

Research Paper

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The effect of temperate climate on potential biocontrol agents for water hyacinth, case study of Rwanda

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Abstract

Water hyacinth is an invasive aquatic plant that has been associated with major negative economic and ecological impacts in water systems worldwide, including Rwanda, since its establishment in the country in the 1960s. While biological control is considered the most sustainable management method, the success of biocontrol agents depends on various abiotic factors, with temperature being critical. This study assessed the suitability of potential water hyacinth biocontrol agents such as: *Neochetina* weevils, *Megamelus scutellaris* Berg (Hemiptera: Delphacidae), and *Cornops aquaticum* Bruner (Orthoptera: Acrididae) for regions with a temperate climate by testing their thermal boundaries. Using thermal physiology limits and CLIMEX modelling, we found that *Neochetina eichhorniae* Warner and *N. bruchi* Hustache (Coleoptera: Curculionidae) had lower thermal minimums (CTmin) of 2.4°C and 2.6°C, respectively, compared to *Megamelus scutellaris* (4.7°C) and *Cornops aquaticum* (6.2°C). CLIMEX modelling predicted the suitability of *Neochetina* weevils and *C. aquaticum* across Rwanda, while *M. scutellaris* appeared unsuitable for the colder northern regions of the country but appropriate for the central and eastern regions. These findings suggest that the historical failure of *Neochetina* weevils introduced to Rwandan water bodies in 2000 was not due to temperature extremes. Rather, other factors such as release numbers or water quality may have played a role. This study provides crucial information for future biocontrol efforts in Rwanda and similar temperate regions, highlighting the importance of pre-release thermal tolerance assessments and climate modelling to predict biocontrol agent establishment and efficacy.

Introduction

Temperature is a critical environmental factor that affects all aspects of living organisms, including their behaviour, physiology, nutrition, growth, development, and distribution in the environment (Clarke, 2014). For insects, temperature influences survival, fecundity, fertility, and overall life span (Zheng *et al.*, 2008), thus impacting their abundance, distribution, and establishment in both native or non-native environments (Yadav and Chang, 2013). Biocontrol agents used to manage invasive alien plant species, particularly insects, are highly dependent on environmental temperature (Morin *et al.*, 2009). Their physiological and behavioural processes, such as metabolic rates, development, feeding, and ultimately survival, reproduction, and fitness, are directly influenced by temperature levels (Morin *et al.*, 2009). These agents are expected to grow, develop, and reproduce sufficiently to reduce the population growth of their target weeds (Barton, 2004). However, as ectothermic organisms, they may fail to establish due to unfavourable temperatures, resulting in minimal damage to the target weed (Goolsby *et al.*, 2005; McClay and Hughes, 2007).

Climate incompatibility is a significant constraint to species' distributions, reducing agent multiplication and ultimately their survival (Byrne *et al.*, 2004). Studies have shown that 44% of biocontrol agents fail to establish due to poor climate compatibility (Harms *et al.*, 2021). Ideally, the distribution of biocontrol agents and their target weeds should have similar climatic tolerances (Hinz and Schwarzlender, 2004). However, this is not always the case, as weeds can often adapt more quickly to new environments than their biocontrol agents (Turner *et al.*, 2015), possibly due to phenotypic plasticity (Broennimann *et al.*, 2007). For example, the biocontrol agent *Gratiana spadicea* Klug (Coleoptera: Chrysomelidae) (tortoise beetle) from South America, released in South Africa in 1994 to control *Solanum sisymbriifolium* Lamarck (Solanaceae), a species native to South America (Hill and Hulley, 1995), failed to control the weed due to its inability to cope with the new climatic conditions of the site (Byrne *et al.*, 2002). Thus, understanding the thermal tolerance or thermal boundaries of biocontrol agents helps to decrease the failure of biocontrol agents due to climatic mismatch (Byrne *et al.*, 2004; May and Coetzee, 2013).

