# SCIENTIFIC OPINION

# Scientific Opinion on the assessment of the potential establishment of the apple snail in the EU[1]

## EFSA Panel on Plant Health (PLH)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

This Scientific Opinion, published on 12 March 2014, replaces the earlier version published on 12 December 2013.*

**ABSTRACT**

EFSA requested the PLH Panel to review the current state of the art of the biology and ecology of apple snails, reported in this opinion, and to perform an environmental risk assessment for validation of the Plant Health environment guidance document, which will be provided in a second opinion. The Panel presents in this opinion the current state of the art of the biology of apple snails, and develops and uses a population dynamics model to assess the potential establishment of apple snails in the EU. A thorough review of the literature on the biology of *Pomacea canaliculata* and *P. maculata* was performed to collect information and data on life history characteristics related to temperature, which was used in the population dynamics model. Although uncertainties on the systematics and taxonomy of the genus *Pomacea* remain, it is now acknowledged that *Pomacea insularum* is a synonym of *P. maculata* and can be undoubtedly differentiated from *P. canaliculata*. Natural spread occurs via rivers and canals, in which the snails crawl, drift, float and raft. Flooding increases spread. In addition, attachment to animals (e.g. birds, cattle, horses, deer and aquatic invertebrates) results in spread. Human assistance results in spread through cultivation transport of rice seedlings, aquaculture, aquaria, boats, and agricultural field machinery. The potential distribution of *P. canaliculata* in Europe was obtained by calculating with the population dynamics model, the average snail abundance per year in each node of a grid of $0.25 \times 0.25$ degrees covering Europe, which resulted in the following conclusions: (1) the area of potential establishment comprises wetlands of southern Europe (i.e. Spain, southern France, most of Italy and Greece) and the Balkans up to the latitude of the Danube river, (2) the potential area of establishment includes the rice production areas in Europe.

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**KEY WORDS**

apple snail, *Pomacea insularum, Pomacea canaliculata, Pomacea maculata,* population dynamics model, establishment

**SUMMARY**

The European Food Safety Authority (EFSA) asked the Plant Health Panel (hereinafter referred to as the Panel) to deliver a scientific opinion on the risk posed to the environment in the European Union (EU) by the apple snail. In this document the Panel presents a review of the current state of the art of the biology and ecology of the apple snail and performs the assessment of the potential for the apple snails to establish in the EU territory using a population dynamics model. The environmental risk assessment of the apple snail for the EU territory, including an evaluation and update of the risk assessment methodology will be presented in a second scientific opinion.

In a previous opinion on *Pomacea* spp. (EFSA Panel on Plant Health (PLH), 2012b) the Panel concluded, based on a preliminary climate matching exercise, that (a) climate is not a limiting factor for spread and further establishment in the risk assessment area, (b) vast areas of Europe have climatic conditions very similar to those in areas within the current distribution of *Pomacea* spp., and (c) the recognised importance of natural spread, as well the availability of suitable habitats other than rice fields and natural wetlands, identifies a great part of southern, as well as parts of central Europe as potentially endangered areas. (d) The Panel also concluded that a high level of uncertainty is associated with the northern limit of the potentially endangered area, which made it difficult to draw a reliable conclusion about this limit. This high uncertainty was mainly related to a lack of biological data. (e) The Panel also stated that with the support of a population dynamics model that can summarise the effect of biotic and abiotic variables on the snail population dynamics, it could be possible to reduce the uncertainty and make more precise projections on the potential northern limit of establishment. (f) Finally, the Panel concluded that the estimates for the potentially endangered area are probably too limited with regard to the climatic suitability and host plant availability in the risk assessment area. As a result of the population dynamics model developed and used in the current opinion, the Panel can now modify or adapt several of the conclusions formulated in the previous opinion. Concerning conclusions (a), (b), (c) and (f), the Panel concludes that a smaller part of the EU is considered as a potentially endangered area. Concerning conclusion (d) and (e), the Panel concludes that based on data collected recently on the biology of *Pomacea canaliculata* and *P. maculata* together with the population dynamics model, uncertainty about the potential northern limit of establishment could be reduced to medium and more precise estimates could be made about areas potentially most sensitive to *P. canaliculata* and *P. maculata* establishment.

In the previous opinion on *Pomacea* spp. (EFSA Panel on Plant Health (PLH), 2012b), the climate comparisons were based on one of the methods for climate matching available in the modelling tool CLIMEX, which has been widely used in the field of plant health risk assessment. The Panel considered whether the published information on the climate responses of *P. canaliculata* contained sufficient data to compile a specific CLIMEX parameter set for the species that could aid in predicting the climatic potential to establish in the EU. However, the Panel realised that this approach would provide little information beyond the predictions of the potential for establishment and indications on climatic factors that may limit the distribution of the species. Therefore, the Panel decided to use a population dynamics modelling approach in order to be able to estimate the population densities of snail eggs, juveniles and adults, and to identify the potential snail hot spots.

**With regard to the biology and ecology of the apple snail**

Although uncertainties on the systematics and taxonomy of the genus *Pomacea* remain, it is now acknowledged that *P. insularum* is a synonym of *P. maculata* and can be undoubtedly differentiated from *P. canaliculata* based on clear genetic differences. Nine recently collected snails in the Ebro Delta, Spain, were all identified as *P. maculata*.

A thorough review of the literature on the biology of *P. canaliculata* and *P. maculata* was performed and revealed a number of important new data compared to data provided in the previous opinion. These data concern the lower development threshold for eggs; the temperature range and optimum temperature for development of eggs, juveniles and adults; egg, juvenile and adult development

related to temperature; reproduction and survival related to temperature; reproduction related to age of adults; cold tolerance of eggs, juveniles and adults; and mating behaviour. The data found for different world locations showed variation, which might be due to the experimental conditions and differences in the genetic composition of *Pomacea* populations.

Natural spread occurs via rivers and canals, in which the snails crawl, drift, raft and float on floating material. Extreme weather events and flooding increase spread. In addition, attachment to other animals such as birds, cattle, horses, deer and aquatic invertebrates results in spread. Snails are further spread by human assistance through cultivation, transport of rice seedlings, aquaculture, aquaria, boats and other means of transportation, agricultural field machinery and irrigation. The risk of spread by human assistance may have decreased recently owing to the Commission Implementing Decision of November 2012, according to which the genus *Pomacea* shall not be introduced into or spread within the EU.

**With regard to the potential establishment of the apple snail in the EU territory using a population dynamics modelling approach**

The limited amount of data on the life history strategies *P. maculata* does not allow developing a population dynamics model on this species. Therefore a model on the population dynamics of the closely related species *P. canaliculata* was developed to enable estimation of the potential density of the snail in a given environment, characterised by specific patterns of air and water temperature fluctuations. The population dynamics of *P. canaliculata* is described through a physiologically based demographic model. First, the model was calibrated acting on the fecundity and mortality of *P. canaliculata*, by using data from several known locations in the southernmost distribution in Argentina. This resulted in the addition of a temperature-independent and density-dependent mortality component. Two values of this additional mortality component have been considered in the subsequent modelling. These two *P. canaliculata* models were used to define two scenarios of potential establishment in Europe, one less favourable to the establishment of the apple snails (PM2.0) and one more favourable (PM1.5). The limited number of locations available for the model calibration did not allow deriving information on the most likely scenario between the two. In addition, the model was tested for the northernmost distribution in Japan and a good fit was obtained for the population dynamics for eggs, juveniles and adults. Finally, the potential distribution of *P. canaliculata* eggs, juveniles and adults for Europe was obtained by calculating the average snail abundance per year in each node of a grid of $0.25 \times 0.25$ degrees covering Europe. These calculations indicate that:

1. Scenario PM2.0 shows that part of Spain, Italy, the Balkans, and a small area in southern France are potentially suitable for the apple snail establishment in Europe. The northern limit of the potential distribution of snails in Europe is in the Po Valley (Italy). Coastal areas are generally more suitable than inland areas. Adult population density is low in most of the suitable areas in Europe. Several locations are potential foci of high infestation or "hot spots" (populations of more than five individuals (juvenile + adults) per square metre).

2. Compared to scenario PM2.0, scenario PM1.5 shows an expansion of snail establishment towards the North comprising part of Spain, southern France, and northern Italy. The area of potential establishment extends in the Balkans up to the latitude of the Danube River and areas far inland from the Mediterranean coast. In addition, the estimated population densities are higher and the number of hot spots is larger.

3. The area of potential establishment of the apple snails comprises only part of southern Europe including the rice production areas in Europe and most of the wetlands of southern Europe and the Balkans up to the latitude of the Danube River.

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## BACKGROUND AS PROVIDED BY EFSA

The Scientific Panel on Plant Health (PLH Panel) provides independent scientific advice on the risks posed by organisms which can cause harm to plants, plant products or plant biodiversity in the European Community. The Panel reviews and assesses those risks with regard to the safety and security of the food chain to assist risk managers in taking effective and timely decisions on protective measures against the introduction and spread of harmful organisms in the European Community. On request, the Panel prepares pest risk assessments and identifies and evaluates the effectiveness of risk reduction options to provide scientific advice to the European Commission in support of protective measures within the European Community to prevent the introduction and further spread of organisms considered harmful to plants or plants products under the Council Directive 2000/29/EC[4].

In 2011 the PLH Panel evaluated a Spanish Pest Risk Analysis[5], and recommended that further study should be performed on the potential consequences of the *Pomacea* genus for the European environment. The assessment of environmental consequences of the *Pomacea* spp.-induced transformation of wetland ecosystems presented in the Spanish pest risk analysis demonstrates some limits in the capacity to predict the interaction of the snails and the receiving communities and ecosystems. These limits affect the possibility of extrapolating the findings in Asia to the EU territory. Given that projections of environmental consequences are based on a scenario exercise, a more structured and ecologically sound approach would strongly contribute to providing insight into the future development of the snail invasion and its consequences. In particular, the following aspects might be considered for the development of an environmental risk assessment for the *Pomacea* snails in the EU territory:

(i)     The snail population density. The environmental consequences of snail invasion have been proven to be dependent on the snail population density. It appears to be essential to project not only the potential area of invasion, based on simple climate matching, but also to produce a scenario of potential impact based on adequate consideration of the snail's biology and its ecology at the basis of population dynamics. The development of a snail population dynamics model may assist the computation of the potential population density over all the suitable areas in continental Europe. This model should include temperature-dependent responses of development, survival and reproduction. The role of host availability and density can also be taken into account in model projections, if data are available.

(ii)    Development of scenarios of potential consequences. To develop a scenario of potential consequences, the following information is required: (a) projection of the potential population density; (b) hypothesis on pest spread potential; (c) information on the distribution of the potentially affected habitats including habitats other than rice fields and natural wetlands that may be invaded by the pest such as rivers, shallow lakes and ponds (these ecosystems are also likely to be affected by the *Pomacea* snails and may serve as reservoirs for the snails and as an infrastructure for spread). Such information can support the estimation of (1) the environmental consequences on natural wetlands and (2) the potential impact on rice fields.

(iii)   Perform a structured evaluation based on biodiversity and ecosystem services. The published guidance on the environmental risk assessment of plant pests[6] provides a structured methodological framework for assessing the environmental risks of plant pests and is very suitable for development of an environmental risk assessment for *Pomacea* snails in the risk assessment area. The guidance emphasises the importance of assessing the consequences on both the structural (biodiversity) and the functional (ecosystem services) aspects of the

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[4] Council Directive 2000/29/EC on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. OJ L 169, 10.7.2000, p. l.
[5] EFSA Panel on Plant Health (PLH), 2012. Scientific Opinion on the evaluation of the pest risk analysis on Pomacea insularum, the island apple snail, prepared by the Spanish Ministry of Environment and Rural and Marine Affairs. EFSA Journal 2012;10(1):2552, 57 pp.
[6] EFSA Panel on Plant Health (PLH), 2011. Guidance on the environmental risk assessment of plant pests. EFSA Journal 2011;9(12):2460, 121 pp.

environment. This new approach includes methods for assessing the environmental effects on both aspects (structural and functional) for the first time in a pest risk assessment scheme and is particularly suitable for a plant pest such as *Pomacea* spp., which represents an important driving force of wetland ecosystems change.

Furthermore in 2012, The PLH Panel has published a statement on the identity of the apple snails[7], and considered that the uncertainties on the invasive potential of the apple snail that are mainly related to the poor knowledge of the biology and ecology of most of the *Pomacea* species that does not allow grading of the invasive potential at species level. The understanding of the invasive potential is also limited by possible change in the invasiveness after establishment.

TERMS OF REFERENCE AS PROVIDED BY EFSA

In this context EFSA requests the PLH Panel to produce an environmental risk assessment of the apple snails for the EU territory. Specifically, the Panel is requested to:

-   Review the current state of the art of the biology and ecology of the apple snails;

-   Perform an environmental risk assessment using the invasive species of *Pomacea* as a case study for validation of the Plant Health environment guidance document

The Panel expects to deliver the scientific opinion in 12 month.

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[7] EFSA Panel on Plant Health (PLH), 2012. Statement on the identity of apple snails. EFSA Journal 2012;10(4):2645.

**ASSESSMENT**

## 1.    Introduction

The island apple snail (previously called *Pomacea insularum* (d'Orbigny, 1835) and now called *P. maculata*) is one of the largest freshwater snails. *P. maculata*, along with other species belonging to the same genus, is highly invasive outside its native distribution range (South America), which might be the result of its polyphagy and high reproductive rate. Apple snails are considered to be serious rice pests and can cause devastating effects on the flora and fauna of natural wetlands. In 2010 the organism invaded rice fields in the Ebro Delta in Spain. Before then, it was not known to occur in the European Union (EU) and was not regulated. Currently the apple snail invasion is still spreading in the Ebro delta despite the control measures to eradicate and/or contain the snail in the rice paddies. Mechanical and chemical control measures are used, as well as inundation of paddies with saline water and other methods. Currently, the snail is not only present in rice paddies but also in some nearby wetlands, and it has been found moving upwards along the Ebro riverbeds.

On request of the European Commission (EC) the Panel on Plant Health (hereinafter referred at as the Panel) provided advice in two different documents regarding the apple snail in 2012:

EFSA Panel on Plant Health (PLH), 2012a. Statement on the identity of apple snails. EFSA Journal, 10(4), 2645.

EFSA Panel on Plant Health (PLH), 2012b. Scientific Opinion on the evaluation of the pest risk analysis on *Pomacea insularum*, the island apple snail, prepared by the Spanish Ministry of Environment and Rural and Marine Affairs. EFSA Journal , 10(1), 2552.

In the scientific opinion the Panel concluded that the Spanish pest risk analysis (Spanish Ministry of Environment and Rural and Marine Affairs, 2011) did not sufficiently address the environmental impact of the snail invasion and recommended that further study should be performed. The Panel was therefore requested by EFSA to perform an environmental risk assessment on the apple snail following the guiding principles presented in the PLH guidance document (EFSA Panel on Plant Health (PLH), 2011). While performing the environmental risk assessment, the Panel was also asked to use this as a case study and evaluate and update the guidance document.

### 1.1.    Purpose of the opinion

In order to perform the environmental risk assessment of the invasive snails, the Panel presents in this opinion a review of the biology and ecology of apple snails and develops a population dynamics model to assess the potential for apple snails to establish in the EU territory in terms of the spatial distribution of the snail's population density.

The environmental risk assessment on the apple snail for the EU territory, including an evaluation and update of the risk assessment methodology, is presented in a second scientific opinion.

### 1.2.    Scope of the opinion

The Panel defines the scope of the evaluation as follows.

(i)    The pest risk assessment area is the EU territory restricted to the area of application of Council Directive 2000/29/EC, which is the EU territory excluding French overseas departments, the Canary Islands and Ceuta and Melilla.

(ii)    Owing to the fact that *P. maculata* and *P. canaliculata* (Lamarck, 1819) are two almost indistinguishable species, and because of the limited studies available on the biology of *P. maculata*, the Panel addresses both species. The common name "apple snail" and the

designation *Pomacea* spp. are used in this opinion to designate two species of freshwater aquatic snails: *P. maculata* and *P. canaliculata*.

(iii)    The evaluation of the areas of concern is focused on natural wetlands in the EU territory. The Panel considers in its evaluation the potential establishment of the two species, *P. maculata* and *P. canaliculata*, in the risk assessment area.

(iv)    Maps of the potential distribution of the snail are obtained by means of a population dynamics model that considers only the air and water temperature and not the availability of water. By adding the information layer related to the wetlands and other suitable habitats to the maps of potential distribution, the area at risk of establishment can be obtained.

Throughout the opinion, the Panel uses the name *P. maculata* since it is the recently accepted synonym of *P. insularum*.

The concern that *Pomacea* spp. snails are a vector of parasites that may cause diseases in humans as well as in animals is not assessed in this opinion.

## 2.    Methodology

### 2.1.    Guidance documents

The Panel assessed the potential establishment and spread of the apple snail following the guiding principles and steps presented in EFSA guidance on the harmonised framework for risk assessment (EFSA Panel on Plant Health (PLH), 2010) and as defined in the International Standard for Phytosanitary Measures (ISPM) No 11 (FAO, 2004).

The Panel conducted the assessment with the current EU plant health legislation in place considering that the apple snail is regulated in the EU by Commission Implementing Decision 2012/697/EU[8].

### 2.2.    Data collection and literature searches

(i)    Specific literature searches, where expert knowledge was not sufficient. This includes information extracted from databases, relevant scientific papers and grey literature. Specific searches have been performed to retrieve relevant information on the temperature thresholds affecting the bio-demographic characteristics of the apple snail, particularly regarding :

a.    development
b.    survival
c.    fecundity

Other literature searches have been performed to find data regarding the temperature gradients in European water bodies.

