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Agents sans frontiers: cross-border aquatic weed biological control in the rivers of southern Mozambique

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Abstract

Biological control is an effective ways of controlling aquatic plants, especially in South Africa. Releases have been limited in Mozambique, where water hyacinth (*Eichhornia crassipes* (Mart.) Solms-Laubach (Pontederiaceae)), water lettuce (*Pistia stratiotes* L. (Araceae)), red water ferns (*Azolla* spp. (Azollaceae)) and salvinia (*Salvinia molesta* D.S. Mitch. (Salviniaceae)) are significant weeds in the south of the country. In 2009 we assessed the status of these weeds in seven rivers across southern Mozambique, and recorded whether any biocontrol agents were present. The weevils *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) and *N. bruchi* Hustache (Coleoptera: Curculionidae) were on water hyacinth, along with the pathogen *Acremonium zonatum*

(Sawada) W.Gams (Hypocreales) and the mite *Orthogalumna terebrantis* Wallwork (Acarina: Sarcoptiformes: Galumnidae). *Pistia stratiotes* supported small numbers of the weevil *Neohydronomus affinis* Hustache (Coleoptera: Curculionidae). The red water fern in the rivers was *A. cristata* Kaulfuss (Azollaceae) not the more widely recorded *A. filiculoides* Lam. (Azollaceae), and it supported small numbers of the weevil *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae). No agents were present on *S. molesta*. Most of these agents are likely to have dispersed from South Africa, and the rivers of southern Mozambique are likely to be benefitting from the trans-national dispersal of these agents.

Key words

Azolla, Trans-national dispersal, *Eichhornia,* Mozambique rivers, *Pistia, Salvinia*

Introduction

Invasive alien aquatic macrophytes pose a threat to natural and man-made water bodies across Africa, where they alter the ecology of aquatic ecosystems, limit their utilization, and can have major economic impacts (Cilliers et al. 2003). Biological control of these plants, using insects introduced from the native ranges of each weed, is often the only viable means of control, and has had some notable successes across the continent (Hill and Coetzee 2017; Zachariades et al. 2017). Biological control is particularly suitable in resource-poor areas of Africa, where other more expensive and short-term interventions are neither affordable, nor appropriate (De Groote et al. 2003).

Although multinational cooperative organisations offer the prospect of coordinated approaches to the challenges that face the continent, most actions in Africa are inevitably initiated at the level of individual countries. South Africa, along with Australia, Canada, New Zealand and the United States, is one of the countries that has been most active in the development and application of weed biological control (McFadyen 1998). Reflecting this, more agents have been released in South Africa, against a wider range of weed species, than anywhere else in Africa. Trade and travel links, and the insects' natural dispersal abilities, favour the spread of agents across natural and geopolitical boundaries, extending the areas where agents may appear, once they have been introduced (Pratt and Center 2012). The consequences can be beneficial if they help control their target host plants in the countries they have colonized (van Lenteren et al. 2006; Paynter and Bellgard 2011). Conversely, although the impacts of most weed control agents on non-target plants are typically small or non-existent, the possibility that novel non-target plants will be encountered is likely to increase when agents spread across international borders (Suckling and Sforza 2014). Mozambique is adjacent to the eastern provinces of South Africa, and southern Mozambique and the South African provinces of KwaZulu-Natal and Mpumalanga in particular share similar climates and watersheds. Releases in South Africa of agents that feed on shared invasive species therefore have the potential to aid control efforts in both countries.

Across Africa as a whole, water hyacinth (*Eichhornia crassipes* (Mart.) Solms-Laub. (Pontederiaceae)) is the most damaging aquatic weed. Water lettuce (*Pistia stratiotes* L. (Araceae)), giant salvinia (*Salvinia molesta* D.S. Mitch. (Salviniaceae)) and water ferns (*Azolla* spp.) are also problematic (Cilliers et al. 2003). These aquatic invasive species, all of which are from South America have been present in Mozambique since the 1940s (Davies et al. 1975; Bond and Roberts 1978; Jacot-Guillarmod 1979).