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Studies on the thermal boundaries of biocontrol agents have received increased attention after observing that they sometimes fail despite host specificity tests showing good performance on the weed (Hakizimana, 2011; Hughes *et al.*, 2011). These thermal boundaries can be assessed using either static or dynamic methods (Hoffmann *et al.*, 2003). Static methods assess insect survival at a certain time after exposure to a stressful temperature, while dynamic methods determine the tolerance of an organism by changing the temperature at a constant rate for a certain period and assessing the temperature at which organisms become weak and eventually reach the knockdown stage (Terblanche *et al.*, 2007). One dynamic technique that has been widely used to resolve extreme temperature thresholds is Critical Thermal limits (CTLs) (Rezende *et al.*, 2011). These thermal limits are defined as the low and high temperatures at which an organism loses its locomotory function. One static technique is Lethal Limits (LT₅₀), set by the temperature at which 50% of the population cannot survive (Overgaard *et al.*, 2011). Lethal temperature is used to describe an insect's survival under stress or extreme temperatures that are common within the insect's natural thermal habitat. It helps to understand insects' thermal physiology (Andersen *et al.*, 2015) and provide information on their geographical distribution (Terblanche *et al.*, 2005).

To increase the success and cost-effectiveness of a biological control programme, the distribution, establishment, and efficacy of biocontrol agents should be predicted and modelled before their release into new areas (Allen *et al.*, 2013; Suckling and Sforza, 2014). However, there are many countries in which biocontrol agents have been released without prior establishment of the suitability of the climatic conditions to the agents. An example is the release of *Neochetina* weevils against water hyacinth in Rwanda in 2000 (Moorhouse *et al.*, 2001), without any prior assessment of the agents' thermal limits or sensitivity to other abiotic factors such as turbidity, nitrogen, and phosphorus. The aim of this study was to provide recommendations for future biocontrol efforts against water hyacinth in Rwanda based on the thermal physiology and climate modelling results. This was achieved by (1) investigating the thermal tolerance limits (CTmax, CTmin, and LTmin) of four biological control agents of water hyacinth: *Neochetina eichhorniae*, *N. bruchi*, *Cornops aquaticum*, and *Megamelus scutellaris*; (2) assessing the climatic suitability of different regions in Rwanda for these biocontrol agents, comparing them with their native ranges and current rearing sites; (3) evaluating whether the temperate climate of northern Rwanda could have been responsible for the failure of *Neochetina* weevils introduced in 2000; (4) assessing the potential for successful establishment of *C. aquaticum* and *M. scutellaris* as new biocontrol agents for water hyacinth in Rwanda.

Methods and materials

Study area

Rwanda is a landlocked country located between 1°S to 3°S latitude and 28°E to 31°E longitude. It is bordered by Uganda to the north, Tanzania to the East, Burundi to the South, and the Democratic Republic of Congo to the West. The country lies at two degrees south of the equator and has a complex topography of high hills, mountains, and valleys, with altitudes ranging from 950 to 4,507 m (Ntwali *et al.*, 2016). This results in a moderate climate (Ntwali *et al.*, 2016; Siebert *et al.*, 2019), with average maximum and minimum annual temperatures of 22.9°C and 14°C, respectively. High altitude regions have an annual average temperature

of 15.9°C (Nshimiyimana *et al.*, 2010), with low altitude regions reaching 8°C in April and May (Haggag *et al.*, 2016). Temperatures at intermediate altitude areas (1350–1550 m) vary between 19°C and 21°C. In the lowlands (east and southwest with an altitude of 900 m) average temperatures vary between 21°C and 22.9°C (Muhire *et al.*, 2015) (fig. 1), with extremes reaching 35°C in July and August (Haggag *et al.*, 2016; Safari, 2012). The effects of these temperature variations on water hyacinth proliferation and on the establishment and performance of its biocontrol agents are still unknown. Understanding the temperature range within which biocontrol agents can survive and reproduce is crucial for predicting their efficacy (May and Coetzee, 2013).

Critical thermal and lethal temperature

The Critical Thermal maximum (CTmax) and Critical Thermal minimum (CTmin) for adult *Neochetina* weevils, *C. aquaticum*, and *M. scutellaris* were assessed using a programmable water bath (Julabo F32-ME, Seelbach, Germany) connected to a Labcon circulator (CPE50). Twelve insects of each species were placed individually into 20 ml glass vials sealed with moist cotton wool to prevent evaporative cooling. Vials were placed in a water bath set at 25°C. The water was cooled or heated at a rate of 0.5°C per minute until critical thermal temperature (when individuals lost the ability to move) was reached. The CTmax and CTmin were recorded when individuals lost their coordination.

