

# Chapter 11

## Biotechnology, Biocontrol and Conservation: Potential Approaches— Harnessing RNAi-Based Sex-Differentiation Manipulations in Decapods

Amit Savaya-Alkalay and Amir Sagi

**Abstract** Invasive species of various taxa, including crustaceans and snails, are harmful to freshwater ecosystems, inflicting a reduction in biodiversity, vast losses for agriculture, alterations in natural ecosystems, and even some human health issues (such as bilharzia). We describe here some destructive invasive species of crustaceans and snails that can be controlled using biotechnological solutions. Specifically, we propose the use of sexually manipulated non-breeding all-male decapod crustacean populations generated through novel techniques that use temporal gene silencing via RNA interference (RNAi), namely, non-genetically modified organisms (non GMO), to control invasive species. The first part of the chapter deals with the control of invasive and destructive freshwater snails using snail-eating freshwater prawns; specifically we propose the use of all-male prawn populations to act as non-invasive and sustainable biocontrol agents. Freshwater prawns have already been shown to act as voracious predators of a few freshwater snail species. Since male prawns grow faster, reach larger size and do not migrate like females, it is likely that they will act as efficient biocontrol agents over snails. The second part of the chapter deals with the proposed control of invasive crustaceans by skewing the sex ratio of the invasive populations by repetitive releases into the invasive populations of neo-females, which bear 100 % male progeny. Since RNAi is becoming widely used and since the commercial use of RNAi-based biotechnologies for the production of neo-females and all-male prawn populations has already been implemented, our proposed solution is readily available for eco-protection applications.

---

A. Savaya-Alkalay · A. Sagi (✉)  
Department of Life Sciences, National Institute for Biotechnology  
in the Negev, Ben-Gurion University, P.O. Box 653, 84105 Beer-Sheva, Israel  
e-mail: sagia@bgu.ac.il

**Keywords** All-male populations • Biocontrol • Decapod crustaceans • Freshwater snails • Invasive species • RNAi

## 11.1 Introduction

The science of conservation biology was born in reaction to the global biodiversity crisis that has been unfolding over the past 100 years, driven mostly by human activities. A key concern in conservation biology is the damage to habitats and native species caused by deliberate or accidental human-mediated introductions of non-native invasive species (NIS) (Sala et al. 2000). Most introduced species fail to become established in their new environments and do not become troublesome invasive species. However, a minority of non-native invasive species do succeed in their new environments, and some of these have become serious threats to global biodiversity and ecosystem stability (Kolar and Lodge 2001). In the present chapter, we discuss some decapod crustaceans and snails—that have become devastating invasive species in freshwater ecosystems. Compared to terrestrial ecosystems, such ecosystems are particularly vulnerable to non-native invasive species due to water-related anthropogenic activities, such as transportation and recreation. The vulnerability of freshwater ecosystems is further exacerbated by the dispersion tendency of freshwater organisms and the relative geographic isolation of some lakes and streams, leading in some cases to location-specific adaptations of indigenous species (Lodge et al. 1998; Gherardi 2000; Sala et al. 2000; Beisel 2001; Gherardi 2006).

Freshwater decapods, such as crayfish, crabs, and shrimps, are among the largest and most long-lived invertebrates, and some act as keystone species due to their diverse diet, on the one hand, and their role as prey for other species, on the other hand (Moyle and Light 1996; Nyström et al. 1996). Non-native decapod crustaceans can have a significant effect on freshwater ecosystems due to their predation activity (Degerman et al. 2001) and their ability to change the physical conditions of the habitat (Anastacio et al. 2005b, c), thereby causing considerable environmental and economic damage (Guan and Wiles 1997). The introduction of freshwater decapods into non-native ecosystems may thus result in irreparable shifts in species diversity (Hobbs et al. 1989). This chapter focuses on invasive crustaceans that have negative impacts on freshwater ecosystems containing these keystone species and suggests that the introduction of non-breeding decapod populations could contribute to the solution of this problem. We describe several potential applications of an RNA interference (RNAi)-based sex-differentiation manipulation in decapods as novel and sustainable approaches to address environmental threats of the type described above. The proposed crustacean biocontrol agents can be applied to challenge invasive snails and crustaceans, thereby reducing the negative impact of the invasive species on freshwater ecosystems.

