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The Red Alien vs. the Blue Destructor: The Eradication of *Cherax destructor* by *Procambarus clarkii* in Latium (Central Italy)

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Abstract: *Cherax destructor* is a crayfish endemic to south-eastern Australia and one of the last alien crayfish to be introduced in Italy. In the Laghi di Ninfa Natural Reserve (Latium region, Central Italy), the species was probably introduced in 1999, but only reported for the first time in 2008. Nearby this area, the most widespread alien crayfish is the red swamp crayfish *Procambarus clarkii*. In the Natural Reserve, between 2008 and 2013 and during spring and summer, crayfish sampling was carried out with baited traps to assess the distribution of *C. destructor* and its possible relationship with *P. clarkii*. *Cherax destructor* was first recorded in 2008; few *P. clarkii* were detected in the cultivation ponds where *C. destructor* was present in 2012 and 2013. Moreover, crayfish plague analyses evidenced a positive result in two out of the 12 sampled *P. clarkii*. *Cherax destructor* is now completely absent from the Natural Reserve, while *P. clarkii* has spread in the area and was probably responsible for this eradication since *C. destructor* is vulnerable to crayfish plague which was also detected in the area. An ecosystem restoration project in the area favoured the spread of. *P. clarkii*; the implications of this intervention are discussed.

Keywords: *Aphanomyces astaci*; crayfish plague; invasive crayfish; red swamp crayfish; yabby

1. Introduction

Freshwater ecosystems are widely recognized as more vulnerable to invasive alien species (IAS) than terrestrial environments due to the highly intrinsic dispersal ability of freshwater species, and the prevalent impacts of human disturbance and climate change on inland waters [1–5]. Despite the frequent and often simultaneous invasion of freshwater ecosystems by several IAS [3], the relationship among those IAS has scarcely been investigated [2]. Crayfish are one of the most introduced taxa in fresh waters with dramatic consequences on the invaded ecosystems [6]; currently, there are 11 established alien crayfish species in Europe and some coexist in the same water body [7].

Italy is one of the European countries heavily affected by the invasion of alien crayfish (reviewed in Aquiloni et al. [8]), which threaten freshwater biodiversity in general and indigenous crayfish in particular. One of the most recent alien crayfish introduced in Italy is the Australian parastacid yabby *Cherax destructor* Clark, 1936 [9]. *Cherax destructor* is a south-eastern Australian crayfish that has successfully colonized many different areas across Australia [10], and it has also been established in Europe, particularly in Spain, in the provinces of Zaragoza, Aragón and Navarra [11,12]. In Italy, *C. destructor* was probably introduced in the Laghi di Ninfa Natural Reserve (Latium region, Central Italy), at the end of the 1980s to foster an experimental aquaculture, and it was reported for the first time in 2008 by Scalici et al. [9]. According to Scalici et al. [9], a large population inhabited six abandoned crayfish cultivation ponds in the Reserve, although the species seemed to be still confined in the ponds without expanding into the several surrounding fresh waters of the area. Until now, the species has only been found in Latium in Italy, but recently a few individuals were sampled in the Costanzo stream in the province of Siracusa, Sicily [13]. *Cherax destructor* is an r-selected species, and is considered a high-risk species by Tricarico et al. [14] due to its high resistance to environmental extremes and its severe impacts on the invaded habitat. Moreover, while Scalici et al. [9] suggested that the low temperature of the surrounding waters of Laghi di Ninfa (12 °C) acts as a barrier against the natural spreading of crayfish, Veselý et al. [15] discovered the ability of *C. destructor* to withstand low winter temperatures by burrowing into the levees.

In the area close to Laghi di Ninfa, the most widespread alien crayfish is the red swamp crayfish *Procambarus clarkii* (Girard, 1852) [16], but up until 2012 the two species were never found in syntopy. In 2012, a few *P. clarkii* specimens and just one *C. destructor* individual were found in the same cultivation pond. Due to a long co-evolutionary history with the crayfish plague agent *Aphanomyces astaci* Schikora, 1906, North American crayfish species such as *P. clarkii* have evolved defence mechanisms against its growth [17]. In contrast, crayfish of European and Australian origin such as *C. destructor* lack efficient immune responses to crayfish plague and are thus considered highly susceptible [18], although Mrugała et al. [19] found that some individuals of *C. destructor* can survive after exposure to the least virulent *A. astaci* strain used in the experiment.