Lower lethal temperature experiments were carried out on adult weevils using a Labcon low temperature water bath (LTB 12/30) with a Labcon circulator (CPE50). Only the lower lethal temperature was investigated because it was found that the low temperatures in the northern province of the country restricted the growth, the survival and establishment of *Neochetina* weevils reared in rearing stations located in northern province (Moorhouse *et al.*, 2001). Ten weevils for each species were placed separately into 20 ml vials, placed in water baths and to target temperatures for one hour. The experiment was repeated with different individuals at temperatures ranging from 2°C to –7°C. The temperature at which 50% of individuals of each species were dead was recorded. Weevils were then placed into petri dishes for 24 hours to recover. Lower lethal temperatures of *C. aquaticum* and *M. scutellaris* were not tested in this study, since they were already tested (Venturi, 2020; Coetzee, unpublished data).

CLIMEX modelling

CLIMEX is a process-based modelling, able to predict the geographical distribution, establishment, and abundance of species (Sutherland *et al.*, 2004). CLIMEX uses meteorological data and climatic responses to predict the probable distribution and relative abundance of ectothermic organisms, including insects, using existing climate data (Kriticos *et al.*, 2015). This enables it to simulate not only where a species can survive, but also whether it can complete its life cycle and establish persistent populations. It provides a more ecologically realistic framework compared to correlative models including Random Forest and MaxEnt that depend only on environmental predictors and species occurrence data to determine habitat suitability (Elith *et al.*, 2011). Thus, current study employed CLIMEX to compare the climatic data of Rwanda with those of the *Neochetina* weevils' native range in Argentina (the origin site of the weevils), the collection sites in Benin (where the *Neochetina* released in Rwanda in 2000 were collected), and current *Neochetina* rearing stations in South Africa. The Rwandan climatic

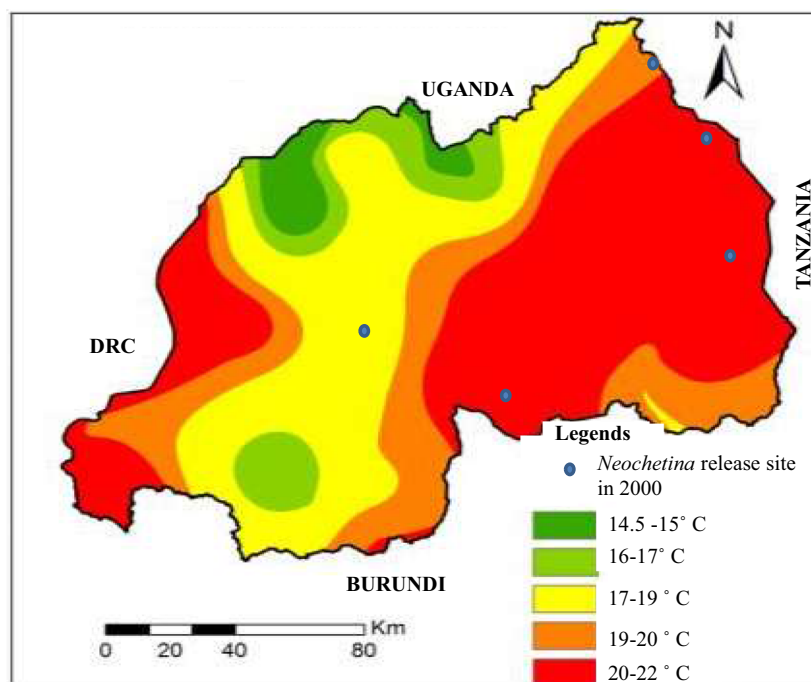


Figure 1. Spatial distribution of mean annual temperatures in different regions of Rwanda (adopted from Muhire *et al.*, 2015).

Table 1. Rwandan sites that reflect the temperatures of the intended areas for potential biocontrol agents of water hyacinth include Gitega, Ruhengeri, Nyamata, and Rusumo, their origin sites in Argentina, collection sites in Benin, and current rearing stations for biocontrol agents in South Africa