## 11.2 Production of All-Male Crustacean Populations for Aquaculture and for Biocontrol of Unwanted Species

All male populations of crustaceans are produced in a two-step procedure that involves sex-reversal of males to neo-females, namely, functional phenotypic females with a masculine genotype (as described in detail below), followed by mating of these neo-females with normal males. The resulting offspring will be male, due to the homogametic sex chromosomes of male malacostracans (Katakura 1989; Sagi et al. 1997; Jiang and Qiu 2013). The main advantage for aquaculture of all-male populations lies in increased commercial yields by virtue of the fact that male prawns grow faster and are larger than females (Nair et al. 2006). An additional advantage of such monosex populations is that they are non-breeding and thus do not pose a threat to the environment. They can therefore be exploited as competitive species for biocontrol of native or invasive undesirable species. These two applications—aquaculture and biocontrol—are discussed below.

### 11.2.1 Production of All-Male Populations via Androgenic Gland Manipulation or RNAi-Based Biotechnology for Aquaculture

Sexual differentiation in crustaceans is governed by the presence or absence of the androgenic gland (AG), an endocrine organ that is unique to males (Charniaux-Cotton 1954; Sagi and Cohen 1990; Sagi et al. 1997) and that controls masculine traits (Touir 1977; Taketomi et al. 1990; Manor et al. 2004). In the commercially important species of freshwater prawn *Macrobrachium rosenbergii*, microsurgical removal of the AG resulted in the production of female characters (Nagamine et al. 1980) and in sex-reversal from males to neo-females. This finding was applied by our group for the commercial production of all-male populations of *M. rosenbergii* (Aflalo et al. 2006). However, microsurgery has a low success rate (~1.3 %), so alternative technologies were sought with the aim to improve the efficiency of the approach (Aflalo et al. 2006). One such technology to produce all-male populations—temporal gene silencing by RNAi (Fire et al. 1998)—exploited the discovery by our group of the main secretory product of the decapod AG, the insulin-like androgenic gland hormone (IAG). This hormone was first discovered in the crayfish *Cherax quadricarinatus* (Manor et al. 2007) and later in other decapods (Ventura and Sagi 2012) (Table 11.1), including a number of economically important species (Manor et al. 2007; Ventura et al. 2009; Chung et al. 2011; Mareddy et al. 2011; Savaya-Alkalay et al. 2014; Vázquez-Islas et al. 2014).

**Table 11.1** Accession numbers of published IAGs of decapod crustaceans

| Species                            | Type     | Accession number |
|------------------------------------|----------|------------------|
| <i>Macrobrachium rosenbergii</i>   | Prawn    | FJ409645         |
| <i>Portunus pelagicus</i>          | Crab     | HM459854         |
| <i>Cherax quadricarinatus</i>      | Crayfish | DQ851163         |
| <i>Fenneropenaeus chinensis</i>    | Shrimp   | JQ388277.1       |
| <i>Macrobrachium nipponense</i>    | Prawn    | KC460325.1       |
| <i>Penaeus monodon</i>             | Shrimp   | GU208677.1       |
| <i>Callinectes sapidus</i>         | Crab     | HM594945.1       |
| <i>Marsupenaeus japonicus</i>      | Shrimp   | AB598415         |
| <i>Palaemon paucidens</i>          | Shrimp   | AB588013.1       |
| <i>Palaemon pacificus</i>          | Shrimp   | AB588014         |
| <i>Macrobrachium lar</i>           | Prawn    | AB579012.1       |
| <i>Cherax destructor</i>           | Crayfish | EU718788         |
| <i>Jasus edwardsii</i>             | Lobster  | AIM55892.1       |
| <i>Scylla paramamosain</i>         | Crab     | AIF30295.1       |
| <i>Sagmariasus verreauxi</i>       | Lobster  | AHY99679.1       |
| <i>Macrobrachium vollenhovenii</i> | Prawn    | KJ524578.1       |