The aim of this paper was twofold: (1) to assess the distribution and abundance of *C. destructor* in the Natural Reserve of Laghi di Ninfa, which is characterized by cold waters, and (2) to evaluate the possible relationship and the consequent coexistence of *C. destructor* with *P. clarkii*.

2. Materials and Methods

The Laghi di Ninfa Natural Reserve (106 ha) is located in Latina Province (Central Italy), and within this reserve there is a Special Area of Conservation (SAC IT6040002 "Ninfa"). Also an ecosystem restoration project has transformed a farm in the wetland area of Pantanello (12 ha), an important site for aquatic birds composed of interconnected ponds, marshes, wet grasslands, and streams (Figure 1).

Figure 1. The study area. Orange dots indicate the localities within the Laghi di Ninfa Natural Reserve where *Procambarus clarkii* was found between 2008 and 2013, the blue dot indicates the only population of *Cherax destructor* present in the area up until 2012. Numbers indicate the different ponds of the wetland area of Pantanello. The circle indicates the area of cultivation ponds.

Pantanello is also connected on the south side to the Pontina plain, where *P. clarkii* is present with an average density of 0.60 ind/m^2 [20]. No individuals of the indigenous *Austropotamobius pallipes* complex have been found in the area since 1986, and it is probably extinct due to pollution and overexploitation.

In the Reserve, close to the entrance, there are six abandoned cultivation ponds where *C. destructor* was found [9] (50 × 8 m, with a muddy bottom and a water depth of 60 to 30 cm). The pond water is provided by a stream (that is connected to the Pantanello area).

Crayfish samplings were carried out between 2008 and 2013 with baited traps during the spring and summer for a total of 16,392 h of sampling. In the cultivation ponds, 10–30 traps were used (minimum six per pond, according to water presence and level). In the outside cultivation ponds, 18–40 traps were used. Population density was estimated using the Catch per Unit Effort (CPUE, the total number of trapped crayfish/(total used traps*hours of sampling)). The collected crayfish were sexed and measured (carapace length). Nine and three individuals of *P. clarkii* found in 2012 and 2013, respectively, and one individual of *C. destructor* found in 2012 were preserved under ethanol 90° and sent to the laboratory of Instituto Zooprofilattico Sperimentale delle Venezie for the detection of *A. astaci*. The analyses were conducted by end-point PCR following Oidtmann et al. [21], with sequencing of the amplified products. Animals were sampled and processed following the standard procedure recommended by the Italian Health Authority and the Research Organization for Animal Health and Food Safety.

3. Results

An individual of *P. clarkii* was found occasionally near the Reserve during 2006, while an abundant population of this species was detected in 2008, outside the Reserve, in the Pontina plain (CPUE = 11). At the same time, in 2008, an abundant population of *C. destructor* was found in the cultivation ponds in Laghi di Ninfa (CPUE = 10.3; Figures 1 and 2). The subsequent samplings highlighted the complete reduction of this species in the study area (to the best of our knowledge no *C. destructor* specimens have been found in the neighboring ditches and areas from 2013 until now) and the exponential increase of the *P. clarkii* population in Laghi di Ninfa (r^2 = 0.84; Figures 1 and 2).

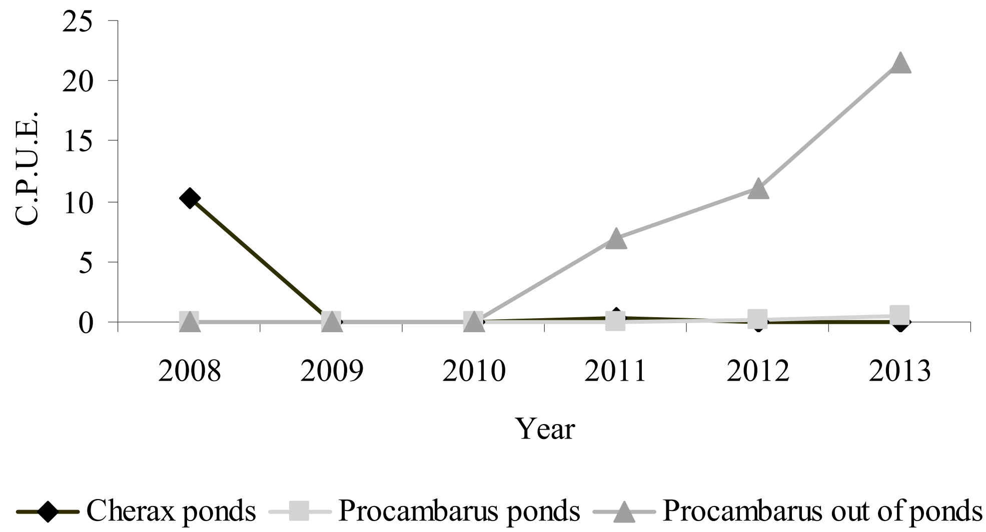

Figure 2. Trend of Catch per Unit Effort (CPUE) of *Cherax destructor* and *Procambarus clarkii* from 2008 to 2013.