Biocontrol agents	Rwandan sites	Matched sites from different countries
<i>Neochetina</i> weevils	Gitega, Ruhengeri, Rusumo and Nyamata	Argentina (original site of <i>Neochetina</i> weevils), weevils collection sites in Benin (Ouémé and Zou Rivers) in the region of Tvedji, and current rearing station at South African Sugarcane Research Institute, rearing site located KwaZulu-Natal, South Africa
<i>Cornops aquaticum</i>	Gitega, Ruhengeri, Rusumo and Nyamata	Manaus, in Brazil (original site of <i>C. aquaticum</i>) Rietondale, Pretoria, South Africa, rearing station of <i>C. aquaticum</i>
<i>Megamelus scutellaris</i>	Gitega, Ruhengeri, Rusumo and Nyamata	Corrientes regions of Argentina, Waainek Research Facility from Rhodes University Grahamstown, South Africa

data were also compared with that of *C. aquaticum*'s native sites in Brazil and its rearing sites at Rietondale, Pretoria, South Africa. Additionally, the Rwandan climatic data were compared and matched with that of *M. scutellaris*' native sites in the Corrientes region of Argentina and its rearing station at the Waainek Research Facility in Grahamstown, South Africa (table 1). CLIMEX comparisons between collection and release sites were based on the mean monthly minimum and maximum temperatures from 2010 to 2019. Rwandan temperature data were obtained from four weather stations: Ruhengeri, Gitega, Nyamata, and Rusumo, that reflect the temperatures of the areas where *Neochetina* weevil rearing stations were established in 2000. Ruhengeri weather station represents climatic data from northern regions; Gitega weather station reflects climatic data from the central region of the country; Nyamata weather station reflects climatic data from Bugesera District and other parts of the eastern region; and Rusumo weather station reflects climatic data from other parts of the eastern region, including Akagera National Park. Temperature data from Argentina were obtained from Servicio Meteorológico Nacional (SMN), Argentina's official national meteorological service, which offers comprehensive weather and climate data, including forecasts, historical records, and real-time observations, accessible at <https://www.smn.gob.ar/>. Climatic data from Benin were obtained

from the World Bank Climate Knowledge Portal. Moreover, temperature data from South Africa were obtained from weather stations of Kwa Zulu Natal, and Graham's town, that reflects climatic data for aforementioned rearing stations of biocontrol agents of water hyacinth. The obtained temperature data were then analysed in CLIMEX to determine the CLIMEX match index (%).

Data analysis

Lethal temperature (LT_{50}), at which 50% of *Neochetina* weevils were predicted to die, was calculated using probit analyses as described by Mitchell *et al.* (1993). In this regard, a probit link function via the R studio interface was used. CLIMEX model for Windows Version 2 (Sutherst *et al.*, 2004) was used to assess the climatic suitability of Rwanda for the biocontrol agents. CLIMEX match index was calculated using the composite match indices by comparing the temperature data of Rwandan sites with the native ranges and current rearing sites of the biocontrol agents. The value ranges from 0% to 100%, with higher values indicating high climate similarity. To compare climatic conditions between intended Rwandan release sites and the biocontrol agents' origin/rearing sites, we used a repeated measures ANOVA to compare means of monthly maximum and minimum temperature data from the

intended Rwandan release sites to those of the biocontrol agents' origin, collection, or rearing sites.

Results

Thermal limits

The biocontrol agents examined in this study demonstrated a wide tolerance to both high and low temperatures, suggesting their potential to establish successfully in Rwanda (table 2). The mean CTmax for all investigated biocontrol agents ranged from $39.14^{\circ}\text{C} \pm 0.42^{\circ}\text{C}$ (SD) to $48.1^{\circ}\text{C} \pm 0.97^{\circ}\text{C}$ (SD). The mean CTmin ranged from $2.4^{\circ}\text{C} \pm 0.81$ (SD) to $6.15^{\circ}\text{C} \pm 0.09^{\circ}\text{C}$ (SD). Furthermore, the mean lethal temperatures for all biocontrol agents investigated in this study ranged from $-3.7^{\circ}\text{C} \pm 0.36^{\circ}\text{C}$ (SD) to $-3.2^{\circ}\text{C} \pm 0.46^{\circ}\text{C}$ (SD).

Climate matching

Generally, the results showed good climatic matching between Rwandan temperatures and those of the biocontrol agents' native ranges. In most Rwandan weevil's release sites, *Neochetina weevils* had matches of over 60% with the Argentinean native habitats, collection sites in Benin, and rearing sites in South Africa (table 3). High matches (above 60%) in minimum and maximum temperatures were observed between Rwandan, Argentinean, and South African climatic conditions, but not with those of Benin (table 3).