Despite considerable research invested in pre-release studies, the outcomes of biological control, especially in developing countries, are seldom quantified (Morin et al. 2009)). In Mozambique, the first recorded presence of biological control agents for aquatic weeds was in the 1970s, when the weevils *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) and *N. bruchi* Hustache (Coleoptera: Curculionidae) were recorded on *E. crassipes*, although their origin remains unknown (Winston et al. 2014). In addition, several other biological control agents may have dispersed to Mozambique from neighboring countries such as South Africa, Zimbabwe and Zambia (Cilliers et al. 2003; Coetzee et al. 2011). In this study we investigated the possible cross-border dispersal of biological control agents of several waterweeds in the rivers in the southern part of Mozambique in 2009.

Materials and methods

Description of study area

The study area comprised seven rivers located in the southern region of Mozambique, situated near main roads and close to residential areas (Figure 1). The agricultural lands in this area are sparse, with no runoff from rural settlements. Continuous reeds and grasses, as well as shrubs and trees occurred on the banks of the rivers. The Maputo and Limpopo Rivers were also situated close to roads, residential areas and farming activities. There was more agricultural activity in the Limpopo River area than along the Maputo River. The Umbeluzi and Incomati Rivers were impacted by abstraction, deposition of sediment and runoff from informal settlements, fruit cultivation (Umbeluzi River), sugarcane cultivation (Incomati River), and extensive residential areas. Further, the Incomati River is situated below Xinavane and Maragra sugarcane refineries and a flood that occurred during a high flow period washed fertilizer and animal manure from cultivated fields, together with aquatic plants, into the river from these refineries.

Sampling procedure of the aquatic weeds and their biological control agents

Invasive aquatic weeds and the presence of associated insects (biological control agents) were quantified twice, once in the dry season (June 2009) and once in the wet season (November 2009). The rivers sampled were the Maputo, Umbeluzi, Incomati, Limpopo, Inharrime, Govuro and Save Rivers, which were each divided into three sites; upper, middle and lower reaches (Figure 1). The extent of the weeds was determined by visually estimating their percentage cover at five localities along a 1000 meters stretch of river for each site. 100 plants of *S. molesta*, *P. stratiotes* and *Azolla* spp. were selected randomly at each site by wading through the mat and removing a plant every few seconds. For *S. molesta* the apical buds were inspected for adults of the biological control agent, *Cyrtobagous salvinae* Calder & Sands (Coleoptera: Curculionidae), or the characteristic holes in the buds left by adult feeding, further the rhizomes were dissected for the presence of larvae. For *P. stratiotes* the prominent veins on the abaxial surface of the leaves were inspected for adults of *Neohydronomus affinis* Hustache (Coleoptera: Curculionidae), and the leaves were searched for the shot holes produced by adult feeding, or larval mines. *Azolla* sp. plants were inspected for adults of *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae), or the obvious black pupal cases. Ten water hyacinth plants were sampled at each site using a standard sampling technique for this weed (see Jones et al. 2018) and inspected for any adults of *N. eichhorniae* and *N. bruchi* found in the base of the plants, characteristic square adult feeding scars on the leaves and larval mines in the petioles. Further, leaves were inspected for mines produced by the mite, *Orthogalumna terebrantis* Wallwork (Acarina: Sarcoptiformes: Galumnidae), or chlorosis caused by three sap-sucking bugs, *Eccritotarsus catarinensis* Carvahlo (Hemiptera: Miridae) and *E. eichhorniae* Henry (Hemiptera: Miridae), and *Megamelus scutellaris* Berg (Hemiptera: Delphacidae). The plants were also inspected for adults and nymphs of the water hyacinth grasshopper, *Cornops aquaticum* Brüner (Acrididae: Orthoptera), and their damage as they remove large sections of the leaves.