(ii)    Expert knowledge in the field has been consulted. When expert judgement and/or personal communication have been used, justification and evidence are provided to support the statements. Personal communications have been considered only when in written form and supported by evidence, and when other sources of information were not publicly available. More specifically scientists from the native area of distribution of the apple snails and from the invaded area in the EU have contributed to this work, as well as experts in the field of ecological functioning of European wetland ecosystems.

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[8]    Commission Implementing Decision 2012/697/EU of 8 November 2012 as regards measures to prevent the introduction into and the spread within the Union of the genus *Pomacea* (Perry) (notified under document C(2012) 7803). OJ L 311, 10.11.2012, p. 14-17.

## 2.3. Modelling approaches

### 2.3.1. Climate matching

The climate comparisons in the previously published opinion EFSA Panel on Plant Health (PLH) (2012b) were based on one of the methods for climate matching available in the modelling tool CLIMEX, which has been widely used in the field of plant health risk assessment. The 'Match Climates' function used previously (EFSA Panel on Plant Health (PLH), 2012b) is limited to a comparison of climates only, based on meteorological data from different places, typically comparing the climate in an area where a species of interest is known to occur with the climate in an area of interest where the species is not known to occur. CLIMEX has also a second and more advanced climate-matching method specifically developed for prediction of the potential geographical distribution of a species based on its climate response parameters (Sutherst and Maywald, 1985). Because the latter method of CLIMEX has also been used frequently to predict the establishment potential of plant pests, it was considered by the Panel following a literature review of the biology of the apple snail, whether the published information on the climate responses of *P. canaliculata* contained sufficient data to compile a specific CLIMEX parameter set for the species that could aid in predicting the climatic potential for its establishment in the EU. However, the Panel is aware that this approach will provide little information beyond the predictions of the potential for establishment and indications on climatic factors that may limit distribution of the species, e.g. no prediction of the expected population density of the species in climatically suitable areas. Therefore, the Panel decided to conduct more advanced studies involving population dynamics modelling in order to obtain more detailed results, both for use in this opinion and for further use in Panel work.

The fact that aquatic survival seems to be the main strategy for this species' survival of the winter in cold locations is a particular challenge for CLIMEX, as it has no dedicated module handling aquatic environmental conditions. Nevertheless, the use of CLIMEX to develop a specific set of model parameters for *P. canaliculata* was further explored (see Appendix A for details).

### 2.3.2. Population dynamics model for *Pomacea canaliculata*

A model on the population dynamics of *P. canaliculata* was developed in order to produce estimates of the potential density of the snail in a given environment, characterised by specific patterns of temperature fluctuation and availability of water. Population abundance, expressed as snail density, is the main variable we use to assess the impact of the snail on the environment. The impact of the snail density is estimated by means of the change in the provision level of the ecosystem services. For the sake of simplicity the role of food availability is considered in the model by means of a density-dependent regulation in the mortality rate functions. To the best of our knowledge, there are no studies reporting bio-demographic functions for *P. canaliculata*. We propose generic formulas for development, survival and fecundity functions that are commonly used for other poikilothermic organisms and we estimate parameters for functions in the generic formulas using data collected from the literature for *Pomacea* species.

The population dynamics of *P. canaliculata* is described through a physiologically based demographic model. Three developmental stages are used: egg, juvenile and reproductive adult (male and female).

Population processes for eggs depend on air temperature as eggs are laid above water, but for juveniles and adults these processes depend on water temperature. Therefore a specific water temperature model was developed. Meteorological data were kindly made available by Professor Luigi Mariani (meteorologist and climatologist at the University of Milan, Italy) who implemented the model for the calculation of water temperatures.

## 3. Biology of the apple snail

Current studies on the systematics and taxonomy of the genus *Pomacea,* acknowledge that there is still a high degree of uncertainty regarding the differentiation of species and their identification, as

previously described in the Panel's statement on the identity of apple snails (EFSA Panel on Plant Health (PLH), 2012a). The Panel therefore aims to make these dynamics as transparent as possible by summarising the most recent literature (see section 3.1). General aspects on the biology of the genus *Pomacea* were provided in the panel's evaluation of the Spanish pest risk analysis (EFSA 2012b). For the purpose of this opinion, which focuses on *P. canaliculata* and *P. maculata,* the taxonomy, life history and spread are presented in greater detail than in the previous opinion. Available information on *P. maculata* is much more limited than that on *P. canaliculata*.

## 3.1. Taxonomy

### 3.1.1. Current status of the taxonomy of *Pomacea canaliculata* and *P. maculata*

Hayes et al. (2012) synonymise *P. insularum* and *P. gigas* (*Ampullaria insularum* d'Orbigny, 1835 and *Ampullaria gigas* Spix, 1827) with *P. maculata* (Perry, 1810) and clearly differentiate the latter from *P. canaliculata*. They designate neotypes for *P. maculata*, *P. canaliculata* and *A. gigas*, as well as a lectotype for *A. insularum*.

The conchological differentiation of *P. maculata* and *P. canaliculata* is difficult since differences in shell morphology are only really obvious in recently hatched juveniles. The shells of adults differ primarily in the angulation of the whorl shoulder and the pigmentation of the inner apertural lip. When present, this pigmentation is a distinctive feature of *P. maculata*. However, sometimes it is lacking or too faint (e.g. in juvenile *P. maculata*) which can result in misidentification (Martín PR, 2013, Universidad Nacional Del Sur, Bahia Blanca, Argentina, personal communication), leading to incorrect information on their biology, spread and impact. Hayes et al. (2012) therefore made another attempt to clarify the taxonomy, describing their morphological and genetically based diagnostics, and re-evaluating their biogeographic ranges. Their results show that the two species differ mostly genetically, with no shared Cytochrome Oxidase subunit I (COI) haplotypes and a mean genetic distance of 0.135.

Some other features described by Hayes et al. (2012) that distinguish the two species are the number of eggs per clutch, which is higher in *P. maculata*, which also has smaller individual eggs. Barnes et al. (2008) found that the average number of eggs in a *P. insularum* egg clutch was 2 064 – almost 10 times higher than the average number of eggs in a *P. canaliculata* egg clutch (Teo, 2004; Martín and Estebenet, 2002). The smaller egg size (determined by egg weight) was confirmed by Matsukura et al. (2013). *P. canaliculata* hatchlings are nearly twice the size of those of *P. maculata*. They also differ in reproductive anatomy. *P. canaliculata* has two distinctive glandular tissues in the apical penial sheath gland, and *P. maculata* has a basal sheath gland instead of a medial sheath gland.

Matsukura et al. (2013) found that *P. maculata* and *P. canaliculata* hybridise both in Southeast Asia and in the lower Río de la Plata bassin, the area identified as the origin of multiple introductions of *P. canaliculata* and *P. maculata* (Hayes et al., 2008), although for the latter, some introductions could have originated from Brasil. Similar origins were suggested for the continental USA by Rawlings et al., (2007). Matsukura et al. (2013) determined two well-supported clades based on sequences of gene coding for the Elongation Factor 1 alpha (EF1a) (Clades C and M), in which both species were represented. Some specimens had sequences of both clades, suggesting that genetic exchange occurs between the individuals belonging to the two clades. A mating experiment between *P. canaliculata* from Clade C and *P. maculata* from Clade M produced viable F1 progeny under laboratory conditions. Genetic exchange was also inferred in some populations collected from Argentina, hinting at hybridisation in the native range.

### 3.1.2. Taxonomic peculiarities of apple snails found in the Ebro Delta

López et al. (2009) genetically identified nine specimens with high variability in shell colour and shape. Some of them were consistent with the morphology of *P. canaliculata*, however all of them showed only one haplotype ("O" haplotype), which was clearly *P. maculata*. This haplotype is attributed to cultivated apple snails.

### 3.2. Life history of *Pomacea canaliculata* (unless otherwise indicated)

### 3.2.1. Development

A lot of recent information from mainland China is available; however, this must be considered with caution regarding the identity of the snails, as the taxonomic situation is complicated and unresolved there. For example, Lv et al. (2013) stated: "Phylogenetic analyses indicate that *P. canaliculata*, *P. insularum* and two cryptic groups, discovered by the present and previous studies, coexist in the mainland of P.R. China. The mosaic distribution and the high diversity found in the collection sites suggests multiple and secondary introductions […]."

When temperature is mentioned in the following sections, this concerns air temperature for eggs and water temperature for juveniles and adults, unless otherwise specified.

#### 3.2.1.1. Eggs

The minimum temperature for the development of eggs of *P. canaliculata* in mainland China is 14.2 °C, according to Huang et al. (2010) and Liu et al. (2011). Seuffert et al. (2012) found ranges from 15.7 to 16.2 °C in *P. canaliculata* from Argentina.

Liu et al. (2011) mentioned the following egg production: 30 °C (4200 eggs)> 25 °C (2350 eggs)> 35 °C (680 eggs)> 20 °C (540 eggs). No eggs were produced at 15 and 40 °C. This indicates that the optimum temperature for spawning *P. canaliculata* is about 30 °C. In contrast to this, the snails in the study of Seuffert and Martín (2013) died without laying eggs when reared at a constant temperature of 35 °C, and those reared at 30 °C laid many egg masses but their hatchability was quite low (Martín PR, 2013, Universidad Nacional Del Sur, Bahia Blanca, Argentina, personal communication). Probably the tolerance of Chinese snails from Zhejiang (30°16′N) to high temperature is a little higher than that of snails from the Southern Pampas (38°S).

The accumulation of degree days until hatch is 137.41 °C days according to Liu et al. (2011) and 152.16 °C days according to Huang et al. (2010). Seuffert and Martín (2012) report 120.7 to 133.8 °C days.

Egg development time is 20.7 days on average (Liu et al., 2012). The longest egg development time found by Liu et al. (2012) was 62 days at temperatures around 16 °C and the shortest was five days at temperatures around 32 °C. In the native range (Argentina) egg development took between 13 and 24 days under mean daily temperatures between 14.9 and 25.5 °C, respectively (Pizani et al., 2005).

#### 3.2.1.2. Juveniles

Juveniles emerge from eggs and change into reproductive adults; however, their size and age at maturity are highly variable depending on food availability. In addition, size and age at maturity differ between the sexes. Tamburi and Martín (2008) reared *P. canaliculata* snails from hatch under a wide range of food availability, between 20 and 100 % of the *ad libitum* ingestion rate, to investigate this effect. They found that males matured at an age of around 13 weeks irrespective of food availability but their size was highly dependent on food availability: 16mm at 20 % and 29mm at 100 %. For females, it was necessary for them to reach sizes of at least 32mm to reproduce. These sizes were attained at very different ages according to food availability: 15 weeks at 100 % and 50 weeks at 20 % of the *ad libitum* ingestion rate.

The optimal temperature for juvenile development according to Liu et al. (2011) is 30 °C. Growth rates increase with temperature from 15 to 25 °C but the growth rates at 30 and 35, although they were a little higher, were not significantly different from those at 25 °C.

Seuffert and Martín (2013) found that at 15 and 20 °C there was no mortality but growth rates were very low. In contrast, at 25, 30 and 35 °C snails grew faster but survival decreased with increasing

temperature. After 10 weeks, the mean shell lengths at temperatures of 30 and 35 °C were not significantly different from those at 25 °C.

### 3.2.1.3. Adults

Liu et al. (2011) considered that the optimum temperature for growth, development and reproduction of *P. canaliculata* is about 30 °C according to their studies. Gettys et al. (2008) indicate 20-30 °C for adult development of *P. insularum*.

At a constant temperature of 35 °C *P. canaliculata* females will not lay eggs (Seuffert and Martín, 2013; Martín PR, 2013, Universidad Nacional Del Sur, Bahia Blanca, Argentina, personal communication on unpublished results).

One to three generations may develop per year, depending on the temperature, especially in winter. The average number of days for females to reach sexual maturity in the south of Hunan Province, where winter temperatures are above 9 °C, was 59.3 for the first generation, 45.4 for the second, and 213.0 for the third, that being the longest as they reach sexual maturity in the year following the winter (Liu et al., 2012).

### 3.2.2. Survival

#### 3.2.2.1. Eggs and juveniles

Yingying et al. (2008) mentioned survival rates for juveniles of 97 % at 30 °C, of 87 % at 33 °C, and of 47 % at 36 °C. At 39 and 42 °C juvenile mortality was 100 %.

The survival rate of juveniles at 15 °C was highest (100 %) and declined to 63 % at 12 °C, while only 7 % of juveniles survived at 9 °C and no snails survived at 6 °C. The shortest survival time was two days and the longest was seven days (average $4.10 \pm 0.24$ days). The median lethal time ($LT_{50}$) at 6 and 9 °C was 4 and 24 days respectively. Young snails survived 1 to 6 days (average survival time: $2.57 \pm 0.32$ days (mean $\pm$ SE) at 42 °C and 1to 13 days at 39 °C (average survival time $6.27 \pm 0.45$ days). The $LT_{50}$ at 36, 39 and 42 °C was 21, 6 and 2 days respectively.

This results in the following ranking of survival of juveniles:

15 °C > 30 °C > 33 °C > 12 °C > 36 °C > 9 °C > 6 °C = 39 °C = 42 °C.

Liu et al. (2011) ranked survival rates of juvenile *P. canaliculata* reared at different temperatures from high to low as: 20 °C > 15 °C > 35 °C > 30 °C > 25 °C > 40 °C. Seuffert and Martín (2013) found a 100 % survival of juveniles at 15 °C and 20 °C after 10 weeks and all snails at these two temperatures were still alive after 21 months (Martín PR, 2013, Universidad Nacional Del Sur, Bahia Blanca, Argentina, personal communication).

The above data are more or less similar to what Yingying et al. (2008) found, but there are also some obvious differences, e.g. 100 % survival at 15 °C found by Yingying et al. (2008) and Seuffert and Martín (2013) and less than 100 % by Liu et al. (2011).

#### 3.2.2.2. Adults

Howells et al. (2006) stated that members of the *P. canaliculata* complex often have a greater tolerance for low temperatures than e.g. *Marisa cornuarietis*, *P. paludosa*, and *P. bridgesii*. Oya et al. (1987) and Mochida (1991) reported that *P. canaliculata* could survive for 15-20 days at 0 °C, for two days at –3 °C, and for six hours at –6 °C. Furthermore, Oya et al. (1987) and Syobu et al. (2001) found that younger and smaller snails tolerated cold better than larger snails. However, most studies in Japan show an opposite trend or an optimum size for tolerance, for instance: "Juveniles of intermediate size were more tolerant of cold temperature than very small juveniles and adults" (Wada and Matsukura, 2007). Field data and laboratory experiments in Southern Japan over seven consecutive years showed

that snails larger than 6.0 mm exhibited greater cold tolerance than small snails (Syobu et al., 2001). Yingying et al. (2008) found that exposure of *P. canaliculata* to 6 °C for seven days caused 100 % mortality with a median $LT_{50}$ of four days, whereas field tests by Yu et al. (2002) showed that their mortality was 100 % when exposed to 1to 2 °C for only one day.

Under laboratory conditions, the supercooling point of cold acclimated and non acclimated snails was not found to differ significantly and was about -7 °C. Snails did not survive freezing and even died under more moderately low temperatures approaching 0 °C (Matsukura et al., 2009). It was found that only one snail out of 80 (1.3 %) without cold acclimatisation survived a 0 °C cold treatment for five days, whereas 98.8 % of snails with cold acclimatisation survived under moist conditions and 93.8 % under aquatic conditions at this temperature. In a desiccation-tolerance test, the survival rate after four weeks after the start of the experiment of non cold acclimated snails (71.3 %) was significantly lower than in two groups of cold acclimated snails (approximately 90 %). The difference in survivorship was even higher after eight weeks (Wada and Matsukura, 2011).

Estebenet and Cazzaniga (1992) recorded a maximal longevity under laboratory conditions of 49.5 519 months at room temperatures fluctuating between 9 and 29 °C. Under a constant temperature of 25 ºC, maximal longevity was 13.5 months.
Under field conditions, *P. canaliculata* has been found in Paso de las Piedras reservoir (38°04'S – 59°18'W), in the South of Buenos Aires province, Argentina, as the most southerly location (Martín et al., 2001), with a mean annual air temperature of 14 ºC and thermal amplitudes of up to 18 ºC (Estebenet and Martín, 2002). In the northern hemisphere, *P. canaliculata* could not survive in mountainous areas of Guangdong province, China, during the winter (MingQian et al., 2012).

According to Liu et al. (2011), survival rates of adult *P. canaliculata* reared at different temperatures can be ranked as 20 °C = 15 °C > 25 °C > 35 °C > 30 °C > 40 °C. When *P. canaliculata* was reared at temperatures of 40 °C, survival rates of both juveniles and adults were lower than 15 % after 10 days, and lower than 5 % after 15 days, and adults died faster than juveniles; the survival rate of adult snails was also lower than that of the young snails at 30-35 °C. Therefore, it can be considered that the high-temperature tolerance of young snails was slightly greater than in adult snails. When *P. canaliculata* was reared at temperatures of 15-35 °C, survival rates among both young and adult snails rose to 70 % or above, and the survival rate of *P. canaliculata* reared at 15-20 °C for 30 days was up to 90 % or above. However, their activity was weak, food intake was low and growth and development was slow, while on the contrary those reared at 25-35 °C developed normally.

Mochida (1991) found a high mortality in water temperatures above 32 °C; however, this could not be confirmed by Ekschmitt and Albrecht (2008). In fact, the effect of temperature on the mortality of this "amphibious" snail depends strongly on water conditions (fouling and oxygen). In aquaria with food but without artificial aeration, survival times were significantly reduced when access to breathable air was blocked by underwater barriers: at 35 °C the mean survival time was less than 2 days (Seuffert and Martín, 2010).