The potential release sites for *Cornops aquaticum* in Rwanda displayed a total index match of above 60% compared to its site of origin (table 4). Moderate climatic matching was found when comparing the Rwandan climate to *C. aquaticum*'s rearing sites in South

Africa (table 4). For *M. scutellaris* climatic matching indicated suitability for introducing it from the Corrientes region in Argentina into Rwandan sites, with an overall match above 60% (table 5). However, a low match (below 50%) in minimum and maximum temperatures was observed when comparing the Rwandan sites to *M. scutellaris*' rearing sites in South Africa (table 5). The mean maximum and minimum temperatures from Argentina, were statistically lower than those from Rwanda (Tmax: $F_{4,5} = 42.2$; $P < 0.01$; Tmin: $F_{4,5} = 52.562$; $P < 0.01$) (fig. 2). Instead, the mean maximum temperatures of Benin and Brazil were not statistically higher than the Rwandan ones, despite being slightly higher ($F_{4,5} = 12.45$; $P > 0.05$; $F_{4,5} = 18.215$; $P > 0.05$) (fig. 2). In contrast, the mean minimum temperatures of Benin and Brazil were significantly higher than the mean minimum temperatures of the Rwandan proposed sites for biocontrol agents release ($F_{4,5} = 27.84$; $P < 0.01$, and $F_{4,5} = 37.142$; $P < 0.01$), respectively (fig. 3).

Discussion

This study investigated the thermal tolerance and potential establishment of four biocontrol agents for water hyacinth in Rwanda. The findings revealed that *Neochetina weevils*, *C. aquaticum*, and *M. scutellaris* are likely to establish and perform well in Rwandan water systems. These results suggest that Rwandan temperatures do not pose a significant threat to the biocontrol agents, highlighting their potential viability for controlling the invasion of water hyacinth in Rwanda.

Thermal tolerance and establishment potential biocontrol agents

Neochetina weevils

The results of this study revealed that *Neochetina weevils* (*N. eichhorniae* and *N. bruchi*) can survive a wider temperature range than initially assumed.

Neochetina weevils are affected by low temperatures in cold regions (Byrne et al., 2010). Before this study, the concern was that *Neochetina weevils* might not survive the low temperatures of Rwanda's northern and eastern regions, respectively, to effectively establish and control water hyacinth.

The Critical Thermal minimum (CTmin) values of 2.42°C for *N. eichhorniae* and 2.6°C for *N. bruchi*, coupled with a lower lethal temperature of -3.7°C , indicate that these species are well-adapted to Rwanda's climate. Even in the country's northern regions, where temperatures can drop to 8°C , these weevils are unlikely to experience cold stress, which typically occurs at temperatures below their CTmin and lower lethal temperatures (Chown and Nicolson, 2004). Although there is no laboratory

Table 2. Thermal limits, such as critical thermal maximum (CTmax), critical thermal minimum (CTmin), and lethal temperature minimum (LTmin), for the biological control agents *Neochetina eichhorniae* and *Neochetina bruchi*, released against water hyacinth in Rwandan water bodies in 2000, as well as *C. aquaticum* (VENTURI, 2020) and *M. scutellaris* (coetzee, unpublished data), which are intended biocontrol agents for future release in Rwanda. Note that data on lethal temperature for *C. aquaticum* were not found

Insects	CTmax	CTmin	LT min
<i>Neochetina eichhorniae</i>	47.3 ± 1.1	2.4 ± 0.81	-3.7 ± 0.36
<i>Neochetina bruchi</i>	48.1 ± 0.97	2.61 ± 1.07	-3.7 ± 0.36
<i>Cornops aquaticum</i>	47.9 ± 0.53	6.15 ± 0.09	No data
<i>Megamelus scutellaris</i>	39.14 ± 0.42	4.7 ± 0.37	-3.2 ± 0.46

Table 3. The temperature match index (TMI, %) obtained by comparing the temperatures of Rwandan sites proposed for *Neochetina weevils* with those of their native origin in Buenos Aires Argentina, collection sites in Benin, and their current rearing sites, Kwa Zulu Natal, South Africa TMI was quantified for both maximum temperatures (max temp) and minimum temperatures (min temp)

	Buenos Aires sites	Beninese sites	KZN, South Africa						
	TMI (%)	Max Temp	Min Temp	TMI (%)	Max Temp	Min Temp	TMI (%)	Max Temp	Min Temp
Rusumo	73	78	68	63	69	52	76	61	82
Ruhengeri	72	83	61	72	63	82	78	85	72
Gitega	74	88	61	63	53	55	66	64	76
Nyamata	71	63	58	68	72	61	64	65	73

