a freshwater species was only collected in a freshwater sampling site as empty shell (( $\left.\left.\mathrm{B}^{\prime}-\mathrm{D}^{\prime}\right) / 14\right)$. These data were used to create Fig.7.


Tab.12. Tis table for each species (Endemic (Alive), Alien (Alive) or Not defined (N.D.) indicates in which sites were collected in the greenhouses. Violet says where the collected samples were only found as enply shells. The species that are underined were only collected as e en (N.D.) species were collected. These data were used to create Fig.8.






## Tab.16: Ecodata of the samples stored in the Naturalis Biodiversity Center's collection from 121 to 160


Tab.17: Ecodata of the samples stored in the Naturalis Biodiversity Center's collection from 161 to 200.