The snails can survive for 15-20 days at 0 °C, two days at -3 °C, but only six hours at -6 °C. In Okinawa, Japan, it has been confirmed that the snails can survive 234 days without water (Mochida, 1991). Yusa et al. (2006) found an even longer survival time: without watering, five large snails (out of 30 individuals; 17 %) survived up to 11 months (approximately 330 days), but no snails survived longer under such dry conditions. Under moist conditions with watering two to three times per month, one medium-sized (out of 30 individuals; 3 %) and two large snails (out of 50; 4 %) survived the entire experimental period of 29 months (approximately 870 days).

The temperature limits for this invasive species have been studied previously by Mochida (1991) who found a high mortality at water temperatures above 32 °C and a lifespan of only 15-20 days at temperatures of 0 °C. Lee and Oh (2006) described the temperature limits as 2 and 38 °C.

### 3.2.3. Fecundity

A mean of 4534.3 eggs/female/year can be estimated from Estebenet and Martín (2002), distributed in 8-57 egg masses. Liu et al. (2012) recorded egg masses containing 42 to around 880 eggs, with the females laying 1-15 egg masses after one copulation. Teo (2004) reported egg numbers per cluster ranging between 92 and 592 (mean 272). In Burela and Martín (2011) the mean values for the total number of eggs and egg masses were clearly lower, although both studies were performed under "optimum conditions" (25 °C, lettuce *ad libitum* and only one couple in a 3 litre aquarium): 2 412.08 ($\pm$ 994.87) and 13.11 ($\pm$ 5.90), respectively. However, in Burela and Martín (2011) the females were older than in Martín and Estebenet (2002) when first allowed to copulate and the oviposition lasted just 8.07 weeks ($\pm$ 3.75) vs 12-20 weeks. The egg-mass laying rate was quite similar in both studies: 1.67 per week ($\pm$ 0.38) vs 1.47-1.73 per week.

In Zixing, Hunan, China, one female was found to spawn around 13 800 eggs, while 6 070 juveniles per female were recorded. This shows a hatchability of 44.1 %, which is close to 37 % as found by Chongqing Aquatic Product Laboratory (1990, cited in Liu et al., 2012). In contrast, 74.6 % and 76.7 % hatchability were recorded under laboratory conditions reported by Lai et al. (2009) and Weng et al. (2008) (both cited in Liu et al. (2012)). According to Mochida (1991) hatchability is between 7 and 90 %. Teo (2004) found that, regardless of clutch size, hatching success ranged from 87 to 100 % (mean 95.8 %). For *P. canaliculata* from Southern Pampas, Tamburi and Martìn (2011) have found an average hatchability of 67 %. In another study (Burela and Martìn, 2011), it was found that the percentage of eggs with embryos was 56.19 % ($\pm$ 27.35) and 61.18 % ($\pm$ 21.21) in two different experiments.

A positive correlation of monthly spawning masses with temperature and water temperature was recorded, but it was also found that the number of egg masses is not only related to temperature but also to reproductive physiological characteristics (Liu et al., 2012). The spawning period found in 2009 and 2010 by Liu et al. (2012) was 251 and 275 days. The annual spawnings for each female snail were related to age, with the higher the age the lower the spawnings. Within a certain physiological period, the average number of eggs contained in each mass between generations of snails was negatively correlated with the age of the snails: the older the snails, the lower the average number of eggs per spawning.

Information regarding fecundity of *P. insularum* is scarce. According to Barnes et al. (2008) the mean number of eggs in one clutch is 2 064, with a hatchability of 70 % (sometimes 80 %) in the field and 30 % under laboratory conditions (the opposite of *P. canaliculata*). A rough estimate of the annual fecundity extrapolated from the authors leads to 26 000 juveniles in Southeastern USA.

Virgin females do not lay eggs (Burela and Martín, 2011). *P. canaliculata* snails are promiscuous, both males and females copulate frequently with multiple partners.

In *P. canaliculata* populations from Japan, sex is determined by a few nuclear genes located on different chromosomes (Yusa, 2007) which results in highly variable sex ratios among the broods of different parents, whereas sex ratios of whole populations are near unity. This sex determination has been studied only in Japan so far. Martín (Martín PR, 2013, Universidad Nacional Del Sur, Bahia Blanca, Argentina, personal communication) found some strongly male biased egg masses in a Southern Pampas' population of *P. canaliculata* but the sex ratio in the wild is not clearly biased.

### 3.2.4. Spread

According to the scientific opinion of the EFSA Plant Health Panel PLH (2012b), both natural and human assisted spread is possible. Human mediated spread generally provides species with their first move out of their natural environment, after which natural dispersal takes over within and outside this new area of introduction (e.g. van der Velde et al., 2010).

After a single copulation a female can store and maintain enough viable sperm to fertilise eggs for her remaining life time. This sperm storage ability has been considered a key feature in the invasiveness of *P. canaliculata* since it greatly increases the potential of a single female to establish a new population, especially when pre-reproductive mortality is high. Therefore, mature, mated females of *P. canaliculata* are probably the key stage for the establishment of new populations after introduction (Jerde et al., 2009), followed by egg masses.

### 3.2.4.1. Natural spread

Rivers and the network of natural and artificial canals connecting water basins as well as flooding events can be considered effective for natural spread (Tu and Hong, 2002; Cowie, 2002; Yusa et al., 2006). As stated in the previous scientific opinion of the EFSA Panel on Plant Health (2012b), the continuity of water habitats in climatically suitable areas may guarantee the interchange of snail populations between the rice fields and the natural wetlands, the two most sensitive habitats where damage has been recorded. While natural spread is generally quite effective at the local and regional level, the expected rate of long distance natural dispersal is considered to be low, i.e. some metres per year activespread by snails creeping, though passive spread could be up to many kilometres per year (e.g. in a river by water, with much greater consequences).

The dispersal of *P. canaliculata* also depends on different mechanisms: when crawling and drifting, *P. canaliculata* does not demonstrate a positive rheotaxis but crawls both upstream and downstream. In short term trials in artificial channels in the laboratory, *P. canaliculata* more often crawled upstream, but at a slower pace, than downstream (Seuffert and Martín, 2012). Active dispersal of freshwater snails is generally less than one kilometre per year (Kappes and Haase, 2011), but may be more for the relatively large *Pomacea* snails. Drifting downstream results in much larger dispersal distances than upstream movement, and downstream dispersal distances in rivers are at least 10-fold those of upstream movement (Kappes and Haase, 2011). Drifting is promoted by the high buoyancy caused by the air in the lung. In standing waterbodies the main vector of displacement for a floating snail is leeward while in floating ones it is downstream (either floating or rolling along the bottom). In fast-flowing rivers, floating as well as rafting on floating material (loose vegetation, driftwood) can cause rapid spread. *P. canaliculata* snails are able to withstand water currents of up to 1.6 metres per second but they frequently detach or lose their foothold at very low water velocities (less than 0.4 metres per second according to Seuffert and Martín, 2012).

Owing to the large size of apple snail eggs, it is not expected that they will be transported by wind, except by hurricanes and cyclones. During flooding of large areas, snails can move actively or can be carried passively and might connect with other snail populations in the wetlands of the flooded area (Van de Meutter et al., 2006). Snails in annually flooded rice fields, for instance, form one large population (Van Leeuwen et al., 2013).

Dispersal of aquatic invertebrates, including snails, also occurs on vectors such as water birds and large mammals such as cattle, horses and deer (Figuerola and Green, 2002; Vanschoenwinkel et al., 2008, 2011; Van Leeuwen et al., 2013). Aquatic snails, including from the *Pomacea* genus, are highly tolerant to desiccation and can easily survive hours out of the water, during which substantial distances can be covered if attached to other animals (Van Leeuwen and van der Velde, 2012). Mud has been shown to be an effective glue, if invertebrates become attached in wet mud that dries. Only upon re-submergence of the mud in water are the attached animals released (Van Leeuwen and van der Velde, 2012). Many animals have been shown to disperse small freshwater clams (Sphaeriidae), as these often have their valves slightly open during resting but will rapidly close their valves upon the touch of a bird feather, insect leg or fish fin potentially facilitating dispersal (Gittenberger et al., 2004). In a similar way, *Pomacea* snails may also close their operculum when in contact with the fur of a mammal or bird feathers. Some aquatic birds (e.g. gulls) fly off with snails and then try to drop them on rocks. If these rocks are close to water and the bird misses the rock, snails can enter a new water body. Internal dispersal, whereby snails survive passage through the gut of animals has not been shown for *Pomacea* snails, although this has not been tested. Egg masses are estimated to be too vulnerable to

successfully pass through the gut. The probability that an aquatic snail will adhere to other animals is linearly related to snail density (Van Leeuwen and van der Velde, 2012). This suggests that once *Pomacea* snail populations have become established and reached very high densities, dispersal by other animals will be greater than at low densities. Dispersal distances achieved by wild boar, cattle and deer species will generally be less than those achieved by waterbirds (Sutherland et al., 2000). As new populations can be established from only one fertilised female, even rare long-distance dispersal events can still have major consequences.

During spring and autumn, millions of waterbirds migrate long distances all over the globe (Wetlands-International, 2007), and large numbers are engaged in regular short-distance flights between feeding and roosting sites. They often make direct flights between wetlands for feeding and roosting, which are often similar wetland habitats providing favorable conditions for snails that they may have carried from other locations. Rice fields are good feeding habitats for many bird species. By this means, the snail could even be introduced to new areas, since birds can fly over hundreds of kilometres. Cattle are frequently transported by humans, and in several national parks there are free-ranging cattle which could spread the snails locally over several kilometres. The combination of adhering in mud or attachment through closure of the operculum makes natural animal vectors potentially an additional means of dispersal to take into account.

### 3.2.4.2. Human assisted spread

In Asia snails are mainly disseminated through human cultivation of snails and by the transport of rice seedlings containing eggs or juveniles (Zhou et al., 2003). In Europe, the release of individuals from aquaculture and aquaria seems to be the major pathway of spread, likewise as has been suggested for spread in the USA (Cowie, 2002; Smith, 2006; Jerde et al., 2009). In the event that snails are already present, it is possible for accidental dispersal to take place through boats or other means of transports. In the Ebro Delta, Spain, snails adhering to boats have already been detected, and egg masses are frequently deposited on the hulls of stranded boats. Another pathway for spread could be agricultural field machinery that is contaminated with egg masses or small inconspicuous snails. Furthermore, the escape of snails from aquaculture or from the aquarium industry might spread the pest. In addition, irrigation and movement of plants intended for propagation (Smith, 2006) could also increase the probability of dispersal, mostly of small, immature snails. Spread over long distances depending on the movement of plants is therefore considered potentially rapid with a low level of uncertainty. However, this type of risk has probably decreased in the EU after the coming into force of the Commission Implementing Decision of November 2012[9] as regards measures to prevent the introduction into and the spread within the Union of the genus *Pomacea* (Perry), according to which the genus *Pomacea* shall not be introduced into or spread within the EU, and that plants from Third Countries have to be free from the snails as well as plants moved from infested areas within the EU. Since surveys have to be done, infestations may be eradicated and contained, reducing the risk of spread.

### 3.2.4.3. Conclusion on spread

Natural spread of *Pomacea* snails occurs via rivers and canals, in which the snails crawl, drift, float and raft on floating material. Extreme weather events and flooding increase spread. In addition, attachment to other animals such as birds, cattle, horses, deer and aquatic invertebrates result in spread. Snails are further spread by human assistance through cultivation, transport of rice seedlings, aquaculture, aquaria, boats and other means of transport, agricultural field machinery and irrigation.

## 3.3. Biodemographic functions

To the best of our knowledge there are no studies available reporting biodemographic functions for *P. canaliculata*. Survival curves and development and fecundity rate functions which are widely used for

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[9] Commission Implementing Decision 2012/697/EU of 8 November 2012 as regards measures to prevent the introduction into and the spread within the Union of the genus Pomacea (Perry) (notified under document C(2012) 7803). OJ L 311, 10.11.2012, p. 14-17.

other poikilotherm species were used and parameters were estimated using information from the literature.

The population dynamics of *P. canaliculata* is described through a physiologically based demographic model. Three developmental stages are used: egg, juvenile and reproductive adult (male and female).

Figure 1 represents the stage structured population dynamics in terms of fluxes of individuals between the stages due to the development $V_i(T(t))$, fecundity $f(T(t))$ and the loss of individuals due to mortality $m_i(T(t))$. These fluxes are represented by development, fecundity and mortality rate functions.
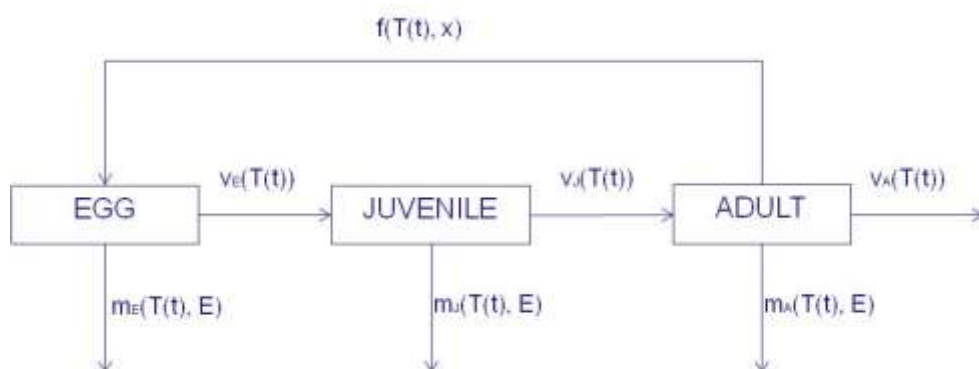


**Figure 1:** Stage structured population dynamics in terms of the three biodemographic functions

### 3.3.1. Development rate function

For the development rate of eggs, we used the function from Brière et al. (1999):

$$v(T(t)) = \begin{cases} - \quad , \quad - \quad \leq \\ \\ 0 \qquad otherwise \end{cases}$$

where $T_{max}$ is the upper temperature development threshold, $T_{min}$ the lower temperature development threshold and $a$ is an empirical constant. Parameters $a, T_{min}, T_{max}$ have to be determined.

For the development rate of juveniles we used a fifth-order polynomial

$$c_1 T^5 + \quad + \quad + \quad + \quad +$$

where $c_1, c_2, c_3, c_4, c_5, c_6$ are constants to be determined. We selected this function because it fits the available data better than the Brière function.

The development rate function for adults is determined following a procedure that links adult development to reproduction. A development rate function for adult females is derived in the next section.

Using data from Liu et al. (2012) and Seuffert et al. (2012) we estimated the development thresholds and the parameter $a$ in the development function for eggs (Table 1). The parameters

$c_1, c_2, c_3, c_4, c_5, c_6$ for the juveniles were estimated with data on growth and feeding reported in Yingying et al. (2008), Seuffert et al. (2010), Liu at al. (2011) and Seuffert and Martín (2013) (Table 2). Figure 2 shows the estimated development rate functions for eggs and juveniles. The development rate is expressed in days$^{-1}$ and is the inverse of the development time at each temperature.

**Table 1:** Parameters for the development rate function for eggs of *Pomacea canaliculata*

| $a$ | $T_{min}$ | $T_{max}$ |
|---|---|---|
| 0.0001327 | 15 | 35 |

**Table 2:** Parameters for the development rate function for juveniles of *Pomacea canaliculata*

| $c_1$ | $c_2$ | $c_3$ | $c_4$ | $c_5$ | $c_6$ |
|---|---|---|---|---|---|
| 0.00000000859 | -0.00000136 | 0.00007085 | -0.001597 | 0.0163 | -0.06187 |



Egg development                  Juvenile development

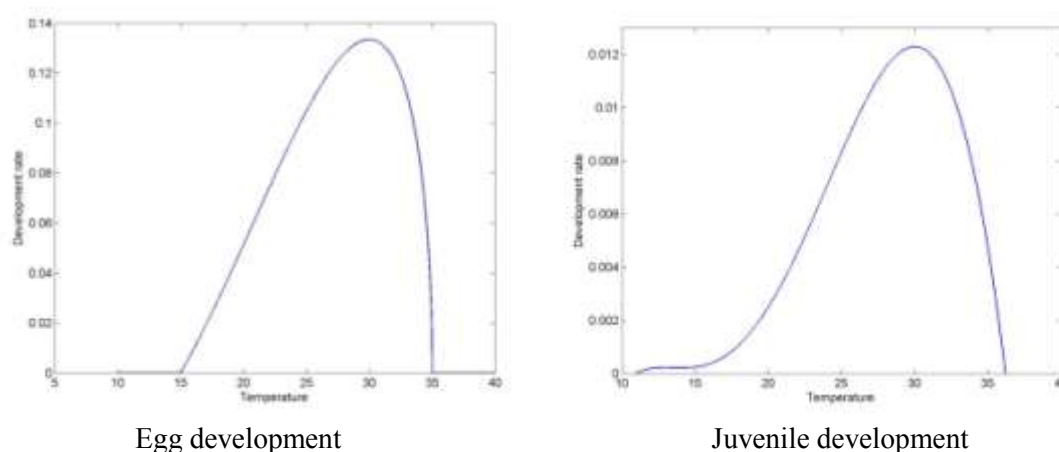**Figure 2:** Development rate function for eggs and juveniles of *Pomacea canaliculata* as function of temperature (°C)

### 3.3.2. Fecundity and adult development rate functions

The number of eggs produced per female per day (i.e. the fecundity rate) is given by the product of a function of the adult physiological age and a function of temperature

$$f\left(T,x\right) = \begin{cases} \cdots & \leq \\ 0 & otherwise \end{cases}$$

where parameters have been estimated based on data for *P. canaliculata* published by Albrecht et al. (1999, 2005) and Burela and Martín (2011). The fecundity rate function is shown in Figure 3.
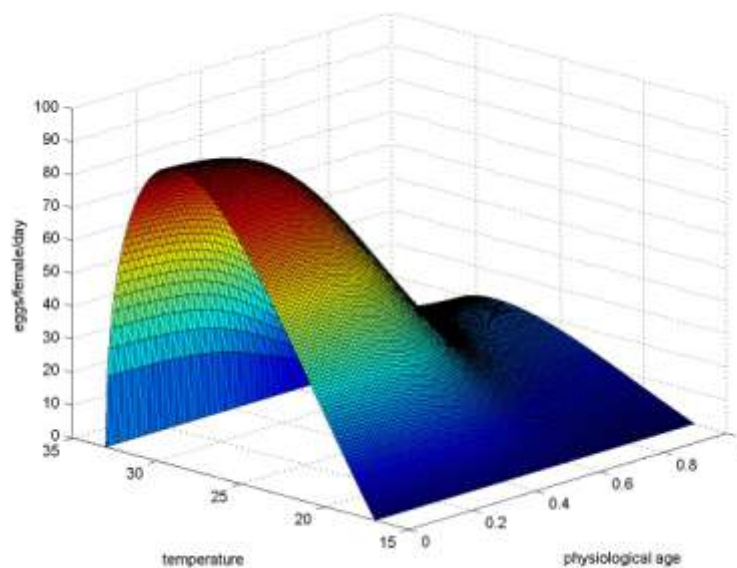
**Figure 3:** Fecundity rate function (number of eggs per female per day) of *Pomacea canaliculata* as a function of temperature (°C) and physiological age (percentage completion of adult lifespan)

The adult development rate is obtained from the fecundity under the following specific assumptions and the procedure described below:

- Each adult female lays 4 500 eggs during her life (total fecundity; Estebenet and Martín, 2002). The instantaneous fecundity rate (eggs/female/day) depends on temperature. We define two extremes – $T_{min}$ = 17 °C and $T_{max}$ = 33 °C – at which fecundity is zero (temperature thresholds for fecundity).
- We use a function describing the instantaneous fecundity rate as function of temperature.
- We run an Individual-based Model (IbM) for a single adult female at a constant temperature describing the linked processes of development and fecundity. Adult physiological age is obtained from the sum of eggs laid up to a certain time *t* with respect to the total fecundity. In particular, at each time *t* the physiological age of the adult is given by: *n° of eggs laid up to t / total n° of eggs to be laid.*
- Development of the adult female ends when she has laid 4 500 eggs, after which she dies. The time at which reproduction ends also represents the end of the development period (i.e. the adult lifespan).
- The development rate at a certain temperature is obtained by taking the reciprocal of the lifespan at that temperature.
- The development rate of adult males at a given temperature is a fixed proportion of the adult female development rate at that temperature.