Since the seminal study on “genetic interference by double-stranded RNA” of Fire et al. (1998), the “biology, mechanisms and applications” of RNAi have been studied in a variety of plant (Agrawal et al. 2003; Qi and Hannon 2005; Sagi et al. 2013) and metazoan species (e.g., Agrawal et al. 2003; Qi and Hannon 2005; Sagi et al. 2013), including crustaceans (Agrawal et al. 2003; Qi and Hannon 2005; Sagi et al. 2013). The strength of RNAi in biological applications lies in the fact that the administration of double-stranded RNA (dsRNA) does not cause genomic modifications [namely, it does not produce genetically modified organisms (GMO)], and therefore it can be applied for aquaculture purposes (Ventura et al. 2012). The first commercial use of temporal gene silencing in aquaculture was demonstrated by our research group (Ventura et al. 2012), with the production of all-male populations of *M. rosenbergii*, without the need for microsurgery (Ventura et al. 2012). In our study, juvenile male prawns were manipulated using dsRNA injections, causing a full and functional sex reversal from males into neo-females that produced 100 % male progeny (Ventura et al. 2012; Ventura and Sagi 2012). This RNAi-based non-GMO biotechnology does not involve hormonal or chemical intervention (Fire et al. 1998; Lezer et al. 2015), rather it relies on a safe temporal intervention at the IAG gene transcriptome level that does not modify the organism’s genome (Lezer et al. 2015). The non-breeding monosex populations that are produced with this technology are suitable for both aquaculture and, as elaborated in the next section, sustainable environmental applications (Aflalo and Sagi 2014).

### 11.2.2 All-Male Populations as Sustainable Biocontrol Agents

The above-described RNAi-based biotechnology to produce all-male crustacean populations was initially developed to meet the needs of the aquaculture sector. However, recently we suggested a novel approach using such all-male populations as non-invasive biocontrol agents (Savaya-Alkalay et al. 2014). It has been demonstrated under laboratory conditions that freshwater prawns of the genus *Macrobrachium* act as efficient predators of species of freshwater snails that serve as vectors for human diseases (Lee et al. 1982; Roberts and Kuris 1990; Sokolow et al. 2013). Preliminary predation studies under laboratory conditions suggest that *M. rosenbergii* prawns are voracious consumers of species of *Pomacea* freshwater snails (Savaya-Alkalay, personal communication). Therefore, we propose that all-male prawn populations should be tested as biocontrol agents—that do not carry the risk of becoming invasive species—against snails in both endemic and exotic habitats.

There are three advantages to using all-male populations of *Macrobrachium* as efficient and sustainable biocontrol agents (Savaya-Alkalay et al. 2014):

- (a) Large size and fast growth. Male prawns grow faster and reach a larger body size than females and consume larger quantities of food (or prey) (Sagi et al. 1986), thereby offering both ecological and economic (aquaculture) advantages.
- (b) Non-migratory behavior. All-male populations of *Macrobrachium* (vs. populations with a 50:50 male:female sex ratio) are preferable as agents for snail control because males do not migrate (while females of *Macrobrachium* spp. must swim downstream to release their larvae in brackish estuarine water) (Bauer 2011).
- (c) Reproductive sterility. All-male populations of *Macrobrachium* cannot reproduce and thus cannot become invasive species following introduction into a new habitat. In addition, if in the long term the all-male prawn populations were found to cause more damage than benefit, they could easily be removed at any time by refraining from restocking.

### 11.3 Controlling Invasive Species by Using Non-reproductive Crustacean Agents

Two different ‘problematic’ groups of species can be controlled by RNAi-manipulated crustaceans: (1) explosive populations of invasive freshwater snails that can be controlled by prawn predation, and (2) explosive populations of invasive crustaceans that can be controlled by changing the sex ratio of these populations towards maleness until the invasive population collapses.

### 11.3.1 *Invasive and ‘Problematic’ Species of Freshwater Snails*

Freshwater snails are distributed worldwide, with negative effects on biodiversity, agriculture, and human health being caused by a number of non-native invasive species. Snails are often overlooked as agricultural pests in the scientific literature, even though they cause major losses for different crops around the globe (Barker 2002). We present here two case studies of invasive and damaging snails from different families for which there is potential for biological control by crustacean species.