Table 4. The temperature match index (TMI, %) obtained by comparing the temperatures of Rwandan sites proposed for *C. aquaticum* release with those of its origin in Manaus, Brazil, and its rearing site in Pretoria, South Africa TMI was quantified for both maximum temperatures (max temp) and minimum temperatures (min temp)

Rwandan sites	Manaus, Brazil			Pretoria, South Africa		
	Overall TMI	Max temp	Min temp	Total Index	Max temp	Min temp
Rusumo	72	74	69	64	62	58
Ruhengeri	68	63	73	68	54	56
Gitega	65	62	56	74	66	79
Nyamata	78	64	80	72	65	86

Table 5. Temperature match index (TMI, %) obtained by comparing the temperatures of Rwandan proposed sites for *M. scutellaris*, with those of its origin in corrientes regions of Argentina, and its rearing site at the Waainek Research Facility, Grahamstown, South Africa TMI was quantified for both maximum temperatures (max temp) and minimum temperatures (min temp)

	Corrientes region of Argentina			Grahamstown, South Africa		
	Total index	Max temp	Min temp	Total index	Max temp	Min temp
Rusumo	77	71	82	65	68	62
Ruhengeri	42	36	47	46	45	38
Gitega	65	58	62	71	75	67
Nyamata	70	71	65	62	72	52