The simulation results obtained by the IbM were used to calculate the adult female reproductive lifespan and development rate at different temperatures. The results are shown in Table 3.

**Table 3:** The calculated lifespan (in days) and the corresponding development rate (days$^{-1}$) at different temperatures (°C) of adult females of *Pomacea canaliculata*

| T (°C) | Reproductive lifespan (days) | Development rate (days$^{-1}$) |
|--------|------------------------------|-------------------------------|
| 18 | 1065 | 0.000938967 |
| 19 | 522 | 0.001915709 |
| 20 | 343 | 0.002915452 |
| 21 | 255 | 0.003921569 |
| 22 | 203 | 0.004926108 |
| 23 | 170 | 0.005882353 |
| 24 | 147 | 0.006802721 |
| 25 | 131 | 0.007633588 |
| 26 | 120 | 0.008333333 |
| 27 | 112 | 0.008928571 |
| 28 | 108 | 0.009259259 |
| 29 | 107 | 0.009345794 |
| 30 | 110 | 0.009090909 |
| 31 | 121 | 0.008264463 |
| 32 | 55 | 0.018181818 |

The development rate function is obtained by fitting data from Table 3 with the Brière function

$$v(T,t) = \begin{cases} 35T\ T- & - & \leq \\ \\ 0 & & otherwise \end{cases}$$

and the resulting development rate curve is shown in Figure 4.
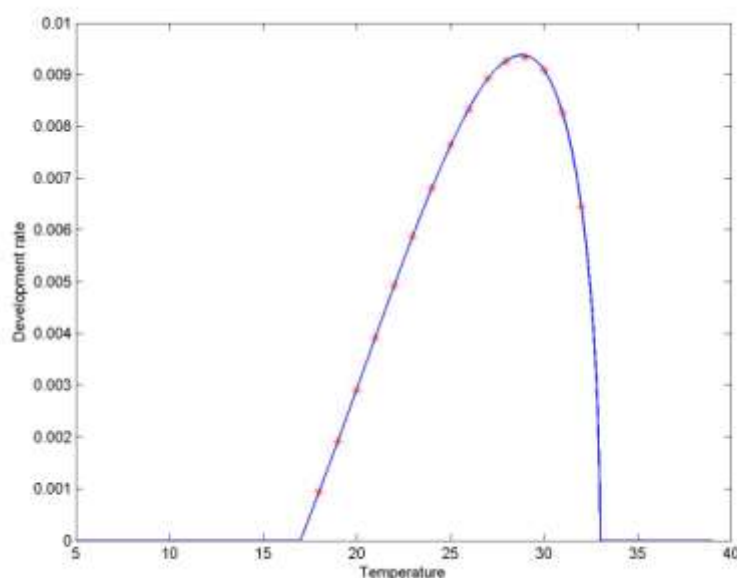


**Figure 4:** Development rate function (day$^{-1}$) as function of temperature (°C) for adult females of *Pomacea canaliculata*

### 3.3.3.  Survival function

Data on egg survival as function of temperature $T$ were published by Liu et al. (2011) and these were fitted by means of a double exponential function of the type

$$s\left(T\right) = \frac{1}{\beta}^{x}$$

resulting in the estimates given in Table 4, and the survival curve presented in Figure 5. The survival function is defined only in the temperature interval for which the development rate function is defined and where data on survival are available.

**Table 4:**  Parameters of the survival rate function for eggs of *Pomacea canaliculata*

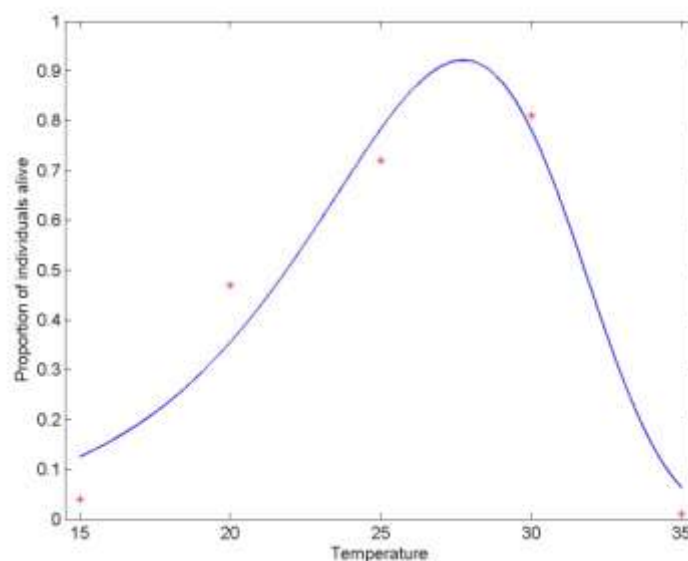|       | $\beta$ | $\mu$ | $\eta$ |
|-------|---------|-------|--------|
| Egg   | 0.3992  | 27.7314 | 4.3279 |



**Figure 5:**  Egg survival (proportion of individuals alive at the end of the stage) as a function of temperature (°C) in *Pomacea canaliculata*. Asterisks are the experimental data and the line represents the interpolated curve

A second order polynomial function for the representation of juvenile and adult survival at different temperatures $T$ could be estimated with data published by Yingying et al. (2008), Liu et al. (2011), Seuffert and Martín (2013):

$$s\left(T\right) = \quad + \quad + \quad$$

where $p_1, p_2, p_3$ are parameters to be estimated; the values of these parameters are given in Table 5 and the estimated survival curves are shown in Figure 6. The survival functions are defined only for the temperature interval for which the development rate is defined.

**Table 5:** Parameters of the survival curve as function of temperature for juveniles and adults of *Pomacea canaliculata*

|  | $p_1$ | $p_2$ | $p_3$ |
|---|---|---|---|
| Juveniles | -0.00275 | 0.1045 | 0 |
| Adults | -0.002912 | 0.1077 | 0 |



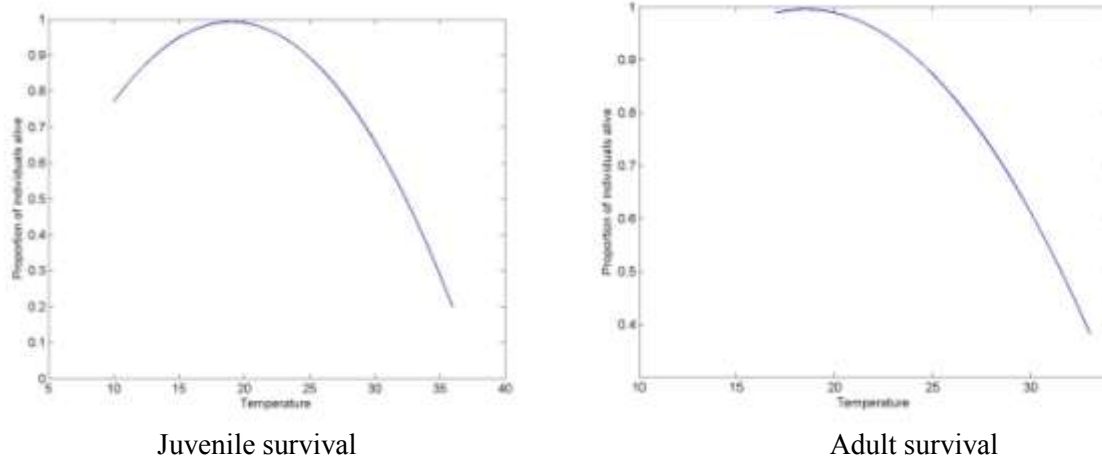Juvenile survival                    Adult survival

**Figure 6:** Survival (proportion of individuals alive at the end of a stage) as a function of temperature (°C) for juveniles and adults of *Pomacea canaliculata*.

### 3.3.4. Mortality rate function

The survival function is used to obtain the instantaneous mortality rate function in days$^{-1}$. The definition of mortality rate function is given in three intervals: the interval between the two development thresholds $\lvert \qquad \rvert$ and two intervals outside the development thresholds, one for temperatures below $T_{min}$ and one for temperatures above $T_{max}$

$$m\ T\ =\ +\begin{cases} \qquad - & \qquad \in \lfloor \qquad \rfloor \\ \qquad - \qquad + & < \\ \qquad - \qquad\ - \qquad \hat{}\ + & > \end{cases}$$

The temperature-dependent mortality rate $m\ T$ depends on the development function within the interval in which development occurs and increases below the minimum or above the maximum development threshold. An additional mortality component $\gamma$ is considered. This mortality component takes into account the effect of abiotic factors other than temperature (e.g., pH, salinity etc.), which become particularly important under temperature stress (low temperature, winter period) (Ito, 2002) and mortality caused by natural enemies.

Density-dependent mortality is obtained by increasing the mortality rate $m(T)$ if the population density is above a given threshold. The total mortality is given by the function

$$\mu = \begin{cases} & \leq \\ & \end{cases}$$

where $N(t)$ is the abundance of juveniles and adults and $\alpha$ and $\varphi$ are positive parameters to be estimated.

Choosing $\gamma = 0$, ⌡. and the values for $T_{\text{inf}}$, $T_{\text{sup}}$, and $\alpha$ given in Table 6, the mortalities represented in Figures 7-9 are obtained.

**Table 6:** Threshold temperatures for the different definitions of mortality for eggs, juveniles and adults of *Pomacea canaliculata*

|  | Egg | Juvenile | Adult |
|---|---|---|---|
| $T_{\text{inf}}$ | 15.0003 | 11.5 | 17.05 |
| $T_{\text{sup}}$ | 34.9998 | 36.15 | 32.96 |
|  | 0.1 | 0.3 | 0.2 |

The values of $\gamma$ will be estimated by a model calibration procedure (see section 4.3).



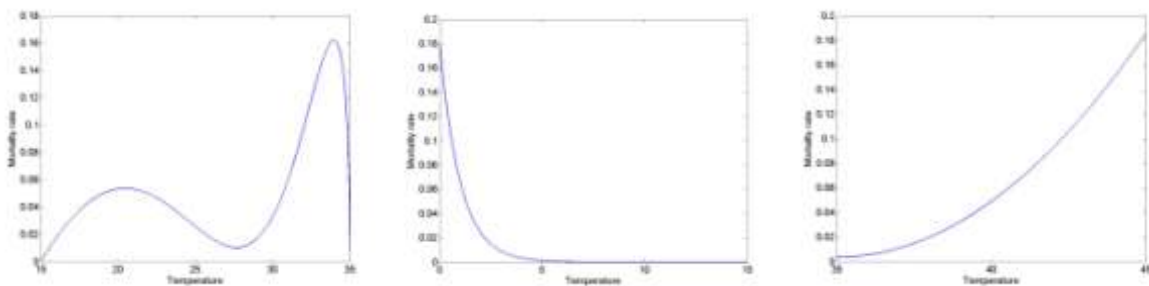**Figure 7:** Egg mortality rate function (days$^{-1}$) as function of temperature (°C) in the interval ⌐ ¦ (left), for $T$ (middle) and for $T$ ---ᵣ (right)



**Figure 8:** Juvenile mortality rate function (days$^{-1}$) as a function of temperature (°C) in the interval ¦ ¦ (left), for $T$ ---- (middle) and for $T$ ---ᵣ (right)

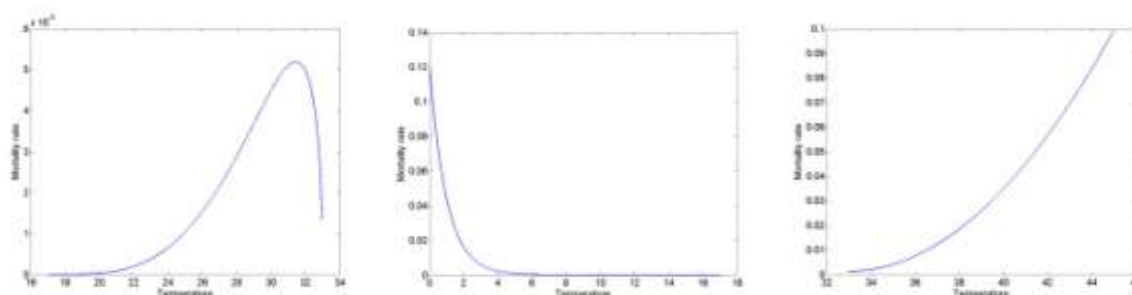**Figure 9:** Adult mortality rate function (days$^{-1}$) as a function of temperature (°C) in the interval ¦ ¦ (left), for $T$ ___ (middle) and for $T$ ___ (right)

## 4. Population dynamic modelling

### 4.1. Introduction

A structured population dynamics model for *P. canaliculata* has been developed to allow prediction of the area of potential establishment. The model also generates an index describing the geographical variation of expected potential snail density, which can be regarded as a representation of snail population abundance. The model deals with the interaction between the invasive species and the relevant ecosystem components of the receiving environments. The model accounts for the influence of abiotic, biotic and management components on snail population abundance, which is regarded as the driving force of ecosystem change (Figure 10). To reduce the mathematical complexity, only a few compartments of Figure 10 are fully developed mathematically, while interactions between compartments have been simplified.

### 4.2. Model components

The mathematical model simulates the population dynamics in a spatial unit. In the model, a spatial unit comprises a spatially defined portion of land characterised by physical (meteorological and hydrological variables), ecological (plants, herbivores and predators) and management subsystems (Figure 10). All these subsystems are viewed in their contribution to facilitate / limit establishment, persistence and growth of the snail populations.
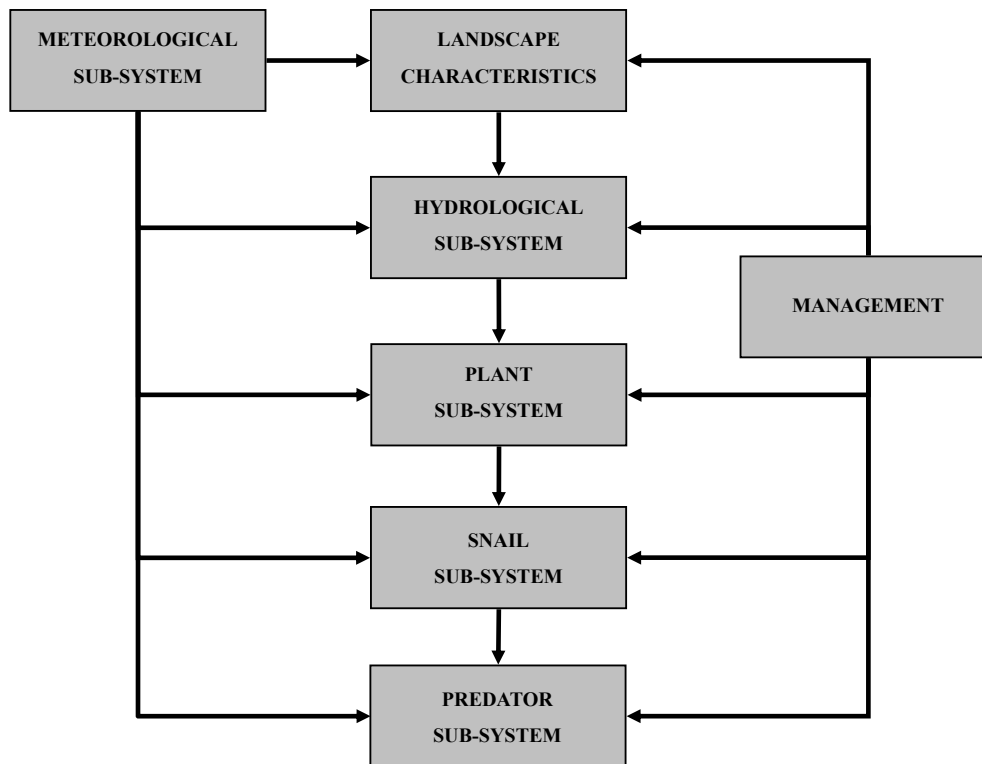
**Figure 10:** A schematic representation of the *Pomacea canaliculata* population system

## Scale aspects and landscape characteristics

The spatial resolution of the meteorological variables is considered the driving aspect determining the scale definition. No spatial heterogeneity is considered within the spatial unit. Scale aspects are also involved in the mobility and spread of the apple snails. The complexity of the hydrological network influencing the natural and continuous spread of the snail and the lack of knowledge of the possible role of zoochoric transport (i.e. dispersal due to mammal and bird vectors) led to the decision not to explicitly model spread.