A few individuals of *P. clarkii* were also detected in the cultivation ponds where *C. destructor* was present in 2012 (n = 1) and in 2013 (n = 3). In 2012, the only specimen of *P. clarkii* found in the ponds was infested by the alien temnocephalan crayfish ectoparasite *Temnosewellia minor* Haswell, 1888 (Figure 3), an ectoparasite always carried by *C. destructor* and described by morphological and molecular investigations in Chiesa et al. [22]. The crayfish plague analyses evidenced a positive result in two out of the 12 sampled *P. clarkii*, the first found in the output canal of the cultivation ponds, the second found in Stagni della Flora (Pantanello area).

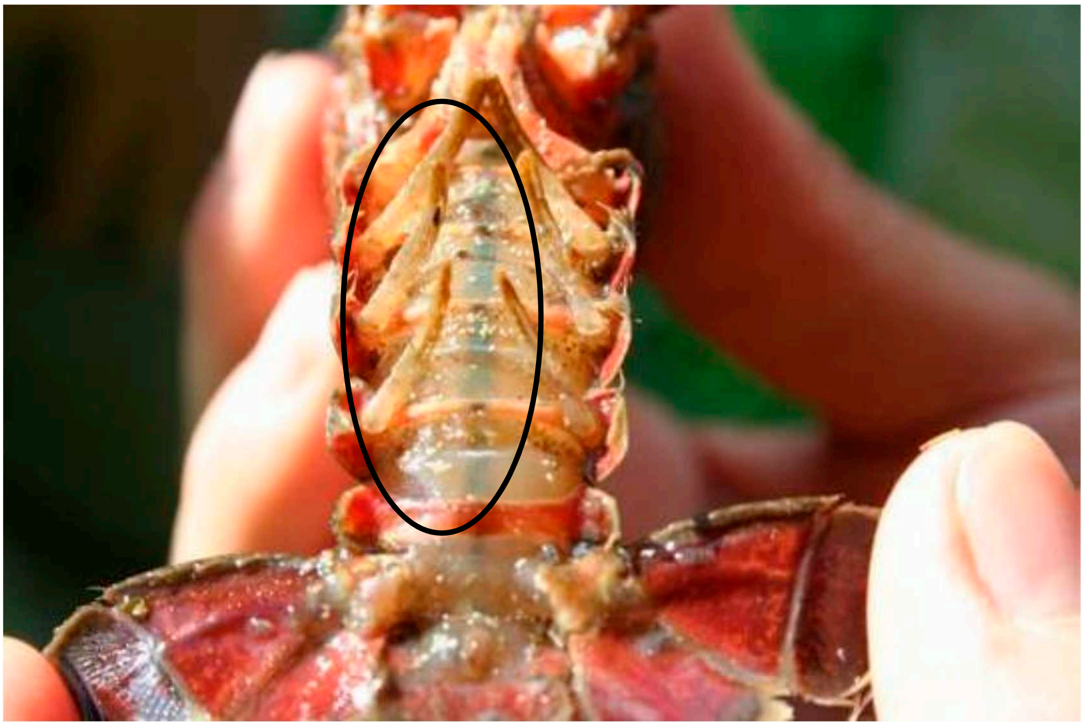

Figure 3. Individual alien temnocephalan crayfish ectoparasite *Temnosewellia minor* on a specimen of *Procambarus clarkii*.