#### 11.3.1.1 **Apple Snails (Ampullariidae)**

Apple snails, the largest freshwater gastropods (reaching a shell height of 155 mm), are distributed mainly in humid tropical and subtropical habitats in South and Central America, Africa, and Southeast Asia (Pain 1960; Burky 1974). Many species of apple snail are amphibious, having both a gill and a lung, and are therefore able to spend long periods of time out of the water—a trait that challenges efforts to control these snails (Prashad 1925; Burky et al. 1972). Apple snails are not only agricultural pests (Naylor 1996), but they are also ecologically destructive and have the potential to alter wetland ecosystems via the impact of their omnivorous diet, their large size, and their amphibious capabilities that help them evade control measures (Naylor 1996; Kwong et al. 2009).

There are a few reports of apple snails causing damage to crops (mostly rice in paddies) in their native habitats, but the major crop damage is caused by apple snails introduced outside of their natural habitats; apple snails have therefore been categorized as one of the world’s 100 worst invasive species (Lowe et al. 2000; Horgan et al. 2014). The global spread of apple snails was triggered by two major commercial activities, namely, the food industry and the aquarium trade (Perera 1996). Apple snails, which are native to South America, were first introduced to Hawaii (in the 1960s) and then to a number of Asian countries (in the 1970s and 1980s) as food items. However, the economic potential of this sector was overestimated, and the market failed due to lack of demand (Naylor 1996; Vitousek et al. 1996). In East Asia, apple snails that either escaped or were released from captivity established themselves as invasive populations. It appears, however, that the aquarium trade market bears the major responsibility for the wide-spread establishment of alien populations of apple snails the world over—Cambodia, Chile, China, Dominican Republic, Egypt, Guam, India, Indonesia, Israel, Japan, Laos, Malaysia, Myanmar, Papua New Guinea, Pakistan, Philippines, Russia, Singapore, South Africa, South Korea, Spain, Sri-Lanka, Taiwan, Thailand, and the USA (Roll et al. 2009; Horgan et al. 2014).

An illustrative case study of invasive apple snails is that of the establishment of the golden apple snail *Pomacea canaliculata* in the delta of the Ebro river in Spain,

first observed in 2009 (López et al. 2010). Since then, none of the efforts to control the snails has been successful. The dominant agricultural crop in the Ebro River delta is rice, whose production has been dramatically reduced since the apple snail invasion. The snails reach high densities, with a biomass of  $1 \text{ kg m}^{-2}$ , and produce more than 5 egg masses  $\text{m}^{-2}$ .

The first introduction of apple snails in Asia caused enormous damage to agricultural production (mostly rice) as well as to natural ecosystems and even to human health (Naylor 1996). Agricultural damage depends on the density and size of the snails as well as on the age of the crop, with losses varying between 20 % (at a density of  $1 \text{ snail m}^{-2}$ ) and 90 % (at a density of  $8 \text{ snails m}^{-2}$ ) (Hirai 1988; Basilio 1991). In Japan, damage due to apple snails caused major reductions to crops such as rice, water spinach, lotus, taro, and mat rush (Mochida 1991), in addition to damage to ecosystems and to humans from the intensive use of molluscicides and insecticides (Laughlin and Lindén 1985; Clark et al. 1988; Hoch 2001). Furthermore, *Pomacea canaliculata* snails act as intermediate hosts for the parasitic worm *Angiostrongylus cantonensis*, which causes human eosinophilic meningitis if not destroyed by sufficient cooking of the snails (Lv et al. 2009). Dozens of cases of human eosinophilic meningitis were detected in 2006 in southern China, where infected apple snails were found to be on sale in markets and restaurants (Lv et al. 2009).

The predation potential of biocontrol agents over populations of *Pomacea* snails was investigated in southern Japan, where 46 species were tested under laboratory conditions as predators of these snails (Yusa et al. 2006). Under these conditions, ducks, rats, turtles and carp were found to be efficient predators of adult *Pomacea canaliculata* snails, and a few species of crustaceans could act as efficient predators of small- to medium-sized snails. However, the only species of *Macrobrachium* tested (*M. formosense*) was a small species (2.4 g average weight), which is significantly smaller than *M. rosenbergii*, suggested here as a biocontrol agent.