## Meteorological determinants influencing snail population dynamics

Meteorological variables are the fundamental driving variables for the snail population dynamics. A grid, using geographical coordinates to cover the whole area of Europe, with a step of $0.25 \times 0.25$ degrees latitude and longitude was used. This resulted in $185 \times 153 = 28\ 305$ grid points, which were then reduced to 14 792 points by excluding the areas of sea. For each of these points hourly air and water temperatures were used. Population processes for eggs depend on air temperature as eggs are laid above water, but for juveniles and adults these processes depend on water temperature. Population abundance is calculated for each node of the grid irrespectively if the node is a suitable habitat for the apple snail. When a map with the distribution of wetlands is superimposed on the map with the potential snail distribution, the expected snail abundance in the wetlands can be derived.

The algorithm used to obtain water temperatures uses hourly air temperatures. Available air temperatures are the daily maximum and minimum temperatures from 5 221 meteorological stations from the NOAA-GSOD dataset (National Oceanic and Atmospheric Administration, National Climatic Data Center, Global Summary Of the Day). A geo-statistical procedure consisting of weighted averages, with weighting inversely proportional to squared distances, is used to reconstruct the daily data on the grid covering Europe. The hourly data of air temperature (AT) are obtained for each grid point by applying the de Wit algorithm (de Wit et al., 1978). Water temperatures at the

surface and at -50 cm have been obtained by applying a semi-empirical model founded on the Fourier equation of heat diffusion to hourly air temperature (Larnier et al., 2010).

### Hydrological sub-system

The physical–chemical characteristics and the temporal persistence of surface water (hydro-period) affect the composition and abundance of vegetation and fauna and drive the establishment and persistence of snail populations. For modelling purposes, these characteristics are not considered. Habitats suitable for survival, feeding and reproduction of snails are treated as simplified water bodies. For the sake of simplicity, the main hydrological processes influencing the water level and composition are also disregarded. A mechanistic simulation model is used to determine the thermal profile related to water and near water temperatures. The model is based on the solution of the equation of the surface energy balance (Confalonieri et al., 2005) and uses as input data only daily values of maximum and minimum temperatures. Other input variables are derived from geographic position and related astronomic variables.

### Plant sub-system

The variation in time and space of plant community composition (edibility) and biomass affects the population dynamics of the snail. As a first approximation, snail potential abundance is calculated assuming that snail population growth is not constrained by plant biomass availability and quality. In accordance with the condition of the plant community, cell-specific scaling factors (coefficients) can be estimated by reducing the potential snail abundance in each cell.

### Snail sub-system

A lattice model was used to describe *P. canaliculata* population dynamics using a grid of 25 × 25 kilometres for Europe. In each node the potential snail population abundance is calculated by a physiologically based demographic model that explains population processes from life history traits described at an individual level (Curry and Feldman, 1987; Metz and Diekmann, 1986; Gutierrez, 1996; Di Cola et al., 1999). Changes in the population abundance are mathematically described in terms of the variation in basic demographic processes and not as a result of integrating independent or correlated variables. Population dynamics is the outcome of the three fundamental demographic processes, which are development, mortality and fecundity. Other processes, such as movement, can also be included. Biodemographic temperature-dependent rate functions were estimated using data from the literature. When the data were contradictory or component of the life history traits were not completely explored in the available literature, information provided by experts on the apple snail gave a comprehensive and univocal representation of the life history traits and their dependence on environmental forcing variables. A *P. canaliculata* population is considered to be stage structured, in which three stages are present: eggs, juveniles and reproductive adults. Stage-structured population dynamics for *P. canaliculata* were simulated for each point of the spatial grid through a discretisation of the Kolmogorov equation (Di Cola et al., 1999). The model simulates *P. canaliculata* population dynamics using a time step of one hour (a small time interval is required to guarantee the convergence of the discretised Kolmogorov equation). The simulated abundance is then adjusted by specific coefficients and functions that represent the role of abiotic and biotic regulating factors (e.g., density-dependent regulation is introduced in the mortality). From the model output an index is derived representing the snail's abundance in each node. The index is computed for each node of the whole of Europe.

### Predator sub-system

The potential impact of the predator community is not well known and is difficult to predict in a newly invaded area. To simplify the model, and also because of a lack of specific data regarding Europe, the

mathematical description of the dynamics of the predator community and its interaction with the snail population were not carried out.

## 4.3. Model results

### 4.3.1. Model calibration

The original formulation of the *P. canaliculata* model (*P. canaliculata* Model version 0 = PM0) considers the set of parameters estimated in section 3.2. Preliminary simulations with PM0 revealed an overestimation of the capability of establishment and difficulties in interpolating data concerning the southernmost distribution in Argentina and the predicted distribution is far more extended to the south than is reported from field survey. Thus, model calibration was required considering variation in the biodemographic functions. Variation in parameters of temperature development rate functions was not needed, because reliable data were available. Model calibration considered only fecundity and mortality, because these components of the life history strategies are less well known and because fecundity and mortality are usually more sensitive to factors not accounted for in laboratory studies.

Model calibration has been implemented by the following procedure:
(i) Sensitivity analysis of the temperature-dependent and density-dependent mortality rate functions
(ii) Sensitivity analysis of the temperature-dependent fecundity rate functions
(iii) Estimation of an additional temperature- and density-independent mortality component.

The models obtained for the three cases have been tested considering the capability to interpolate the southernmost distribution in Argentina, as defined by the following information on locations and population density:

Natural populations:
    Pigué stream                                     37°27′1.68″S / 62°28′8.40″W
    Curamalal stream,                          37°14'31.85"S / 62° 8'5.78"W
    Corto stream                                   37°25'31.59"S / 62°22'35.81"W
    El Fuerte reservoir                       37°20'44.23"S / 59° 7'50.43"W
    La Manuela pond                       37°22'33.16"S / 63° 1'58.60"W

Introduced populations:
High-density populations
    El Durazno stream, Miramar town:        38°15'45.47"S / 57°49'57.03"W
    Artificial ponds, Punta Mogotes beach:    38°3'53.49"S /   57°32'40.19"W
Low-density, non-spreading populations
    Paso de las Piedras reservoir:            38°24'47.46"S / 61°41'43.87"W
    Los Chilenos lake:                        38°1'50.10"S /   62°28'38.82"W
    Quequén Grande river:                  38°11'53.62"S / 59° 7'1.85"W
Failed massive introduction to control aquatic weeds
    CORFO drainage channels:             39°34'57.24"S / 62°24'58.63"W

In the group "natural populations" and in the first two sites of the group "introduced populations" the population density is supposed to be high. The locations with low-density, non-spreading populations and the site where the massive introduction of apple snail to control aquatic weeds failed are considered to be located at the edge of the potential distribution. For model calibration purposes, the population abundance is considered to be very low and there is a high risk of local extinction in these locations.
The estimated models have been further tested to simulate the northernmost distribution in Japan (Kasumigaura, 36°06′02.44″N / 140°22′38.86″E), where data on population density are available and can allow a preliminary validation of the predicted density (Ito, 2002).

Because of the overestimation of the distribution in Argentina obtained with model PM0, a sensitivity analysis has been performed for the cases (i)-(ii) by considering a reduction in the fecundity rate and an increase in the mortality rate. The fecundity has been multiplied by a factor 0.8 and the mortality by a factor 1.2. In the less favourable case of simultaneous perturbation of fecundity and mortality a reduction in population abundance is observed but the model is still unable to interpolate the southernmost distribution in Argentina.

Only option (iii) considerably improved results in the interpolation of the distribution in Argentina. There is no unique value of the additional mortality factor optimising the model performance for all the locations tested. Therefore an interval has been defined in which the model interpolated better all the population patterns in the southernmost locations in Argentina. The interval is between 1.5 and 2.0 and two versions of the model were developed for these two extremes: Pomacea model 1.5 (PM1.5) and Pomacea model 2.0 (PM2.0). The model calibration with the locations used for the interpolation of the southernmost distribution of apple snail in Argentina for both versions PM1.5 and PM2.0 are presented in Figures 11 and 12 respectively.

These two models were used to define two scenarios of potential establishment in Europe, one less favourable to apple snail establishment (PM2.0) and one more favourable to apple snail establishment (PM1.5). The limited number of locations available for the model calibration did not allow designating which of the two scenarios could be more likely.

Accordingly, two values for the additional mortality component were obtained by multiplying the peaks of the stage-specific temperature-dependent mortality rates in the interval between the two development thresholds by a coefficient 1.5 (PM1.5) and 2.0 (PM2.0) (Figures 7-9, section 3.3.3). Other values of the multiplying coefficient have been considered but values lower than 1.5 and higher than 2.0 gave unrealistic population distributions and densities.

Simulated data on apple snail dynamics and distributions were obtained by averaging population densities in the last three years of a period of 10 years of simulation. This allowed avoidance or minimising of the effects of the initial population density used for the simulations. In all the simulations the initial population density is equal to one adult per square metre.
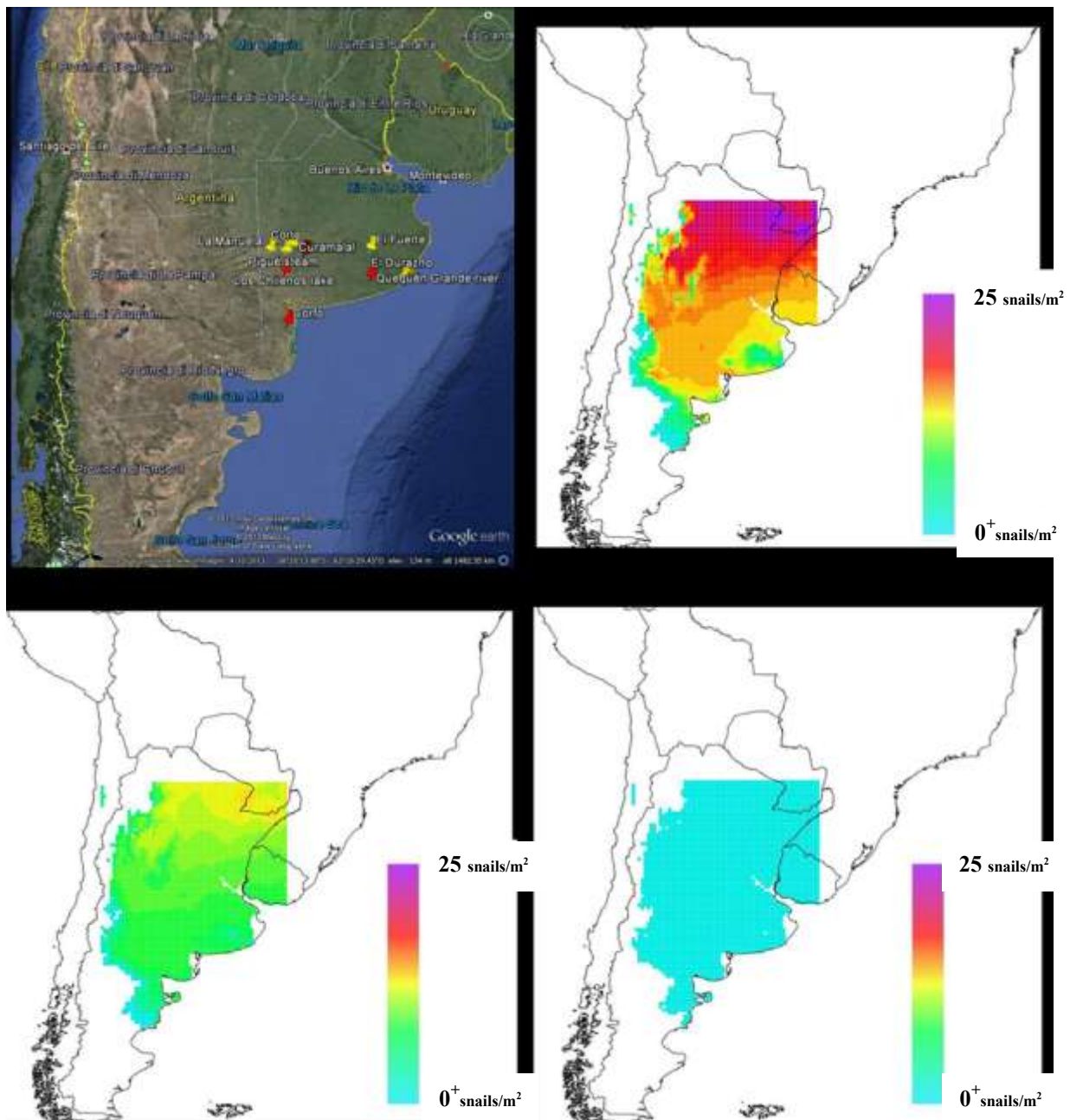
**Figure 11:** *Pomacea canaliculata* model calibration (version PM1.5). The top left section of the Figure indicates the locations used for the interpolation of the southernmost distribution of apple snail in Argentina. The points marked in yellow have high population densities and are locations where it is considered that the apple snail is well established. The points marked in red are locations with low densities, non-spreading populations and considered to be at the edge of the potential area of distribution of apple snail. The other three pictures represent the population densities obtained with the model PM1.5 for eggs (top right), juveniles (bottom left) and adults (bottom right). The colour code in the legend corresponds to density values above 0.
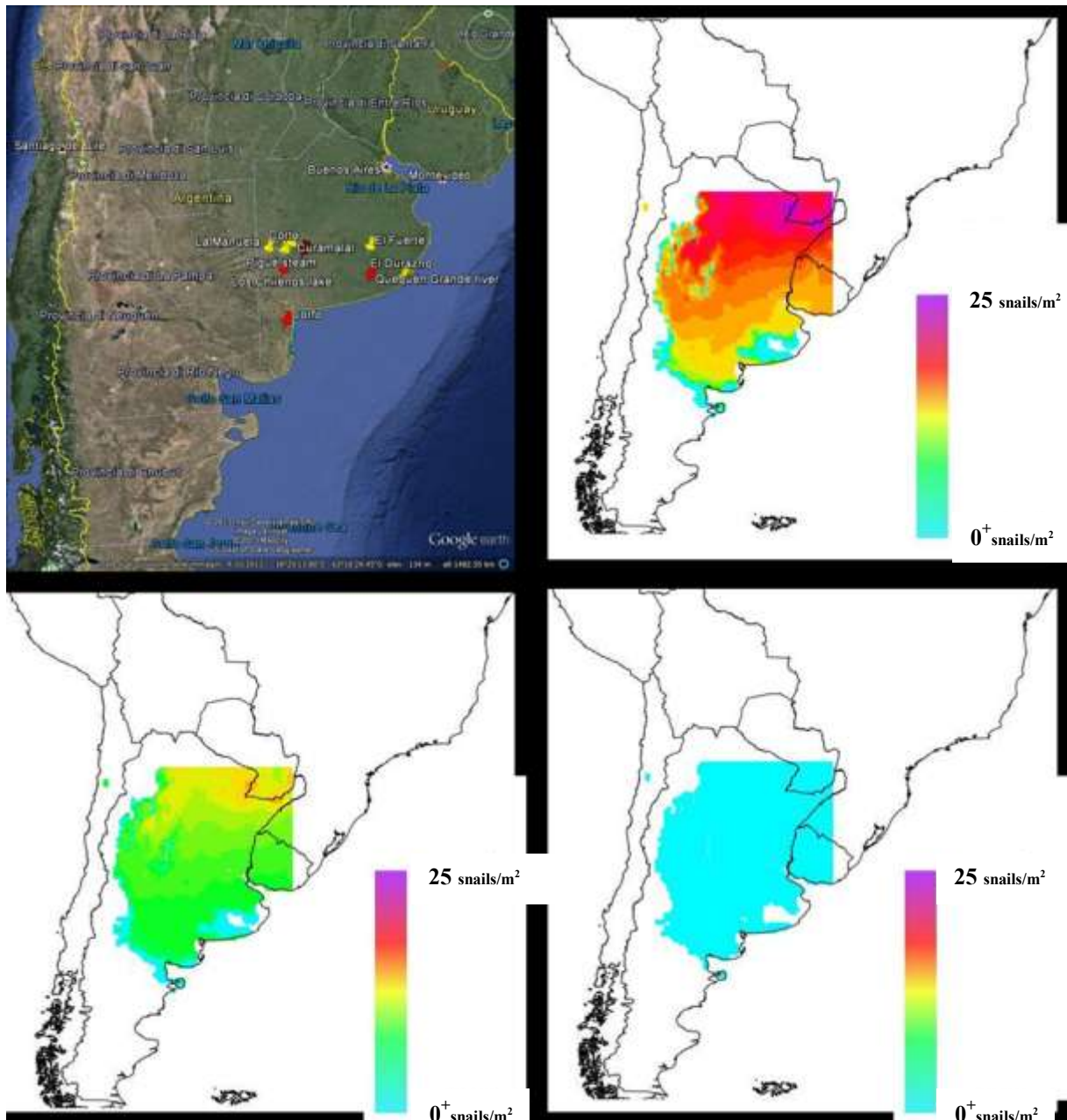
**Figure 12:** *Pomacea canaliculata* model calibration (version PM2.0). The top left section of the Figure indicates the locations used for the interpolation of the southernmost distribution of apple snail in Argentina. The points marked in yellow have high population densities and are locations where it is considered that the apple snail is well established. The points marked in red are locations with low densities, non-spreading populations and considered to be at the edge of the potential area of distribution of apple snail. The other three pictures represent the population density obtained with the model PM2.0 for eggs (top right), juveniles (bottom left) and adults (bottom right). The colour code in the legend corresponds to density values above 0.