4. Discussion and Conclusions

The results of our study show that *C. destructor* is now completely absent from the Laghi di Ninfa Natural Reserve, while *P. clarkii* is widespread in the area and was probably responsible for this eradication. In 2011, only 11 specimens of *C. destructor* were found in the cultivation ponds while *P. clarkii* was absent from the same ponds. In 2012, remains of dead *C. destructor* individuals (and only one alive) were found in the cultivation ponds together with a few *P. clarkii*, while in 2013 no specimens of *C. destructor* were found. Thus, the decline occurred before they came in syntopy in 2012, supporting the hypothesis of the crayfish plague. Indeed, *Cherax destructor* is vulnerable to the crayfish plague that is present in the area, as evinced by the positive outcomes on sampled *P. clarkii*. This case clearly demonstrated how an IAS can lead to the eradication of another IAS, not due to direct competition or predation but due to transmission of pathogens. In the Laghi di Ninfa area, all the water bodies are interconnected, and this allowed not only the spread of *P. clarkii* that reached the cultivation ponds where *C. destructor* was present but also the spread of the crayfish plague. Similarly, an episode of mortality was reported in farmed red claw crayfish, *Cherax quadricarinatus* (von Martens, 1868), reared in Sicily (Italy), due to *A. astaci* probably being carried by *P. clarkii* reared in the same facility [23]. As the native river crab *Potamon fluviatile* Herbst, 1785, protected under the regional law 18/88, is present in the area (and was even found in the same trap with *P. clarkii*), the presence of alien crayfish carrying plague elicits great concern for the conservation of this species. Other crustaceans known to be susceptible to infection by *A. astaci* are the Chinese mitten crab (*Eriocheir sinensis*) (Benisch, 1940) [24], the crab *Potamon potamios* (Olivier, 1804) [25], and the Asian shrimp *Macrobrachium dayanum* (Henderson, 1893) and *Neocaridina davidi* (Bouvier, 1904) [26], thus, we cannot discard the hypothesis that *P. fluviatile* could be affected.

The connectivity of the Natural Reserve has been increased by the ecosystem restoration project in Pantanello, which was planned to create an area of conservation interest for several species without considering its potential for favouring the dispersal of *P. clarkii*. However, the risk of invasion should be addressed at the earliest possible stage of the planning of protected areas or any other intervention, starting from the earliest design or management plan for any new protected area [27]. The landscape configuration of the geographic context in which a protected area is established, and the natural corridors connecting the protected area with surrounding areas, affect not only the interconnectivity which is vital for sustaining biodiversity but also the permeability of the protected area, and are crucial in determining the future patterns of invasions [28]. A recent review on biological invasions in conservation planning evidenced that alien species were considered to be of concern for conservation in only 46% of the analysed cases (70) while mitigation measures were proposed in only 13% of the cases. Most of the studies (73%) ignored alien species in conservation planning even if their negative impacts were recognized [29]. Thus, there is an urgent need to tackle this issue as protected areas are planned to maintain and increase the local biodiversity. Similarly, ecosystem restoration projects, often undertaken by or within protected areas, should consider the risk of causing or facilitating further IAS invasions, especially in aquatic habitats, and should adopt risk assessment protocols and a precautionary approach when data relating to biosecurity are lacking. However, limiting aquatic connectivity for IAS can disproportionately reduce potential connectivity restoration for desirable species [30]. It is necessary to carry out a cost-benefit analysis (including the potential cost of IAS management) to assess the feasibility and effectiveness of a restoration intervention. Up to now, the spread of *P. clarkii*, favoured by the ecosystem restoration in the area of Pantanello has led to contrasting results. In addition to the eradication of *C. destructor*, an increase in the number of birds, especially aquatic birds, has been reported (from 709 in 2007 to 1159 in 2013, A. Monaco, pers. comm.) Aquatic birds mainly include the small grebe *Tachybaptus ruficollis* Pallas, 1764, the cormorant *Phalacrocorax carbo* (Linnaeus, 1758), the cattle egret *Bubulcus ibis* Linnaeus, 1758, the grey heron *Ardea cinerea* Linnaeus, 1758, the teal *Anas crecca* Linnaeus, 1758, the mallard *Anas platyrhynchos* Linnaeus, 1758, the common moorhen *Gallinula chloropus* (Linnaeus, 1758) and the coot *Fulica atra* Linnaeus, 1758), and this could be due to the abundant population of *P. clarkii*, as already observed in other areas highly invaded by this species (e.g., Massaciuccoli Lake, Tuscany). Conversely, *P. clarkii* may cause negative impacts on the local biodiversity, especially within the SAC area, where the fish *Sarmarutilus rubilio* (Bonaparte, 1837), endemic to Central Italy and classified as Near Threatened, is present. Further monitoring activities are thus necessary to assess the long-term effects of the restoration activities, particularly focussing on the spread of *P. clarkii* in the Laghi di Ninfa Natural Reserve.