Whilst only one pathogen, *Cercospora piaropi* Tharp. (= *Cercospora rodmanii* Conway) has been released as a classical biological control agent for *E. crassipes* in southern Africa, several others, including *Acremonium zonatum* (Sawada) W. Gams, *Alternaria alternata* (Fr.) Keissler, and *Alternaria eichhorniae* Nag Raj and Ponnappa (Hypocreales) occur naturally in Africa and can contribute to some level of control (Kenfack-Voukeng et al. 2019), and any lesions were noted and identified. Only presence or absence data for the agents on water hyacinth is expressed.

Statistical analyses

Statistical analyses were carried out using R 3.6.0 and RStudio 1.2.1335-1 (R Core Team, 2019). The percentage of plants at a site with their respective biological control agents, or damage, was compared between the three reaches of each river, and the wet and dry seasons using a 3x2 Chi-squared contingency table.

Results

Pistia stratiotes was distributed along the Govuro, Incomati and Umbeluzi Rivers. The percentage cover at all three sites in the Govuro River was less than 5% in both the dry and wet seasons. The cover on the Incomati River was 25% in the dry season while in the wet season cover increased to 40% in the middle and lower sites. The Umbeluzi River had less than 50% cover at all three sites in the dry season, which increased to more than 60% in the wet season (Table 1). Adult and larval damage of *N. affins* was found on *P. stratiotes* leaves in the Incomati and Umbeluzi Rivers during the dry and wet seasons, but not in the Govuro River (Table 2). There were no significant differences in the percentage of plants with *N. affinis* in all sections of the Incomati River during the dry season ($\chi = 6.231$, df = 2, $P = 0.342$) and the wet season ($\chi = 8.268$, df = 2, $P = 0.082$), although there were many more plants with the weevil during the wet season (Table 2). The Umbeluzi River supported a higher percentage of weevil infested *P. stratiotes* plants than the Incomati River in the dry season, but there were no significant differences between the reaches of the river in the dry season ($\chi = 7.605$, df = 2, $P = 0.101$), but there were significantly fewer plants with the weevil in the lower reaches of the river in the wet season ($\chi = 26.332$, df = 2, $P < 0.001$) (Table 2).

Azolla spp. occur throughout Africa, but the most dominant species is the exotic *A. filiculoides* L. (Azollaceae) (Hill and Cilliers 1999; Madeira et al. 2016). Two indigenous species have been recorded in southern Africa, *Azolla pinnata* subsp. *africana* (Desv.) R.K.M. Saunders and K. Fowler (Azollaceae) and *A. nilotica* De Caisne Ex. Mett. (Azollaceae) (Hill et al. 2008; Crouch et al. 2011) but surprisingly during these surveys none of the three species were recorded. The only *Azolla* species recoded was the relatively new invader *A. cristata* Kaulfuss (Azollaceae) (Madeira et al. 2013), which was present in five of the seven rivers (Table 1). This weed was absent from the Govuro and Inharrine Rivers, and in low abundance in the Save and Maputo rivers, where there was about 5% cover in the dry and wet season. In the Limpopo River cover varied between 5% and 25%, while the Incomati River had 50% cover in both seasons, and the Umbeluzi had up to 95% cover in both seasons (Table 1). The biological control agent, *Stenopelmus rufinasus*, that was released for the control of *A. filiculoides* in South Africa, but will feed and develop on *A. cristata* (Madeira et al. 2016), was recorded in low numbers in the Umbeluzi, Incomati and Limpopo Rivers (Tables 1 and 3). *Stenopelmus rufinasus* adults or pupae were recorded on low numbers of plants through all three reaches and over both the wet and dry seasons and these were not significantly different in the Incomati (wet: $\chi = 5.747$, df = 2, $P = 0.218$; dry: $\chi = 2.036$, df = 2, $P = 0.729$), Limpopo (wet: $\chi = 6.852$, df = 2, $P = 0.132$; dry: $\chi = 6.036$, df = 2, $P = 0.771$) and the Umbeluzi Rivers (wet: $\chi = 9.338$, df = 2, P = 0.102; dry: $\chi = 8.762$, df = 2, P = 0.097), despite the percentage of plants being higher in the wet season in the Umbeluzi River (Table 3).