The population dynamic patterns of apple snail in the locations at the edge of its potential distribution in South America have been analysed using the models PM1.5 and PM2.0. The population dynamics of apple snail in the Quequén Grande river (38°11'53.62"S / 59°7'1.85"W) and in the Paso de las Piedras reservoir (38°24'47.46"S / 61°41'43.87"W) are shown here as examples. The average temperature profiles in the period 2000-2013 used for the simulation are shown in Figure 13. The population dynamics for the three stages of the apple snail obtained by the PM1.5 and PM2.0 versions of the model are shown in Figures 14 and 15 for the Quequén Grande river and Figures 16 and 17 for Paso de las Piedras reservoir.
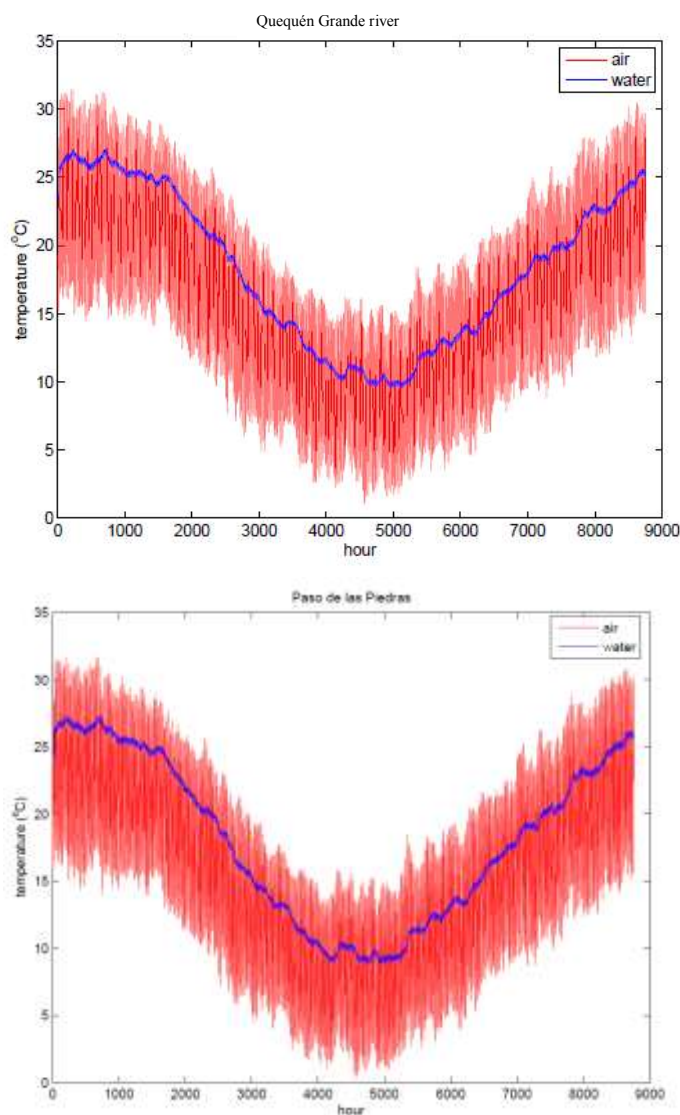


**Figure 13:** Mean hourly temperature profile for the period 2000-2013 for the location Quequén Grande river, Argentina (38°11'53.62"S / 59° 7'1.85"W), above, and Paso de las Piedras reservoir, Argentina (38°24'47.46"S / 61°41'43.87"W), below. Air temperature (in red) and water temperature (in blue) derived from air temperature for a depth of 50 cm by the procedure described in section 4.2. The starting point on the *x*-axis is 1 January, which is mid summer in Argentina, and the final point is 31 December.
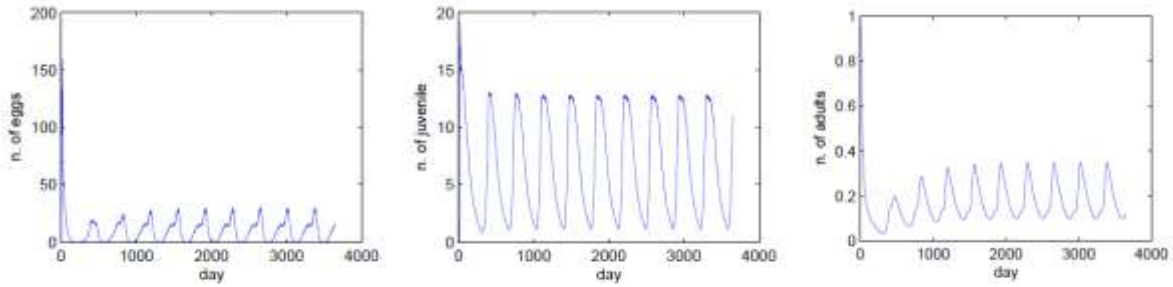
**Figure 14:** Population dynamics of *Pomacea canaliculata* obtained for the location Quequén Grande river, Argentina (38°11'53.62"S / 59° 7'1.85"W), with model PM1.5



**Figure 15:** Population dynamics of *Pomacea canaliculata* obtained for the location Quequén Grande river, Argentina (38°11'53.62"S / 59° 7'1.85"W), with model PM2.0
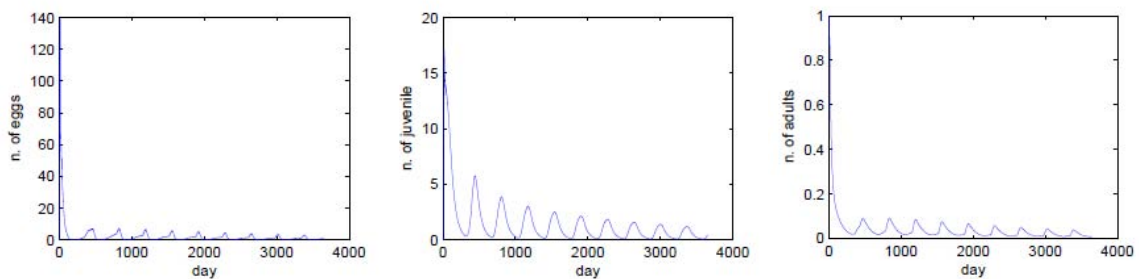


**Figure 16:** Population dynamics of *Pomacea canaliculata* obtained for the location Paso de las Piedras reservoir, Argentina (38°24'47.46"S / 61°41'43.87"W), with model PM1.5
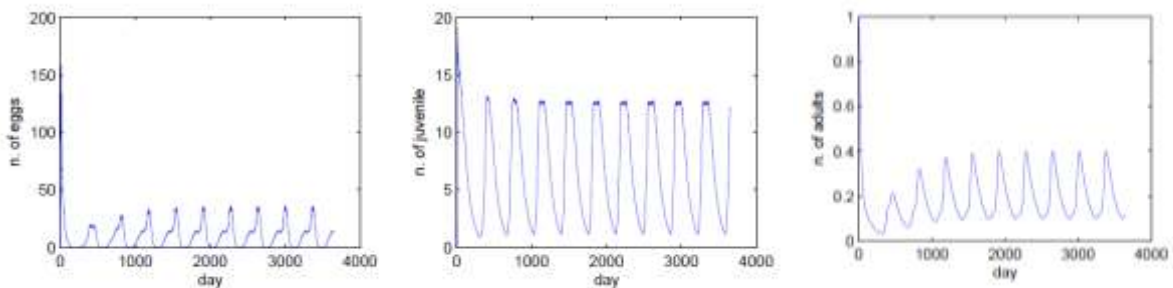


**Figure 17:** Population dynamics of *Pomacea canaliculata* obtained for the location Paso de las Piedras reservoir, Argentina (38°24'47.46"S / 61°41'43.87"W), with model PM2.0
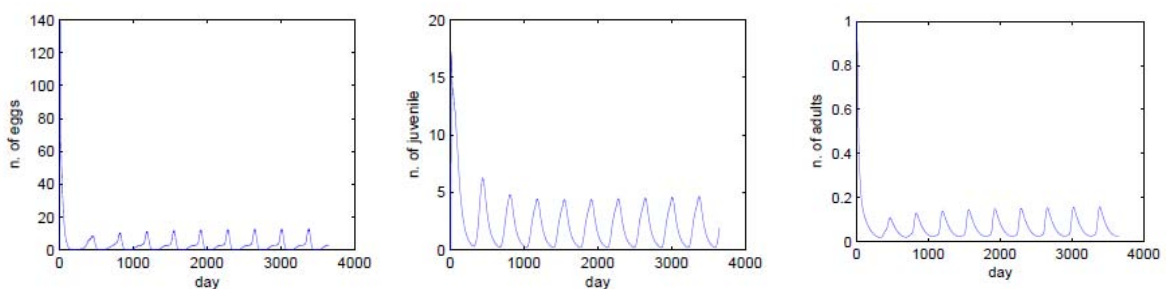
The comparison of the results obtained for the potential distribution in the simulated area of South America and the data on population status at the edge of the area (Figures 11 and 12) results in the conclusion that version PM2.0 gives slightly better results than PM1.5. This is further confirmed by the comparison of the snail population dynamics at each location. For the majority of the populations, the additional mortality coefficient 1.5 does not result in a risk of local extinction or low population density, such as was found for the Quequén Grande river example (Figures 14 and 15) and Paso de las Piedras (Figures 16 and 17). The reported population status in the Quequén Grande river and Paso de las Piedras (classified as a low-density, non-spreading population) is better predicted using the additional mortality coefficient 2.0 (Figure 15).

### 4.3.2. Model validation

Models PM1.5 and PM2.0, calibrated with data from Argentina, were tested considering independent data from the northernmost distribution in Japan (Ito, 2002). The fact that Ito (2002) presents data on population densities made it possible to validate the density predicted by the simulation. The average temperature profile in the period 2000-2013 used for the simulation for the location Kasumigaura (36°06'02.44"N / 140°22'38.86"E) is shown in Figure 18.



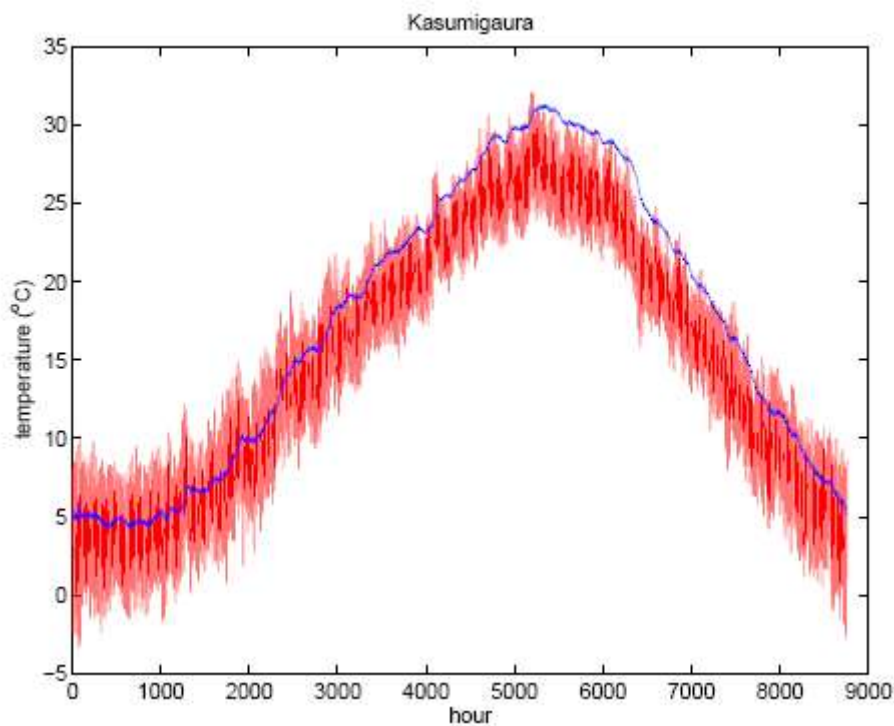**Figure 18:** Mean hourly temperature profile for the period 2000-2013 for the location Kasumigaura, Japan (36°06'02.44"N / 140°22'38.86"E). Air temperature (in red) and water temperature (in blue) derived from air temperature for a depth of 50 cm by the procedure described in section 4.4. The starting point on the x-axis is 1 January, which is mid winter in Japan, and the final point is 31 December.
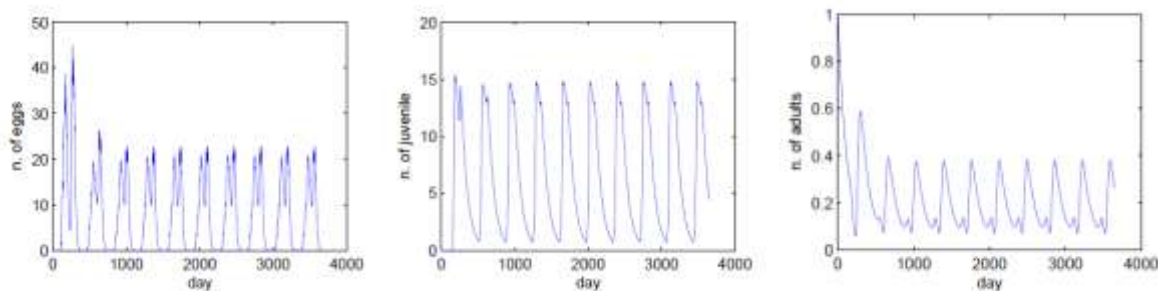
**Figure 19:** Population dynamics of *Pomacea canaliculata* obtained for the location Kasumigaura, Japan (36°06'02.44"N / 140°22'38.86"E) with model PM1.5
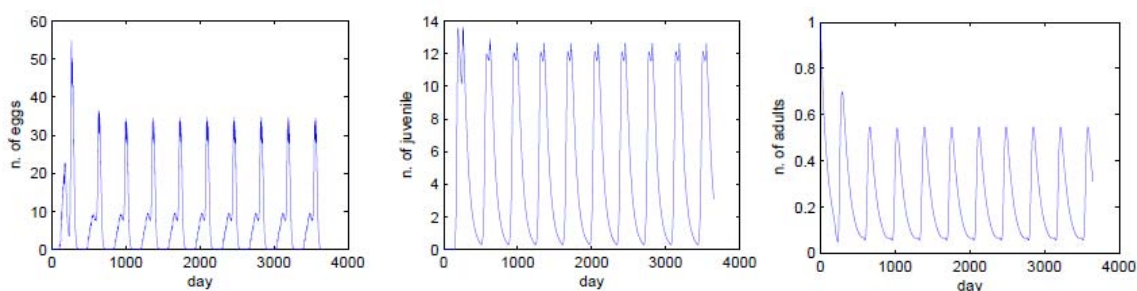


**Figure 20:** Population dynamics of *Pomacea canaliculata* obtained for the location Kasumigaura, Japan (36°06'02.44"N / 140°22'38.86"E) with model PM2.0

The population dynamic patterns for the three stages obtained with models PM1.5 and PM2.0 are shown in Figures 19 and 20 respectively. Using the model calibrated with data from Argentina results in realistic population dynamics for the site near Kasumigaura. An average density of 7.3 eggs/m$^2$, 6.3 juveniles/m$^2$ and 0.18 adults/m$^2$ were found for the last three years of a period of 10 years of simulation for the PM1.5 model. The average densities with the PM2.0 model are 6.7 eggs/m$^2$, 4.9 juveniles/m$^2$ and 0.2 adults/m$^2$. In both cases the population in Kasumigaura appears to be not at risk of extinction, and the high densities suggest that a further expansion towards the North is highly probable in Japan. Another important result is that the combined population density of juveniles and adults fluctuates between a minimum of less than one individual/m$^2$ to a maximum of 13 individuals/m$^2$ for model PM1.5 and between 0.5 individuals/m$^2$ to a maximum of 13 individuals/m$^2$ for model PM2.0. Both these ranges are very similar to the range of population density—minimum 1.8 and maximum 12.3 juveniles + adults/m$^2$—for the same site found by Ito (2002). In conclusion, in an area of recent colonisation and with an on-going process of expansion northwards the performance of PM1.5 and PM2.0 are quite similar. The models' performance decreases for values of the additional mortality coefficient lower than 1.5 or higher than 2.0.

### 4.3.3. Potential distribution in Europe

The potential distribution of the apple snail *P. canaliculata* in Europe is obtained by calculating the average abundance per year in each node of the grid covering Europe. The scale of population density varies between 0 and 12.5 individuals/m$^2$.

The predictions of the potential distribution of *P. canaliculata* for eggs, juveniles and adults obtained with model PM1.5 are shown in Figures 21-23. Model parameters are based on the estimation procedure described in section 3.2, and the results of model calibration using data from the distribution in Argentina and population distribution and density in Kasumigaura, Japan.