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References

1. Gherardi, F.; Gollasch, S.; Minchin, D.; Olenin, S.; Panov, V.E. Alien invertebrates and fish in European inland waters. In *Handbook of Alien Species in Europe*; Springer: Dordrecht, The Netherlands, 2009; pp. 81–92.
2. Strayer, D.L.; Dudgeon, D. Freshwater biodiversity conservation: Recent progress and future challenges. *J. N. Am. Benthol.* **2010**, *29*, 344–358. [CrossRef]

3. Havel, J.E.; Kovalenko, K.E.; Thomaz, S.M.; Amalfitano, S.; Kats, L.B. Aquatic invasive species: Challenges for the future. *Hydrobiologia* **2015**, *750*, 147–170. [CrossRef]
4. Tricarico, E.; Junqueira, A.O.; Dudgeon, D. Alien species in aquatic environments: A selective comparison of coastal and inland waters in tropical and temperate latitudes. *Aquat. Conserv.* **2016**, *26*, 872–891. [CrossRef]
5. Mazza, G.; Tricarico, E. *Invasive Species and Human Health*; CABI Invasives Series 10; CPI Group: Preston, UK, 2018; 186p, ISBN 13 978-1-78639-098-1.
6. Lodge, D.M.; Deines, A.; Gherardi, F.; Yeo, D.C.; Arcella, T.; Baldridge, A.K.; Barnes, M.A.; Lindsay Chadderton, W.; Feder, J.L.; Gantz, C.A.; et al. Global introductions of crayfishes: Evaluating the impact of species invasions on ecosystem services. *Annu. Rev. Ecol. Evol. Syst.* **2012**, *43*, 449–472. [CrossRef]
7. Kouba, A.; Petrusek, A.; Kozák, P. Continental-wide distribution of crayfish species in Europe: Update and maps. *Knowl. Manag. Aquat. Ecosyst.* **2014**, *413*, 5. [CrossRef]
8. Aquiloni, L.; Tricarico, E.; Gherardi, F. Crayfish in Italy: Distribution, threats and management. *Int. Aquat. Res.* **2010**, *2*, 1–14.
9. Scalici, M.; Chiesa, S.; Gherardi, F.; Ruffini, M.; Gibertini, G.; Marzano, F.N. The new threat to Italian inland waters from the alien crayfish "gang": The Australian *Cherax destructor* Clark, 1936. *Hydrobiologia* **2009**, *632*, 341–345. [CrossRef]
10. Coughran, J.; Daly, G. Potential threats posed by a translocated crayfish: The case of *Cherax destructor* in coastal drainages of New South Wales, Australia. *Crustac. Res.* **2012**, *7*, 5–13. [CrossRef]
11. Souty-Grosset, C.; Holdich, D.M.; Noel, P.Y.; Reynolds, J.D.; Haffner, P. *Atlas of Crayfish in Europe*; Patrimoines Naturels 64; Muséum National d' Histoire Naturelle: Paris, France, 2006.
12. Holdich, D.M.; Reynolds, J.D.; Souty-Grosset, C.; Sibley, P.J. A review of the ever increasing threat to European crayfish from nonindigenous crayfish species. *Knowl. Manag. Aquat. Ecosyst.* **2009**, *11*, 394–395.
13. Deidun, A.; Sciberras, A.; Formosa, J.; Zava, B.; Insacco, G.; Corsini-Foka, M.; Crandall, K.A. Invasion by non-indigenous freshwater decapods of Malta and Sicily, central Mediterranean Sea. *J. Crustac. Biol.* **2018**. [CrossRef]
14. Tricarico, E.; Vilizzi, L.; Gherardi, F.; Copp, G.H. Calibration of FI-ISK, an invasiveness screening tool for non-indigenous freshwater invertebrates. *Risk Anal.* **2010**, *30*, 285–292. [CrossRef] [PubMed]
15. Veselý, L.; Buřič, M.; Kouba, A. Hardy exotics species in temperate zone: Can "warm water" crayfish invaders establish regardless of low temperatures? *Sci. Rep.* **2015**, *5*, 16340. [CrossRef] [PubMed]
16. Scalici, M.; Pitzalis, M.; Gibertini, G. Crayfish distribution updating in central Italy. *Knowl. Manag. Aquat. Ecosyst.* **2009**, *6*, 394–395. [CrossRef]
17. Cerenius, L.; Bangyeekhun, E.; Keyser, P.; Söderhäll, I.; Söderhäll, K. Host prophenoloxidase expression in freshwater crayfish is linked to increased resistance to the crayfish plague fungus, *Aphanomyces astaci*. *Cell. Microbiol.* **2003**, *5*, 353–357. [CrossRef] [PubMed]
18. Quaglio, F.; Pretto, T.; Dundon, W.; Zambon, M.; Giustinelli, A.; Manfrin, A. First occurrence of *Aphanomyces astaci* epidemic infection in cultured yabby *Cherax destructor* (Clark, 1936) in Northern Italy. In Proceedings of the 19 Symposium International Association of Astacology, Innsbruck, Austria, 26–31 August 2012.
19. Mrugała, A.; Veselý, L.; Petrusek, A.; Viljamaa-Dirks, S.; Kouba, A. May *Cherax destructor* contribute to *Aphanomyces astaci* spread in Central Europe? *Aquat. Invasions* **2016**, *11*, 53–64. [CrossRef]
20. Gherardi, F.; Aquiloni, L.; Bertocchi, S.; Brusconi, S.; Inghilesi, A.F.; Mazza, G.; Scalici, M.; Tricarico, E. Un contributo multidisciplinare alla conoscenza dei gamberi alloctoni del Lazio. In *Alieni: La Minaccia Delle Specie Alloctone per la Biodiversità del Lazio*; Monaco, A., Ed.; Palombi Editori: Roma, Italy, 2014.
21. Oidtmann, B.; Geiger, S.; Steinbauer, P.; Culas, A.; Hoffmann, R.W. Detection of *Aphanomyces astaci* in North American crayfish by polymerase chain reaction. *Dis. Aquat. Organ.* **2006**, *72*, 53–64. [CrossRef] [PubMed]
22. Chiesa, S.; Scalici, M.; Lucentini, L.; Marzano, F.N. Molecular identification of an alien temnocephalan crayfish parasite in Italian freshwaters. *Aquat. Invasions* **2015**, *10*, 209–216. [CrossRef]
23. Marino, F.; Pretto, T.; Tosi, F.; Monaco, S.; De Stefano, C.; Manfrin, A.; Quaglio, F. Mass mortality of *Cherax quadricarinatus* (von Martens, 1868) reared in Sicily (Italy): Crayfish plague introduced in an intensive farming. *Freshw. Crayfish* **2014**, *20*, 93–96. [CrossRef]
24. Schrimpf, A.; Schimdt, T.; Schulz, R. Invasive Chinese mitten crab (*Eriocheir sinensis*) transmits crayfish plague pathogen (*Aphanomyces astaci*). *Aquat. Invasions* **2014**, *9*, 203–209. [CrossRef]