### 11.3.1.2 Snail Hosts of *Schistosoma*

Schistosomiasis (bilharzia), a debilitating chronic disease that infects more than 240 million people around the world, is caused by parasitic flatworms of the genus *Schistosoma* (WHO 2013a). These parasitic worms require two hosts to complete their life cycle, a freshwater snail intermediate host (a species of *Biomphalaria* or *Bulinus*) and a vertebrate definitive host (usually a mammal) (WHO 2013a). The distribution of schistosomiasis is intimately linked to the distribution and abundance of the snail hosts, and control efforts are focusing on snail distribution as a major target in breaking the disease cycle.

A few species of crustaceans have been tested as potential biocontrol agents for snails that transmit parasitic diseases to humans (Mkoji et al. 1999; Sokolow et al. 2013). Until recently, the invasive crayfish *Procambarus clarkii* was the only species that has been studied under field conditions (Hofkin et al. 1991a, b; Ibrahim et al. 1995; Mkoji et al. 1999; Lodge et al. 2005). Later studies with

*M. vollenhovenii* are discussed below. We review here two different parts of Africa where schistosomiasis affects large numbers of people: firstly, Kenya (East Africa), where more than 25 % of the population (more than 11 million people) is in danger. Secondly, the Senegal River basin (West Africa), in northern Senegal, whose damming has been associated with the fastest recorded spread of human schistosomiasis; more than 30 % of Senegal's population (about 4 million people) requires preventive chemotherapy against the disease (Southgate 1997; Southgate et al. 2001; Sow et al. 2002; WHO 2013b).

In Kenya, the North American crayfish or red swamp crayfish *Procambarus clarkii* was originally introduced for aquaculture (Oluoch 1990), but escaped crayfish have now spread out, and *Procambarus clarkii* has become a well-established invasive species with a significant impact on native ecosystems (Mkoji et al. 1999). Scientists have examined the efficiency of *Procambarus clarkii* as a biocontrol agent of snails infected with *Schistosoma* under field conditions (Mkoji et al. 1999) following laboratory experiments that supported the hypothesis that these crayfish could decrease the prevalence of schistosomiasis in human populations (Hofkin et al. 1991a, 1992). Indeed, under certain environmental conditions, the introduction of *Procambarus clarkii* significantly reduced the abundance of *Schistosoma*-infected snails and the rate of infection among human residents of the study area (Mkoji et al. 1999). However, the impact of *Procambarus clarkii* on aquatic ecosystems is an issue of major concern from a conservation point of view, because the appetite of these crayfish for aquatic plants is large enough to alter the biotic and physical conditions of the invaded habitats (see Sect. 3.2.2). Therefore, it is essential to carefully consider the environmental costs and benefits of using an invasive crustacean species as a biocontrol agent to reduce populations of damaging invasive snails.

Another conservation approach has been to reintroduce endemic species that have been extirpated from their natural environment by anthropogenic environmental changes. For example, the man-made Diama dam on the Senegal River created favorable conditions for freshwater snails but unfavorable conditions for its natural predator, the freshwater African river prawn *M. vollenhovenii* (Savaya-Alkalay et al. 2014). The females of *M. vollenhovenii*, being catadromous, need to migrate down the Senegal River to release their larvae in saltwater habitats, but they are prevented from so doing by the Diama dam downstream on the river. As a result, the present distributional range and population levels of *M. vollenhovenii* are greatly reduced from their former sizes, and the reduction of this predator has coincided with a population explosion of freshwater snails, which are vectors of schistosomiasis. Reintroduction of *M. vollenhovenii* to the Senegal River is an appealing idea but would require careful management, because its sustainability is dependent on a constant prawn stocking strategy together with the building of 'fish ladder' channels (Gowans et al. 1999) at the dam to allow the prawns to navigate the river upstream and downstream. A recent field study in the Senegal River basin demonstrated that African river prawns as biological control agents had a significant effect on snail density and hence on human infection rates and disease burden (Sokolow et al. 2015). These findings provide support for the



suggested solution of using all-male African river prawns as sustainable and efficient biocontrol agents (Savaya-Alkalay et al. 2014).