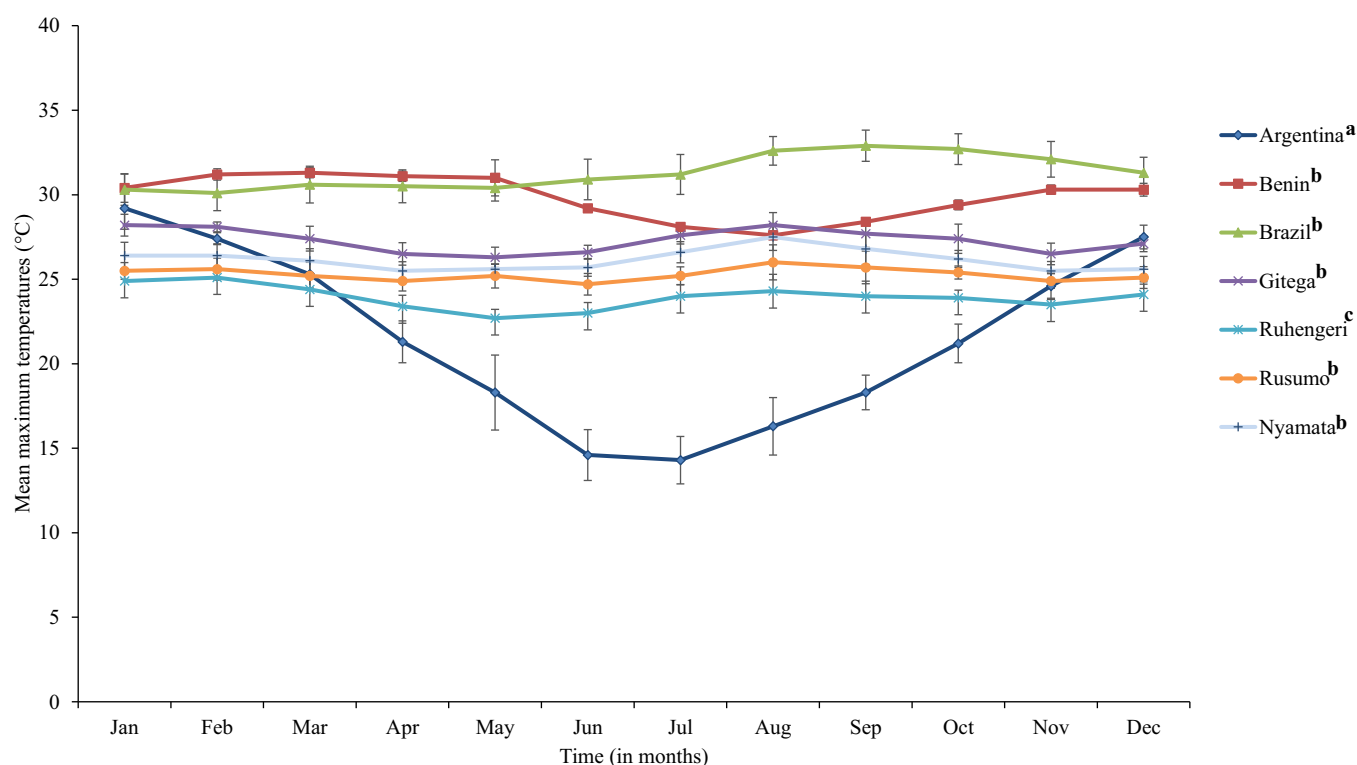


Figure 2. Monthly mean maximum temperatures for a period ranging from 2010 to 2019 for Argentinean (Buenos Aires), Beninese (tevedji), Brazilian (manaus) collection range, and the Rwandan proposed sites such as Gitega, Ruhengeri, Rusumo, and Nyamata for release of *Neochetina* weevils, *C. aquaticum*, and *M. scutellaris*. Repeated measures ANOVA were used to determine the statistical significance in mean maximum temperatures between the different sites at $P < 0.05$. The same lower-case letters indicate not statistically significant differences in maximum temperature between sites.

nor field study on varying temperatures across Rwanda impact survival, growth, and establishment of *Neochetina* weevils, studies by Bokotomba (2017) and Reddy *et al.* (2018) revealed that cold temperatures generally hinder *Neochetina* weevils' activity, development, and survival, while warmer temperatures within

their optimal range promote feeding, development, survival, and reproduction.

The climate matching evidence analysis further confirmed the suitability of *Neochetina* weevils for use in Rwanda, though the level of matching varies across regions. A high matching index

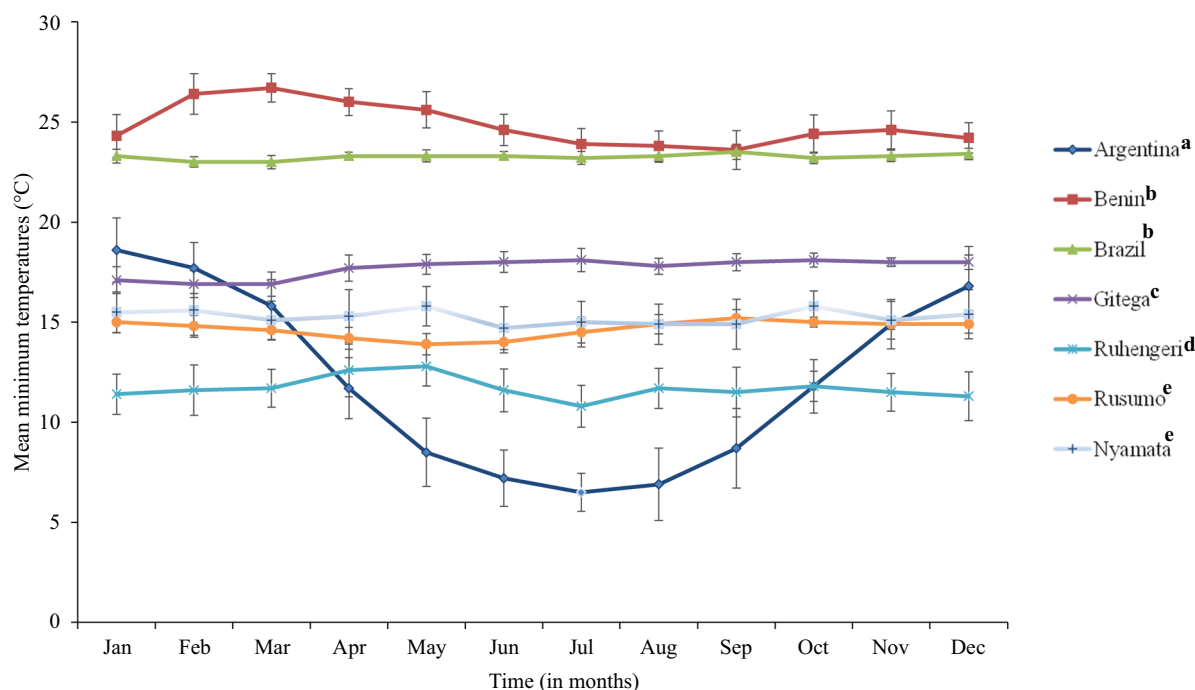


Figure 3. Monthly mean minimum temperatures for a period ranging from 2010 to 2019 for Argentinean (Buenos Aires), Beninese (tevedji), Brazilian (manaus) collection range, and the Rwandan proposed sites such as Gitega, Ruhengeri, Rusumo, and Nyamata for release of *neochetina* weevils, *C. aquaticum* and *M. scutellaris*. Repeated measures ANOVA were used to determine the statistical significance in mean maximum temperatures between the different sites at $P < 0.05$. The same lower-case letters indicate not statistically significant differences in maximum temperature between sites.