Salvinia molesta was confined to the Incomati and Umbeluzi Rivers with a low percentage cover of about 5% in both seasons (Table 1a, 1b). No damage was recorded on these plants and no *C. salviniae* were recorded on *S. molesta*.

Water hyacinth was present at all of the sites except the Save River. Small variations in percentage cover were recorded between the wet and dry seasons. This plant was less dominant in the Maputo, Govuro and Inharrime Rivers where less than 10% of the water surface area was covered. In the Limpopo River up to 30% cover was recorded at some sites, while in the Umbeluzi and Incomati Rivers up to 75% and 95% cover respectively were recorded (Table 1a,b). Only three (*N. eichhorniae*, *N. bruchi* and *O. terebrantis*) of the eight arthropod and one pathogen species that had been intentionally introduced to South Africa (Hill and Coetzee 2017) were recorded on *E. crassipes* in the rivers in southern Mozambique. Adult *N. eichhorniae* and *N. bruchi* were found in the Umbeluzi and Incomati Rivers, but not in the Govuro, Inharrime, Maputo and Limpopo Rivers (Table 4). In the Maputo and Limpopo Rivers, no weevils were found but there was some evidence of them through the presence of characteristic scars on the leaves. The indigenous pathogen *Acremonium zonatum* (Sawada) W.Gams (Hypocreales) was only found on water hyacinth plants in the Incomati River, while the mite *O. terebrantis* and the cosmopolitan fungus *Alternaria eichhorniae* Nag Raj & Ponnappa (Ascomycota: Pleosporaceae) were found on the leaves of *E. crassipes* in the Incomati, Umbeluzi and Limpopo Rivers (Table 4).

Discussion

The most invasive aquatic macrophytes in southern Mozambique were *E. crassipes* and *A. cristata* because they had a high percentage cover at most sites, and in most rivers. *Eichhornia crassipes* was found in all rivers except the Save River and *A. cristata* was found abundantly in all the rivers except the Govuro and Inharrime Rivers. *Pistia stratiotes* and *S. molesta* were less abundant. *Pistia stratiotes* was recorded in only three rivers, Umbeluzi, Incomati and Govuro and *S. molesta* was found only in the Umbeluzi and Incomati Rivers. The heavily infested sections of the rivers were all situated downstream of an international river and/ or suffered from eutrophic water quality and this could explain the more severe invasion in the Umbeluzi and Incomati Rivers, followed by Limpopo and Maputo Rivers, and why fewer aquatic invasive plants were recorded in the less impacted Inharrime, Govuro and Save Rivers. The Govuro and Inharrime Rivers have their entire catchments within Mozambique and are thus not subjected to the dispersal of waterweeds through shared watersheds with South Africa, where these weeds are abundant (Hill and Coetzee 2017).

In addition to the importation of waterweeds, Mozambique has also been the recipient of a number of biological control agents that have been released in Zimbabwe and South Africa. The water hyacinth biological control agent populations varied between sites, and although this study was not designed to determine the relative efficacy of the arthropods that feed on these aquatic weeds, observations of the amount of feeding damage and the number of individuals suggest that, in particular, the *E. crassipes* weevils, would be damaging. The number of *N. eichhorniae* and *N. bruchi* feeding scars on the most recently opened *E. crassipes* leaf was higher on the Umbeluzi compared to the Incomati River. *Neochetina eichhorniae* is listed as having been intentionally released onto *E. crassipes* on Cahora Bassa dam in 1972, although its origin is recorded as being unknown (Winston et al. 2014). This would mark a very early release for this insect, which was only released in the surrounding counties in the mid 1980s (Hill 2003), and the first release outside of South America was in 1972 into the USA (Winston et al. 2014). *Neochetina bruchi* and the mite, *O. terebrantis* and the agents on the other waterweeds were not intentionally released into Mozambique, but are likely to have dispersed with the weeds down the Letaba, Komati and Zambezi Rivers from South Africa and Zimbabwe/Zambia where they have all been released (Cilliers et al. 2003).