**Figure 21:** Projection of the potential distribution of *Pomacea canaliculata* eggs in Europe obtained with model PM1.5. The colour code in the legend corresponds to density values above 0



**Figure 22:** Projection of the potential distribution of *Pomacea canaliculata* juveniles in Europe obtained with the model PM1.5. The colour code in the legend corresponds to density values above 0

**Figure 23:** Projection of the potential distribution of *Pomacea canaliculata* adults in Europe obtained with the model PM1.5. The colour code in the legend corresponds to density values above 0

The predicted potential distributions of *P. canaliculata* in Europe for eggs, juveniles and adults obtained with model PM2.0 are shown in Figures 24-26. Model parameters are based on the estimation procedure described in section 3.2, and the results of model calibration using data from the distribution in Argentina and population distribution and density in Kasumigaura, Japan.
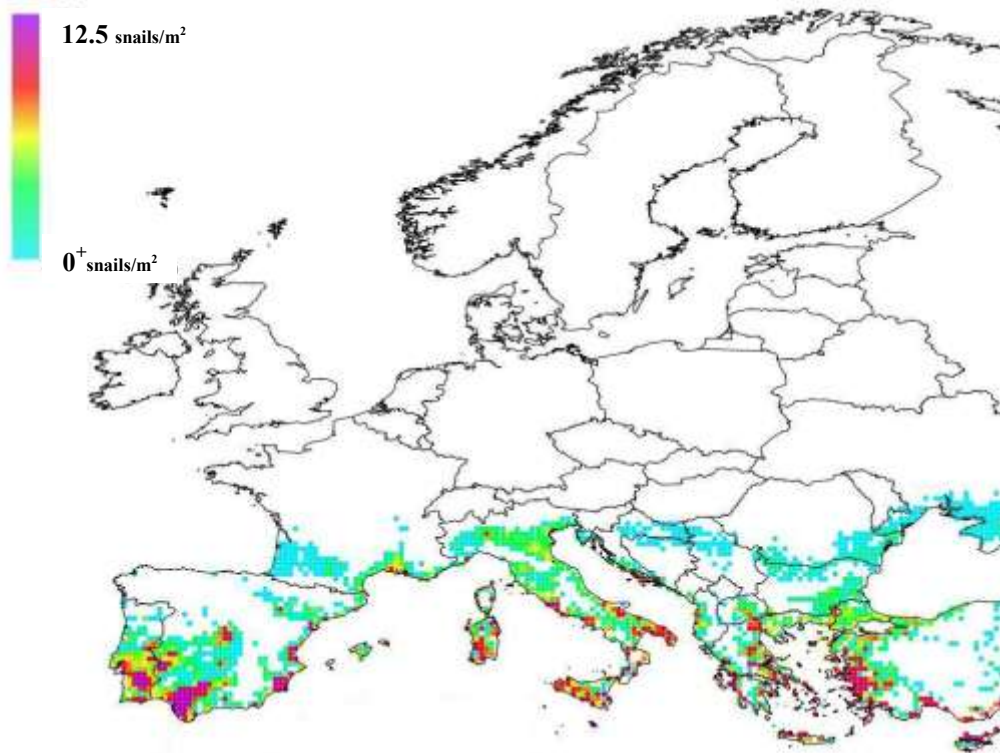


**Figure 24:** Projection of the potential distribution of *Pomacea canaliculata* eggs obtained with model PM2.0. The colour code in the legend corresponds to density values above 0

**Figure 25:** Projection of the potential distribution of *Pomacea canaliculata* juveniles obtained with model PM2.0. The colour code in the legend corresponds to density values above 0



**Figure 26:** Projection of the potential distribution of *Pomacea canaliculata* adults obtained with model PM2.0. The colour code in the legend corresponds to density values above 0

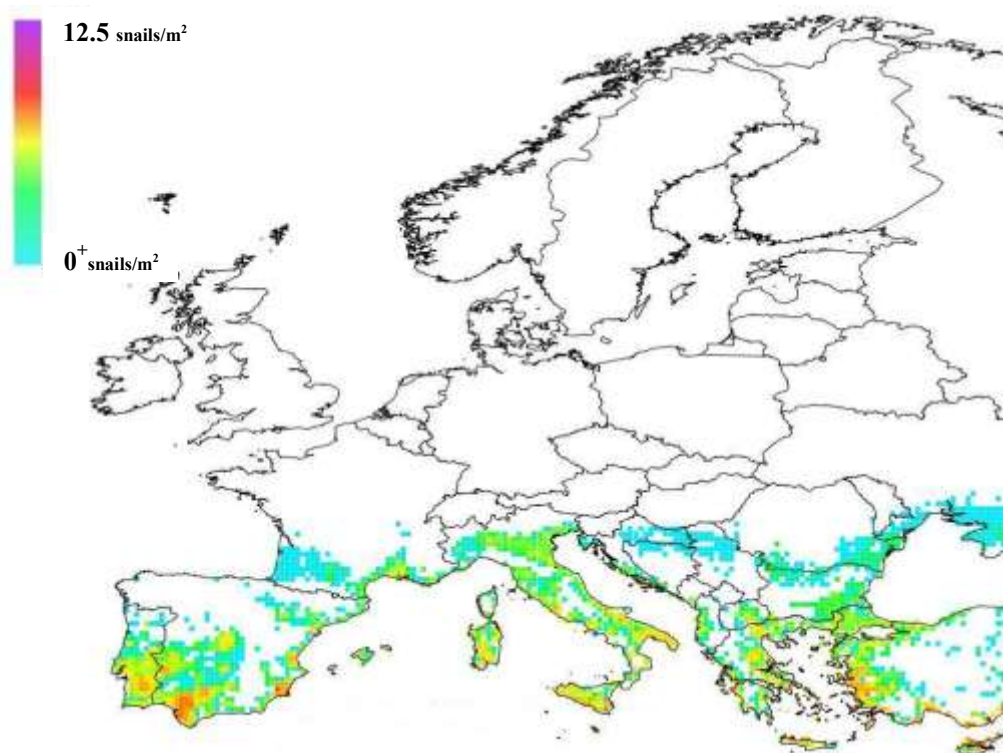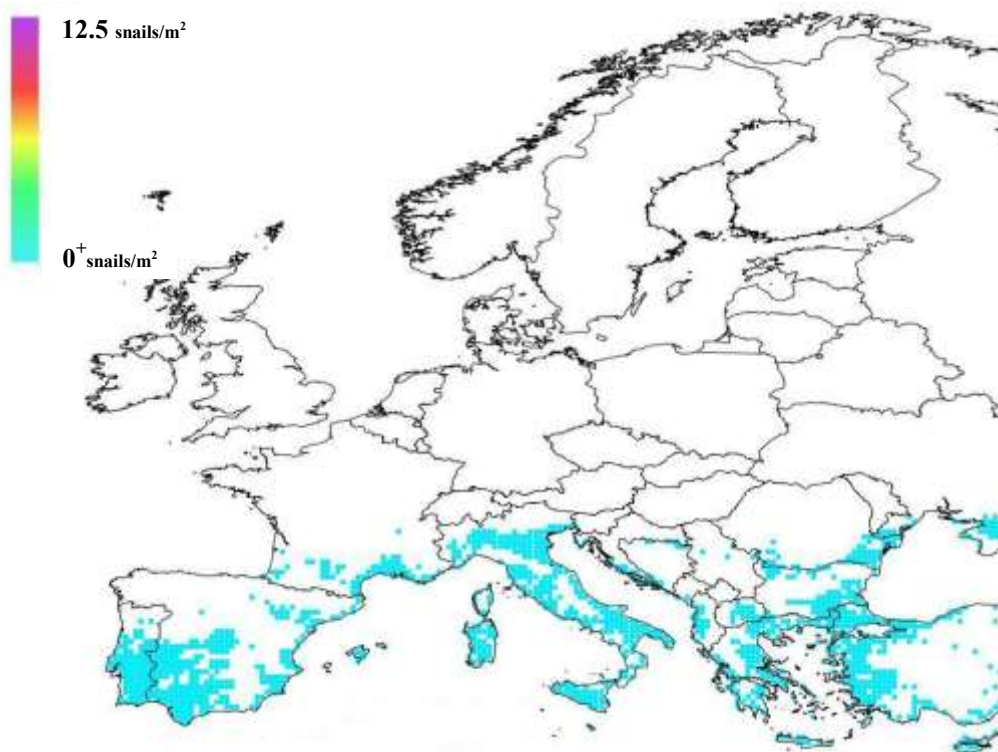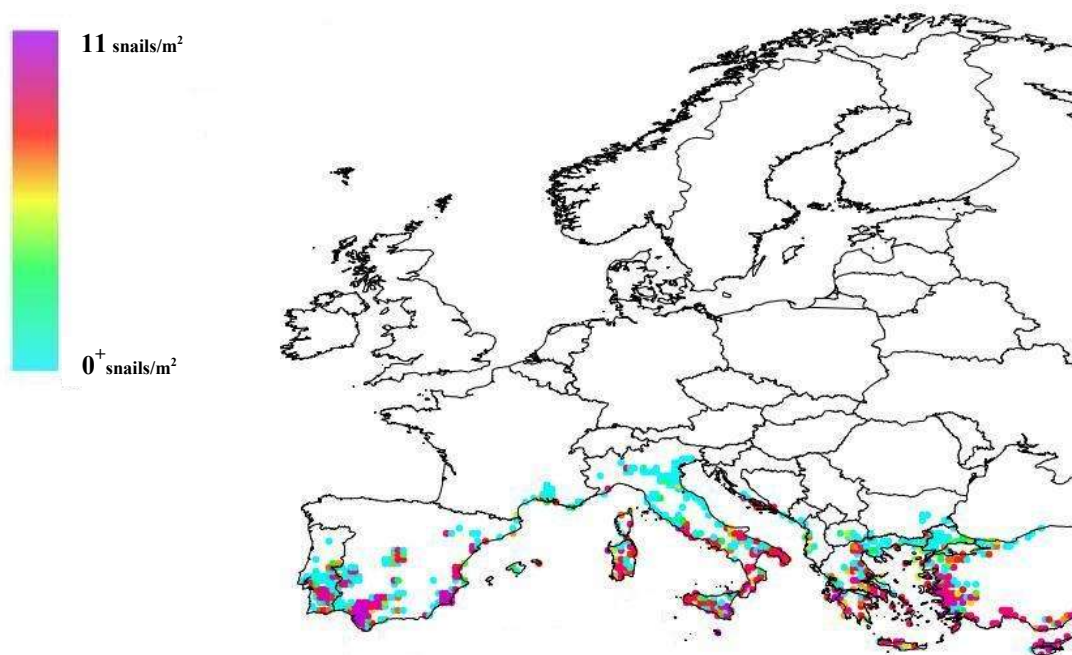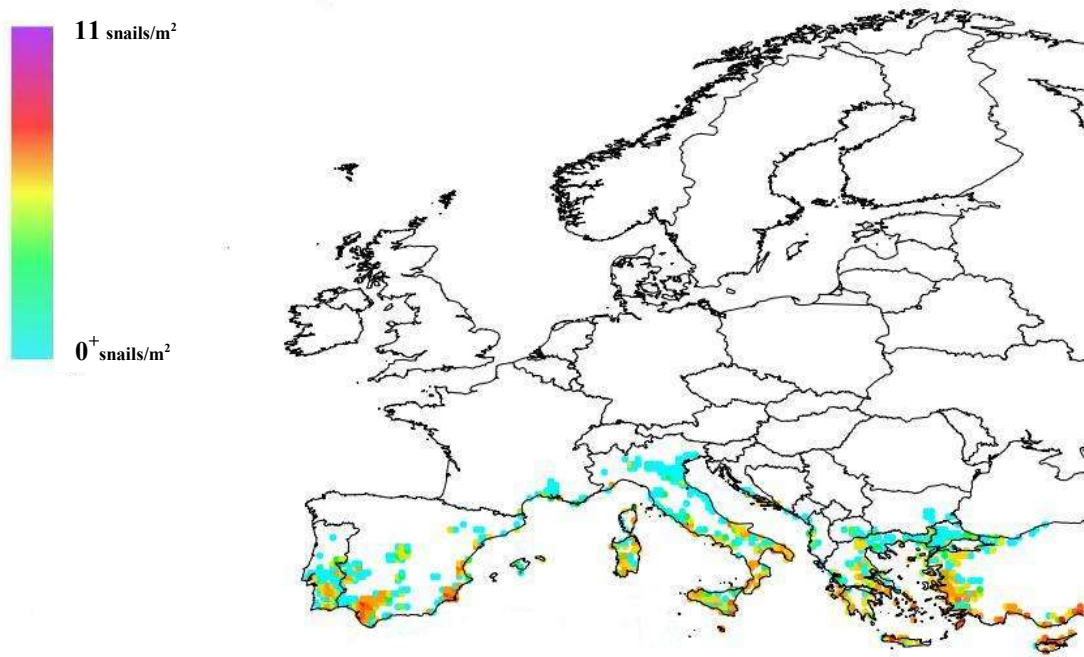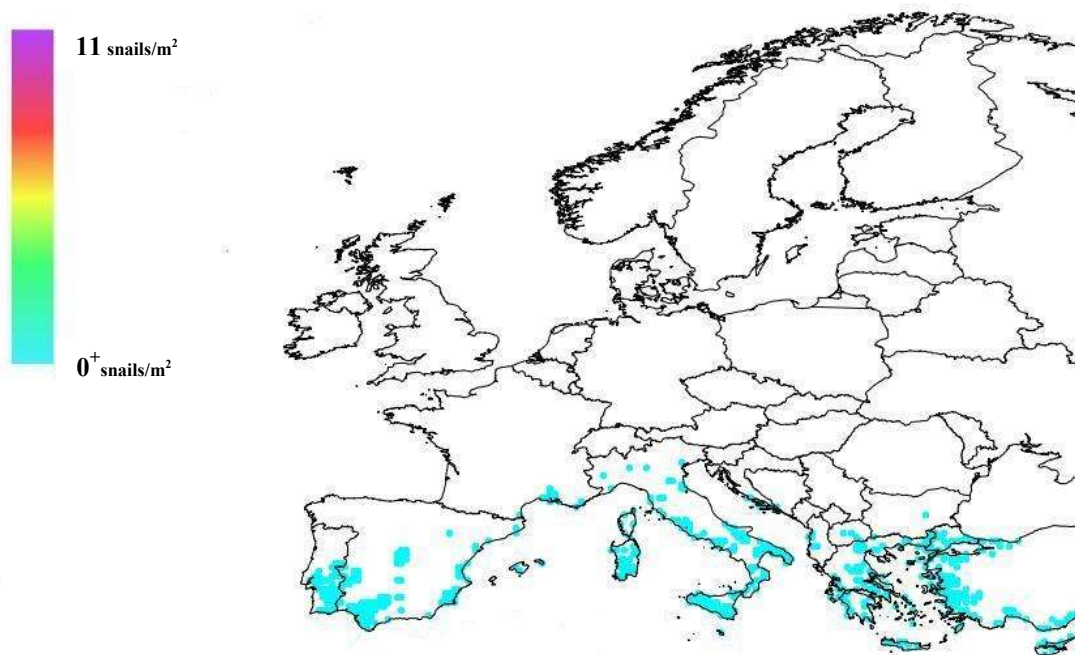The comparison of the two scenarios obtained with models PM1.5 and PM2.0 allows us to draw several important conclusions concerning the potential distribution of the apple snail over Europe:

1. Scenario PM2.0 shows that part of Spain, Italy, the Balkans, and a small area in southern France are potentially suitable for the apple snail establishment in Europe. The northern limit of the potential distribution of snails in Europe is in the Po Valley (Italy). Coastal areas are generally more suitable than inland areas. Adult population density is low in most of the suitable areas in Europe. Several locations are potential foci of high infestation or "hot spots" (populations of more than five individuals (juvenile + adults) per square metre).

2. Compared to scenario PM2.0, scenario PM1.5 shows an expansion of snail establishment towards the North comprising part of Spain, southern France, and northern Italy. The area of potential establishment extends in the Balkans up to the latitude of the Danube River and areas far inland from the Mediterranean coast. In addition, the estimated population densities are higher and the number of hot spots is larger.

3. The area of potential establishment of the apple snails comprises only part of southern Europe including the rice production areas in Europe and most of the wetlands of southern Europe and the Balkans up to the latitude of the Danube River.

## 4.4.    Uncertainties

The uncertainties were rated following the rating descriptors Table presented in Appendix B.

Temperature is the main driver of the population dynamics model. Other abiotic and biotic factors may also limit the potential area of establishment of the apple snail. To account for other factors not explicitly included in the estimated biodemographic functions, the model was calibrated with the southernmost distribution of the apple snail in Argentina and the northernmost distribution in Japan. In view of the results of the model calibration, two values of an additional mortality factor were considered. Accordingly, the Panel proposes two different scenarios of potential establishment and concluded that the most likely scenario falls between these two cases. Because of the good results obtained in describing the population density and pattern of distribution at different locations in Argentina and Japan, the Panel is confident in the reliability of the interval of values of the additional mortality factor. However, the limited dataset available for the model calibration and the uncertainties affecting these data suggest associating a medium level of uncertainty to the estimated values of the additional mortality factor.

Few data are available for the *P. maculata* life history strategies and therefore the biodemographic functions estimates used in the model were developed considering the data available for *P. canaliculata*. However, the species invading the Ebro Delta in Spain is *P. maculata* (previously known as *P. insularum*). Considering the similarities between the two species and the unclear taxonomy of the *Pomacea* genus, the Panel assumes that the population dynamics model results apply to *P. maculata* and the results of the potential establishment of the apple snail in the EU, in terms of snail abundance and geographical distribution, are therefore discussed for both species *P. maculata* and *P. canaliculata*. The Panel estimates the uncertainties on the results linked to this assumption as medium.

For estimating the biodemographic functions of *P. canaliculata*, data on the relationship between stage-specific development, survival and fecundity rates were collected. However some data were not available in the published scientific literature, and expert knowledge was used to estimate this missing information. The Panel considers the uncertainty on the results linked to the data gaps as medium.

As far as the uncertainty in the environmental forcing variables is considered, a low level of uncertainty affects meteorological daily fields of maximum and minimum temperature (pixel 0.25 $\times$ 0.25 degrees latitude and longitude). This is because they are obtained from data measured by synoptic weather stations over a period of 12 years, from 1 January 2000 to 31 December 2012, and spatialised by means of standard and commonly accepted procedures. A low level of uncertainty

affects the simulation model of water temperature driven by the daily maximum and minimum from the temperature dataset. This simulation model is simple and reliable because (a) it is based on the resolution of the Fourier equation of heat propagation in an isotropic mean, parameterised to describe the thermal behaviour of wetlands at a given depth, and (b) it has been validated with a long-time series of a subsurface lake temperatures representative of European environments.