25. Svoboda, J.; Strand, D.A.; Vrålstad, T.; Grandjean, F.; Edsman, L.; Kozák, P.; Kouba, A.; Fristad, R.F.; Koca, S.B.; Petrusek, A. The crayfish plague pathogen can infect freshwater-inhabiting crabs. *Freshw. Biol.* **2014**, *59*, 918–929. [CrossRef]
26. Svoboda, J.; Mrugała, A.; Kozubíková-Balcarová, E.; Kouba, A.; Diéguez-Uribeondo, J.; Petrusek, A. Resistance to the crayfish plague pathogen, *Aphanomyces astaci*, in two freshwater shrimps. *J. Invertebr. Pathol.* **2014**, *121*, 97–104. [CrossRef] [PubMed]
27. Meyerson, L.; Pyšek, P. Manipulating alien plant species propagule pressure as a prevention strategy for protected areas. In *Plant Invasions in Protected Areas: Patterns, Problems and Challenges*; Foxcroft, L.C., Pyšek, P., Richardson, D.M., Genovesi, P., Eds.; Springer: Dordrecht, The Netherlands, 2013; pp. 473–486.
28. Foxcroft, L.C.; Jarošík, V.; Pyšek, P.; Richardson, D.M.; Rouget, M. Protected-area boundaries as filters of plant invasions. *Conserv. Biol.* **2011**, *25*, 400–405. [CrossRef] [PubMed]
29. Mačić, V.; Albano, P.G.; Almpanidou, V.; Claudet, J.; Corrales, X.; Essl, F.; Evagelopoulos, A.; Giovos, I.; Jimenez, C.; Kark, S.; et al. Biological invasions in conservation planning: A global systematic review. *Front. Mar. Sci.* **2018**, *5*, 178. [CrossRef]
30. Milt, A.W.; Diebel, M.W.; Doran, P.J.; Ferris, M.C.; Herbert, M.; Khoury, M.L.; Moody, A.T.; Neeson, T.M.; Ross, J.; Treska, T.; et al. Minimizing opportunity costs to aquatic connectivity restoration while controlling an invasive species. *Conserv. Biol.* **2018**, *32*, 894–904. [CrossRef] [PubMed]