Azolla cristata was distributed in five rivers but was not found in the Govuro and Inharrime Rivers, this is possibly due to the fact that these have their entire catchments within Mozambique. The biological control agent *S. rufinasus* that was released against *A. filiculoides* in South Africa in 1997 (Hill 1998), was present on *A. cristata* in the Incomati, Limpopo and Umbeluzi Rivers. This agent has been shown to be an excellent disperser, having been recorded over 300km from initial release sites in South Africa (McConnachie et al. 2004). The fairly low densities of this agent in Mozambique is probably because *A. cristata* is not the natural host of *S. rufinasus* (Madeira et al. 2016), but *S. rufinasus* will still reduce populations of the weed (Hill and Coetzee 2017).

In the study area *P. stratiotes* was confined to the Incomati, Umbeluzi and Govuro Rivers. Surveys in these areas showed that the weevil, *N. affinis,* was found in two rivers only, but at densities too low to effectively control the weed. Once again, the weevil was not intentionally introduced to Mozambique, but it is found in many rivers that are located upstream of Mozambique's borders. Successful biological control of water lettuce has been recorded in Zimbabwe (Chikwenhere and Keswani 1997), and South Africa (Coetzee et al. 2011). This weevil is not considered a good disperser and thus is most likely to have been introduced on infested plants.

The infestation of *S. molesta* was not abundant in the studied rivers and was only present in the Incomati and Umbeluzi Rivers. Surprisingly the highly successful biological control agent, *Cyrtobagous salviniae* was not found. This could be because *S. molesta* is very successfully controlled in South Africa (Hill and Coetzee 2017) and Zimbabwe (Cilliers et al. 2003) and thus there is little propagule pressure downstream that could facilitate the movement of the agent. Furthermore, this agent is regarded as a very poor disperser (Martin et al. 2018).

Given the success of the biological control agents on aquatic weeds throughout Africa (Cilliers et al. 2003), there is no doubt that Mozambique is benefitting from cross-border dispersal of these agents. The range expansion described above offers Mozambique the opportunity to mass rear and release these agents into areas where they have not yet been recorded, or are in low numbers. Furthermore there are additional agents, in particular for *E. crassipes* which have been tested and released in southern Africa that would also be appropriate for release in Mozambique (Hill and Coetzee 2017).

Conclusion

This study has shown that insects that have been released for the biological control of aquatic weeds in South Africa and Zimbabwe have been able to disperse into Mozambique. Most of these species, with the exception of *S. rufinasus*, are considered to be poor dispersers and thus it is likely that these agents arrived in Mozambique as hitchhikers on their host plants during periods of high flow. Cross border dispersal of biocontrol agents on aquatic weeds in Africa has also been reported from Cameroon, where *N. eichhorniae* and *N. bruchi* have established on *E. crassipes* despite not having been released in that country (most likely having dispersed from neighbouring Nigeria (Kenfack-Voukeng, 2017), and *S. rufinasus* dispersed from *A. filiculoides* sites in northern South Africa into Zimbabwe where it effectively controlled the weed (McConnachie et al. 2004).