Considering the uncertainties in model calibration and the estimation of biodemographic functions the Panel concludes that there is a medium uncertainty on the results of the population dynamics model of the apple snail using temperature as the main driver of the model.

**CONCLUSIONS**

The European Food Safety Authority (EFSA) asked the Plant Health Panel (hereinafter referred to as the Panel) to deliver a scientific opinion on the risk posed to the environment in the European Union (EU) by the apple snail. In this document the Panel presents a review of the current state of the art of the biology and ecology of the apple snail and performs the assessment of the potential for the apple snails to establish in the EU territory using a population dynamics model. The environmental risk assessment of the apple snail for the EU territory, including an evaluation and update of the risk assessment methodology will be presented in a second scientific opinion.

In a previous opinion on *Pomacea* spp. (EFSA Panel on Plant Health (PLH), 2012b) the Panel concluded, based on a preliminary climate matching exercise, that (a) climate is not a limiting factor for spread and further establishment in the risk assessment area, (b) vast areas of Europe have climatic conditions very similar to those in areas within the current distribution of *Pomacea* spp., and (c) the recognised importance of natural spread, as well the availability of suitable habitats other than rice fields and natural wetlands, identifies a great part of southern, as well as parts of central Europe as potentially endangered areas. (d) The Panel also concluded that a high level of uncertainty is associated with the northern limit of the potentially endangered area, which made it difficult to draw a reliable conclusion about this limit. This high uncertainty was mainly related to a lack of biological data. (e) The Panel also stated that with the support of a population dynamics model that can summarise the effect of biotic and abiotic variables on the snail population dynamics, it could be possible to reduce the uncertainty and make more precise projections on the potential northern limit of establishment. (f) Finally, the Panel concluded that the estimates for the potentially endangered area are probably too limited with regard to the climatic suitability and host plant availability in the risk assessment area. As a result of the population dynamics model developed and used in the current opinion, the Panel can now modify or adapt several of the conclusions formulated in the previous opinion. Concerning conclusions (a), (b), (c) and (f), the Panel concludes that a smaller part of the EU is considered as a potentially endangered area. Concerning conclusion (d) and (e), the Panel concludes that based on data collected recently on the biology of *Pomacea canaliculata* and *P. maculata* together with the population dynamics model, uncertainty about the potential northern limit of establishment could be reduced to medium and more precise estimates could be made about areas potentially most sensitive to *P. canaliculata* and *P. maculata* establishment.

In the previous opinion on *Pomacea* spp. (EFSA Panel on Plant Health (PLH), 2012b), the climate comparisons were based on one of the methods for climate matching available in the modelling tool CLIMEX, which has been widely used in the field of plant health risk assessment. The Panel considered whether the published information on the climate responses of *P. canaliculata* contained sufficient data to compile a specific CLIMEX parameter set for the species that could aid in predicting the climatic potential to establish in the EU. However, the Panel realised that this approach would provide little information beyond the predictions of the potential for establishment and indications on climatic factors that may limit the distribution of the species. Therefore, the Panel decided to use a population dynamics modelling approach in order to be able to estimate the population densities of snail eggs, juveniles and adults, and to identify the potential snail hot spots.

**With regard to the biology and ecology of the apple snail**

Although uncertainties on the systematics and taxonomy of the genus *Pomacea* remain, it is now acknowledged that *P. insularum* is a synonym of *P. maculata* and can be undoubtedly differentiated from *P. canaliculata* based on clear genetic differences. Nine recently collected snails in the Ebro Delta, Spain, were all identified as *P. maculata*.

A thorough review of the literature on the biology of *P. canaliculata* and *P. maculata* was performed and revealed a number of important new data compared to data provided in the previous opinion. These data concern the lower development threshold for eggs; the temperature range and optimum temperature for development of eggs, juveniles and adults; egg, juvenile and adult development related to temperature; reproduction and survival related to temperature; reproduction related to age of adults; cold tolerance of eggs, juveniles and adults; and mating behaviour. The data found for different world locations showed variation, which might be due to the experimental conditions and differences in the genetic composition of *Pomacea* populations.

Natural spread occurs via rivers and canals, in which the snails crawl, drift, raft and float on floating material. Extreme weather events and flooding increase spread. In addition, attachment to other animals such as birds, cattle, horses, deer and aquatic invertebrates results in spread. Snails are further spread by human assistance through cultivation, transport of rice seedlings, aquaculture, aquaria, boats and other means of transportation, agricultural field machinery and irrigation. The risk of spread by human assistance may have decreased recently owing to the Commission Implementing Decision of November 2012, according to which the genus *Pomacea* shall not be introduced into or spread within the EU.

**With regard to the potential establishment of the apple snail in the EU territory using a population dynamics modelling approach**

The limited amount of data on the life history strategies *P. maculata* does not allow developing a population dynamics model on this species. Therefore a model on the population dynamics of the closely related species *P. canaliculata* was developed to enable estimation of the potential density of the snail in a given environment, characterised by specific patterns of air and water temperature fluctuations. The population dynamics of *P. canaliculata* is described through a physiologically based demographic model. First, the model was calibrated acting on the fecundity and mortality of *P. canaliculata*, by using data from several known locations in the southernmost distribution in Argentina. This resulted in the addition of a temperature-independent and density-dependent mortality component. Two values of this additional mortality component have been considered in the subsequent modelling. These two *P. canaliculata* models were used to define two scenarios of potential establishment in Europe, one less favourable to the establishment of the apple snails (PM2.0) and one more favourable (PM1.5). The limited number of locations available for the model calibration did not allow deriving information on the most likely scenario between the two. In addition, the model was tested for the northernmost distribution in Japan and a good fit was obtained for the population dynamics for eggs, juveniles and adults. Finally, the potential distribution of *P. canaliculata* eggs, juveniles and adults for Europe was obtained by calculating the average snail abundance per year in each node of a grid of 0.25 × 0.25 degrees covering Europe. These calculations indicate that:

1. Scenario PM2.0 shows that part of Spain, Italy, the Balkans, and a small area in southern France are potentially suitable for the apple snail establishment in Europe. The northern limit of the potential distribution of snails in Europe is in the Po Valley (Italy). Coastal areas are generally more suitable than inland areas. Adult population density is low in most of the suitable areas in Europe. Several locations are potential foci of high infestation or "hot spots" (populations of more than five individuals (juvenile + adults) per square metre).

2. Compared to scenario PM2.0, scenario PM1.5 shows an expansion of snail establishment towards the North comprising part of Spain, southern France, and northern Italy. The area of potential

establishment extends in the Balkans up to the latitude of the Danube River and areas far inland from the Mediterranean coast. In addition, the estimated population densities are higher and the number of hot spots is larger.

3. The area of potential establishment of the apple snails comprises only part of southern Europe including the rice production areas in Europe and most of the wetlands of southern Europe and the Balkans up to the latitude of the Danube River.

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**Appendix A.    Climate matching modelling**

## 1.    Introduction

The Panel explored the CLIMEX approach for prediction of potential geographical distribution for *P. canaliculata* applying the 'Compare locations' function. This approach is based on estimation of a set of parameter values either from experimental data on the species' responses to climatic conditions and/or from climate conditions in areas where the species is known to occur. In the parameter selection process, only climate data from the areas where *P. canaliculata* occurs outside Europe was used. Two climate databases were used, both covering the period 1961–1990, with two different spatial resolutions of 0.5º and 0.1º latitude × longitude, respectively, based on the climatology CRUCL1.0 developed by New et al. (1999) and CRUCL2.0 by New et al. (2002). The selected parameter values for *P. canaliculata* are presented in Table 1.

## 2.    Results

**Table 1:**    Parameter values for *Pomacea canaliculata* used as input to the "Compare Locations" function of CLIMEX

| Index | Parameter | Meaning | Parameter values | Units[a] |
|---|---|---|---|---|
| Temperature | DV0 | Limiting low temperature | 15 | ºC |
| | DV1 | Lower optimum temperature | 25 | ºC |
| | DV2 | Upper optimum temperature | 32 | ºC |
| | DV3 | Limiting high temperature | 35 | ºC |
| Moisture[b] | SM0 | Limiting low soil moisture | 0.0 | |
| | SM1 | Lower optimum soil moisture | 0.01 | |
| | SM2 | Upper optimum soil moisture | 5.0 | |
| | SM3 | Limiting high soil moisture | 10.0 | |
| Cold Stress | TTCS | Temperature threshold (weekly avg.) | 4 | ºC |
| | THCS | Stress accumulation rate | -0.042 | Week$^{-1}$ |
| Heat Stress | TTHS | Heat stress temperature threshold | 35 | ºC |
| | THHS | Heat stress temperature rate | 0.0045 | Week$^{-1}$ |
| Thermal sum for species persistence[c] | PDD | Annual thermal sum above DV0 | 1000 | Degree days |

(a): Values without units are dimensionless index of a 100 mm single bucket soil moisture profile
(b): Optionally explored.Moisture parameter values are based on estimates used in a CLIMEX analysis for the aquatic plant alligator weed (*Alternanthera philoxeroides*) by Julien et al. (1995), also known to co-occur with Pomacea spp. (Meza-Lopez and Siemann, 2012)

### 2.1.    Soil moisture

According to the CLIMEX user guide the modelling of moisture in the environment, through the 'Moisture Index (MI)', is based on the assumption that "soil moisture is the dominant factor determining the moisture content of vegetation and hence the microclimatic conditions". The Panel noted that this assumption of CLIMEX and its emphasis on soil moisture indicates that CLIMEX is primarily intended for prediction of establishment for organisms under terrestrial conditions. Considering the published CLIMEX study, by Julien et al. (1995), for the alligator weed (*Alternanthera philoxeroides*), a partially aquatic organism, , the Panel decided to use the same set of moisture parameters for *P. canaliculata* as it assumed that these soil moisture population growth parameters were appropriate.

## 2.2. Temperature

The lower temperature limit for development (DV0) is set to a value of 15 °C. In the literature, various estimates are reported for the lower temperature limit for development of the egg, juvenile and adult stages (see section 3.2.1). The parameter value for lower and higher temperature optimum range for population growth (DV1 - DV2) was set to the temperature 25 and 32 °C respectively. The limiting high temperature for population growth (DV3) was set to 35 °C. These values were also inferred from the review of the literature on climate responses of *P. canaliculata* (see section 3.2.1).

## 2.3. Cold stress

In CLIMEX three options are available for capturing cold stress in the 'Compare locations' function. Alike the other stress indices, the cold stress indice in CLIMEX is composed of a threshold value and an exponential accumulation rate value. While the first option aims to capture the situation when daily thermal accumulation is too low to maintain metabolism, the second two options aims to capture mortality due to exposure to excessively low temperatures. One of the latter two options is, according to the CLIMEX user guide, designed for modelling cold stress for the situation in which "a species may spend the winter in locations where it is buffered from extreme minima and maxima". With this option activated, cold stress accumulation is calculated from weekly average air temperatures (°C) below the cold stress temperature threshold, instead of the weekly average minimum air temperature (°C). The Panel considers that this is the only option in CLIMEX that can contribute to capture the observations from field studies that winter survival of *P. canaliculata* mainly occurs in the aquatic environment in areas with cold winters (e.g. Ito, 2002). The much higher aquatic than terrestrial survival rate during winter in cold locations, is assumed to be caused by the cold stress mortality occurring at the lower temperature recorded in the terrestrial environment (Ito, 2002). In his field experiment, Ito (2002) measured both temperature in the paddy soil and drainage channel water.

Alternative parameter values for cold stress temperature threshold and cold stress accumulation rate were tested in several CLIMEX 'Compare locations' simulation runs, based on the data from observed mortality at various low temperature levels and durations reported for *P. canaliculata* in the literature (section 3.2.1). When used in the CLIMEX simulations, most parameter settings for cold stress resulted in a prediction of 100 % winter mortality, for areas at the highest and lowest latitudes within the current distribution of the species. Only when using cold stress parameter values in the stronger end (least sensitive to cold) among the reported cold tolerances, the result indicated possibility for winter survival in all known areas of occurrence (outside Europe). This result was obtained based on the observation reported by Mochida (1991) of survival up to 20 days at 0 °C. Of particular interest, the resulting cold stress accumulation allowed also winter survival in the known northernmost location of occurrence of the apple snail in the world, at Lake Kasumigaura in Japan. In the observed range of occurrence of the species (outside Europe) the cold stress index parameters for CLIMEX was estimated to be 4 °C for the cold stress temperature threshold and with a cold stress accumulation rate of -0.042 per week.

## 2.4. Heat stress

Less data about heat stress for *P. canaliculata* was found in the literature than for cold stress. The heat stress temperature threshold (TTHS) was set to 35 ºC, which is equal to the value used as limiting high temperature for population growth (DV3). The heat stress temperature accumulation rate was set quite low, taking into account that the aquatic environment will offer a delayed heating compared to the terrestrial environment. However, further studies are required to determine more precisely the value of this parameter by delimiting *P. canaliculata* distribution in hot climates.

## 2.5. Annual thermal sum as a constraint to species persistence in an area

An additional option of CLIMEX allows introducing a constraint to species persistence in an area beyond the stress indices accumulating during unfavourable season(s). This is the concept of minimum thermal accumulation above the development temperature threshold (PDD). This parameter is an

annual temperature sum calculated above the minimum temperature threshold for development (DV0). The reasoning behind this additional option is that persistence for poikilothermic species in an area will require a minimum amount of thermal accumulation during the growth season in order complete a minimum of growth, development and/or reproduction. CLIMEX addresses this constraint by the optional parameter PDD (minimum amount of thermal accumulation above the development temperature threshold) which also affects the 'Ecoclimatic Index (EI)' setting it to 0 if the required parameter value set for PDD is not met for an area/location. The Panel explored also this option by looking at the results from CLIMEX for the known locations of occurrence of *P. canaliculata*. The location with the lowest thermal sum above the minimum temperature sum for development (DV0 = 15), was again Lake Kasumigaura with a thermal sum of 1 038 degree-days. The Panel produced a second set of maps with this option activated by setting the value of PDD parameter equal to 1 000 day-degrees.

## 2.6. Predictions of climatic suitability for *Pomacea canaliculata* in Europe and the rest of the World

The predictions of areas of climatic suitability for *P. canaliculata* in Europe indicate optimal climate conditions along the Mediterranean coastline and islands in Greece, Malta and Italy. For Portugal and Spain in particular, regarding the climate conditions, the predicted suitable areas extend inland, and cover most of the South and Eastern coasts of Spain.

## 3. Discussion

Great caution should be taken with respect to the reliability and the potential use of the above results. The lack of correspondence between data on cold stress mortality reported for *P. canaliculata* in the literature, compared to CLIMEX output for parameter settings based on this information, is likely to be due to several factors reducing the reliability of the model results. The most obvious reason is that CLIMEX is not performing the calculation of the accumulated cold stress for an aquatic environment. Air temperature, even if using the weekly average air temperature, seems not to be a usable proxy for water temperature, without further modelling of physical relationships between the air and the water temperatures. What is actually calculated by CLIMEX is the cold stress accumulation under a weekly average air temperature regime. Generally, this is likely to result in an over-estimation of the cold stress, compared to the cold stress occurring under aquatic conditions, and could explain why the attempts to use scientific data showing less cold tolerance, resulted in the above mentioned results.

This limitation of the CLIMEX model is also confirmed by Dr Kriticos D in a personal communication (2013, Principal Research Scientist at CSIRO Ecosystem Sciences), "*in the case of modelling winter survival of the apple snails in cold environments, it is recommended to constrain the CLIMEX simulation of winter survival based on cold stress accumulation to terrestrial environmental conditions, as CLIMEX does not have a module for simulating water temperature conditions. Addition of a post-CLIMEX analytical step could be based on non-standard meteorological data, such as water temperatures or other relevant parameters for the aquatic environment.*"

The climate data used also affects the results. The presented analysis is based on climate data covering the 1961–1990 period. On the other hand, the warmest time period during the last 100 years are the years the 1961–1990 period. This might be a second cause of over-estimation of cold stress, because some areas have simply become warmer after the time period of 1961-90. The two main reasons for use of the 1961–1990 period in this analysis, are (1) that the dataset is readily available for CLIMEX and (2) because this period is still regarded by climatologists to be the most recent real climate period available, as the next period i.e. the 1991–2020 "normal period", will not be available until 2021.

## Appendix B.    Rating Table for uncertainties

| Rating | Descriptors |
|---|---|
| *Low* | No or few information or data are missing, incomplete, inconsistent or conflicting. No subjective judgement is introduced. No unpublished data are used. Where models are used: <br><br> i) input data are clearly described and contain only minor measurement errors; <br><br> ii) model assumptions, structure, methods, algorithms, and limitations are clearly described; <br><br> iii) output is clearly described with sensitivity and uncertainty analysis. |
| *Medium* | Some information or data are missing, incomplete, inconsistent or conflicting. Subjective judgement is introduced with supporting evidence. Unpublished data are sometimes used. Where models are used: <br><br> i) input data are not clearly described and/or contain measurement errors; <br><br> ii) model assumptions, structure, methods, algorithms, and limitations are not clearly described; <br><br> iii) output is not clearly described and neither sensitivity nor uncertainty analysis is available. |
| *High* | Most part of information or data are missing, incomplete, inconsistent or conflicting. Subjective judgement may be introduced without supporting evidence. Unpublished data are frequently used. Where models are used: <br><br> i) input data are not described and/or contain measurement errors; <br><br> ii) model assumptions, structure, methods, algorithms, and limitations are not described; <br><br> iii) output is not described and neither sensitivity nor uncertainty analysis is available. |