(above 60%) between the climate of Rwanda's northern and eastern regions and those from Benin suggests good potential for establishment in these areas. However, the lower matching index (58%) for the central region of the country indicated that the establishment might be less successful there. These findings align with Firehun *et al.* (2015), who showed that *Neochetina* weevils are effective biocontrol agents for water hyacinth in Western and Eastern Africa's warm and temperate climate. Thus, our results suggest that water temperature was not the primary cause of the failed establishment of *Neochetina* weevils released in Rwandan water bodies in 2000. Instead, factors such as low release numbers in lakes (Moorhouse *et al.*, 2001), and high turbidity levels in the rivers (Mukarugwiro *et al.*, 2023) likely contributed to their failure.

Cornops aquaticum

The critical thermal minimum (CT_{min}) of 6.15°C for *C. aquaticum* suggests that it can establish in Rwanda, where the lowest recorded temperature is 8°C (Siebert *et al.*, 2019). Unlike countries that experience low temperatures and frost, like South Africa, where biocontrol agents may experience a 'chill coma' lasting for approximately three to four hours during winter (Byrne *et al.*, 2010), Rwanda's climate appears favourable for *C. aquaticum* establishment. The high match index (60% and above) between Rwandan, Brazilian, and South African climates further support this conclusion, suggesting that *C. aquaticum* could establish in all proposed released sites across Rwanda.

Megamelus scutellaris

While *M. scutellaris* has been primarily used as a biocontrol agent in cooler, high-altitude regions of South Africa due to its native range being in high-altitude regions of Argentina and Peru

(Tipping *et al.*, 2008), our study shows its potential for both warm and temperate climates in Rwanda. The CTmin (4.7°C) and lethal temperature (−3.2°C) are well below the Rwanda's lowest temperature. However, CLIMEX results suggest that *M. scutellaris* could establish in all proposed Rwandan release sites except in the northern regions where temperature can drop to 8–12°C in April and *M. scutellaris* cannot grow in an environment with temperatures below 15°C (Grodowitz *et al.*, 2017). The study by Miller *et al.* (2021) showed that low winter temperatures negatively affected *M. scutellaris*'s density, and thus hindering its success to control water hyacinth in South Africa.

Conclusion

This study investigated the thermal tolerance and climatic suitability of four potential bio control agents for water hyacinth invasion in Rwandan water bodies such as *Neochetina eichhorniae*, *N. bruchi*, *C. aquaticum*, and *M. scutellaris*. Laboratory measurements of critical thermal limits (CTmax, CTmin) and lower lethal temperatures (LT50), together with CLIMEX modelling, revealed that all four biocontrol agents are compatible with Rwandan climatic conditions. Results of this study suggest that thermal constraints are implausible to have influenced the failure of *Neochetina* weevils in Rwanda, declaring instead other factors such as biocontrol agents's release strategies and other environmental factors. Although, *C. aquaticum* and *M. scutellaris*, have not yet introduced, they show strong potential for successful establishment, except *M. scutellaris* which showed to be affected by low temperatures from northern regions of the country. This study points out the importance of integrating physiological tolerance data with species distribution modelling to guide the selection of climatically compatible biocontrol agents. Future research should focus on: (i) investigating

the influence of synergistic and antagonistic interactions between temperature and other environmental factors such as water nutrients, turbidity, pH, dissolved oxygen, and water flow on biocontrol agent performance; (ii) testing other life stages such as eggs, larvae, and pupae or thermal tolerance to determine thermal sensitivities that may contribute to the establishment success of biocontrol agents; (iii) conducting robust post-release monitoring to optimize biocontrol outcomes; and (iv) incorporating field-based ecological assessments, long-term monitoring, and multifactorial models that integrate both abiotic and biotic factors to improve the predictive accuracy and effectiveness of water hyacinth biocontrol strategies in Rwanda.

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Competing interests. The authors declare that there are no conflicts of interest relevant to this research paper.

Informed consent. In this research, there were no animals' subjects or human necessitating ethics were used. Thus, issues of informed consent were not provided.

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