Winston et al. (2014) produced a world catalogue of biological control agents and their target weeds and list 131 biological control agent species that have established in an adventive range, but that had not been deliberately released there. Most of the species listed have low- to medium impacts on the target weeds while some 23 species were regarded as having high impacts (Winston et al 2014). These high impact species include agents on waterweeds that have dispersed throughout Africa, usually through shared watersheds, and have had significant beneficial impacts. Pratt and Center (2012) asked whether the geographical footprint of a biological control agent should be considered prior to release as they do not respect geopolitical boundaries. This question is especially relevant to continents such as Africa where there are several organizations based in western, eastern and southern Africa that have very active weed biological control programmes. The African Union established the Inter-African Phytosanitary Council (IAPSC) in 1954 to develop regional strategies against the introduction and spread of plant pests; its mandate does not address invasive alien weeds and their possible biological control. There is a need to develop best practices to ensure the safe use of biological control in sub-Saharan Africa (Suckling and Sforza 2014).

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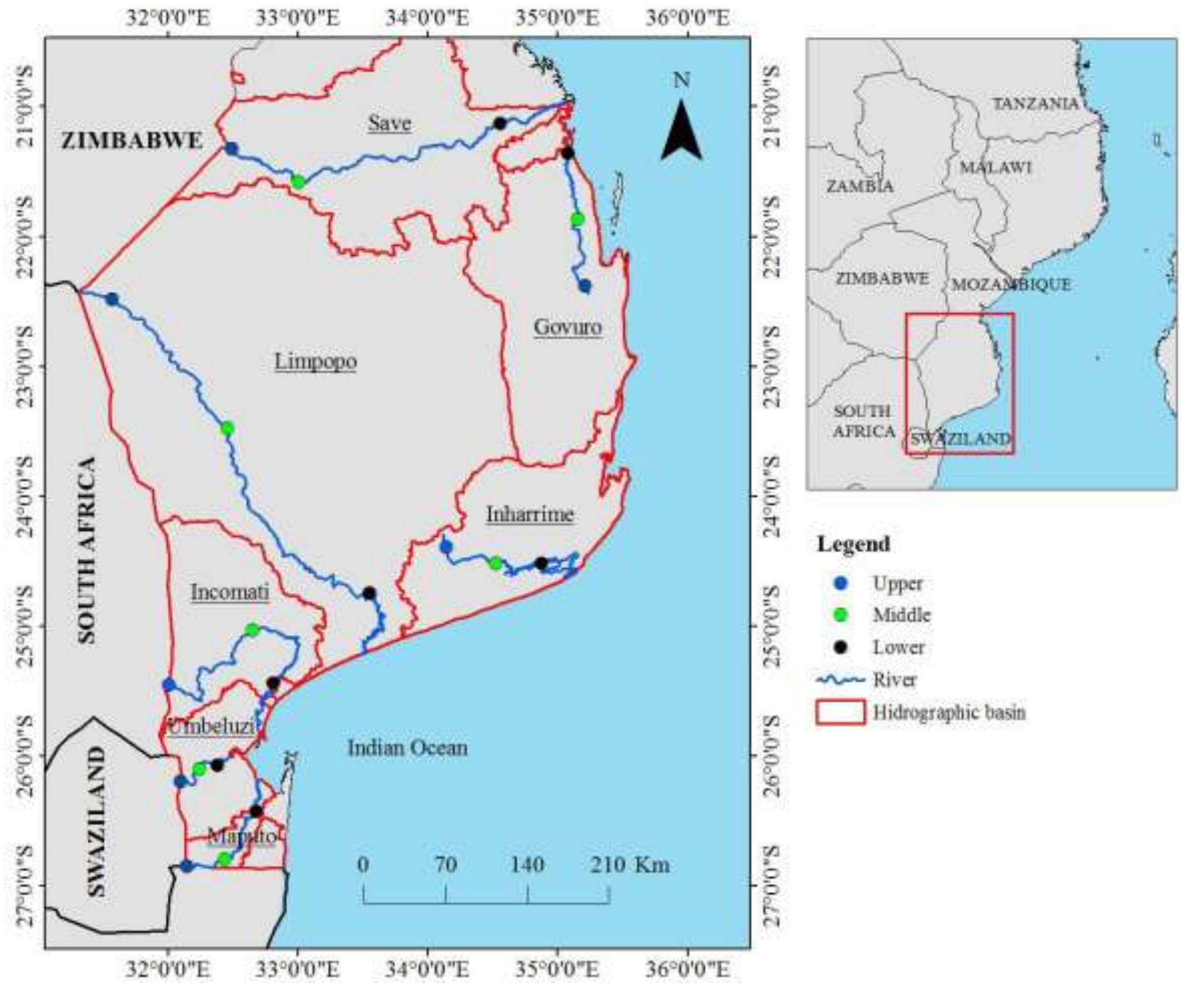

Figure 1. Sampling localities for aquatic weeds in the southern Mozambique rivers

Table 1 The percentage cover of invasive aquatic weeds at sites in southern Mozambique in the dry season (a.) and the wet season (b).

a.

	Percentage water cover											
	Eichhornia crassipes			*Azolla cristata*			*Pistia stratiotes*			*Salvinia molesta*		
	U	M	L	U	M	L	U	M	L	U	M	L
Save	0	0	0	0	5	5	0	0	0	0	0	0
Govuro	0	0	0	0	0	0	5	5	5	0	0	0
Inharrime	0	0	5%	0	0	0	0	0	0	0	0	0
Limpopo	10	20	20	10	15	20	0	0	0	0	0	0
Incomati	65	65	70	50	50	50	25	25	25	0	5	0
Umbeluzi	50	65	60	75	95	95	30	30	45	5	5	0
Maputo	5	5	5	0	0	5	0	0	0	0	0	0

b.

	Percentage water cover											
	Eichhornia crassipes			*Azolla cristata*			*Pistia stratiotes*			*Salvinia molesta*		
	U	M	L	U	M	L	U	M	L	U	M	L
Save	0	0	0	0	5	0	0	0	0	0	0	0
Govuro	0	0	5	0	0	0	5	5	5	0	0	0
Inharrime	0	0	5	0	0	0	0	0	0	0	0	0
Limpopo	10	30	30	5	20	25	0	0	0	0	0	0
Incomati	75	75	95	50	50	50	25	40	40	0	5	0
Umbeluzi	10	70	75	95	95	95	65	50	65	5	5	0
Maputo	0	5	5	0	0	5	0	0	0	0	0	0

Table 2. The percentage of *Pistia stratiotes* plants with *Neohydronomus affinis* adults, larvae or damage at three sites in three rivers in southern Mozambique in the wet and dry seasons. Percentage values followed by * indicate a significant difference.

River	Dry season			Wet season		
	Upper	Middle	Lower	Upper	Middle	Lower
Incomati	5	26	29	90	76	77
Guvuro	0	0	0	0	0	0
Umbeluzi	59	40	30	56	59	21*

Table 3. The percentage of *Azolla cristata* plants with *Stenopelmus rufinasus* adults or pupae at three sites in three rivers in southern Mozambique in the wet and dry seasons.

	Dry season			Wet season		
River	Upper	Middle	Lower	Upper	Middle	Lower
Incomati	6	9	13	5	5	3
Limpopo	0	0	6	6	2	5
Umbeluzi	6	9	18	59	40	36

Table 4. The presence of *Eichhornia crassipes* biological control agents in southern Mozambique during dry and wet season. Absent (-), present (+).

	Control agents presence/absence									
	Neochetina eichhorniae		*Neohetina bruchi*		*Acremonium zonatum*		*Alternaria eichhorniae*		*Orthogalumna terebrantis*	
	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet
Govuro	-	-	-	-	-	-	-	-	-	-
Inharrime	-	-	-	-	-	-	-	-	-	-
Limpopo	-	-	-	-	-		-	+	+	+
Incomati	+	+	+	+	+	+	+	+	+	+
Umbeluzi	+	+	+	+	-	-	+	+	+	+
Maputo	-	-	-	-	-	-	-	-	-	-