### 11.3.2 *Invasive Crustaceans as a Conservation Challenge*

Decapod crustaceans are successful invaders of freshwater ecosystems due to their efficient adaptive behavior. Factors that contribute to the high adaptability of crustaceans that are invasive species include a high reproductive rate, a tolerance to environmental fluctuations, and a diverse diet (Gherardi 2006). All these characteristics result in high fitness and contribute to the successful invasion of new habitats, especially in combination with opportunistic behavior. The presence of invading species impacts the habitat through alterations of vegetation patterns and food resources that may increase the vulnerability of endemic species to extinction. In addition to their negative impact on natural ecosystems, invasive species also frequently cause damage to the agricultural and aquaculture sectors (Anastacio et al. 2005a, b, d).

Biological control via altering the reproductive success of a species has been widely utilized by the agricultural sector to manage insect pests (Dyck et al. 2005). The technique is based on releasing sterilized males that compete with normal males for females, which eventually decreases fecundity through unsuccessful mating. Here, we propose that this concept be exploited in a novel technique that relies on RNAi-based biotechnological approaches to eradicate invasive crustacean species by creating and releasing neo-females that produce all-male offspring. This approach is based on the premise that successive releases of neo-females would compete with the population of normal females for mates; the all-male progeny of the neo-females would skew the sex ratio of the population towards males, with the obvious consequent reduction in the population size.

The proposed technique could be applied for controlling invasive shrimps, crayfish and prawns, as discussed below. Examples of crustacean pests are the burrowing shrimps *Neotrypaea californiensis* and *Upogebia pugettensis*, whose activities soften the intertidal substrate where oysters are grown. The soft sediment particles clog up the oysters' filter-feeding and respiratory channels, leading to oyster mortality and consequent economic losses to the oyster industry (Dumbauld et al. 2006). Controlling sexual differentiation in shrimp populations such as these could pave the way for safely skewing their sex ratio, thereby controlling the size of the populations and hence minimizing their negative effect.

Crayfish are probably the most dangerous crustaceans in terms of invasion impacts due to their high densities, relatively long life spans, and large size (Gherardi 2006). Crayfish are keystone species due to their dominant role in the food chain and thus invasions by crayfish species may cause significant alterations to freshwater food webs (Lodge et al. 1998; Covich et al. 1999). Crayfish invasions reduce biomass and the species richness of the organisms comprising their diet, and this causes an accompanying reduction in the recruitment of commercial fish

species (Lodge and Lorman 1987; Nyström 1999). One of the most damaging invasive crustacean species is the North American red swamp crayfish *Procambarus clarkii*, which has been commercially cultured since the 1950s in a number of southern US states. The species is endemic from northeastern Mexico to the south-central USA but is now found in every continent, excluding Australia and Antarctica (Huner 2002). The success of *P. clarkii* as an invasive species lies in a number of biological characteristics that aid its colonization of new habitats. This species exhibits an *R*-selected reproductive strategy (producing large numbers of offspring), a small body size, early sexual maturity, a fast growth rate, and the ability to incubate eggs all year round (Lindqvist and Huner 1999; Paglianti and Gherardi 2004). In addition, *P. clarkii* has a very high tolerance to extreme environmental conditions, such as polluted habitats and temporary streams that dry up seasonally (Gherardi et al. 2000). Another important aspect of *P. clarkii*'s survival potential is its ability to carry but not be infected by the pathogenic fungus *Aphanomyces astaci*, which causes crayfish plague in native species (Dieguez-Uribeondo and Söderhäll 1993). Additional ecological damage is caused by *P. clarkii*'s burrowing activities, which alter water quality and sediment characteristics and may hence severely affect the agricultural and aquaculture sectors (Souty-Grosset 2006). One solution to the problem would be to release neo-female crayfish into the invaded habitats so that sex-ratios would be skewed toward males and the invading population would be extirpated over a few generations.

A few years ago, invasive populations of the Asian freshwater prawn *M. rosenbergii* were found in freshwaters in northern Brazil. *M. rosenbergii* was originally introduced into northern Brazil for aquaculture in the late 1970s, and its escape from captivity has resulted in the establishment of wild breeding populations in a number of locations, including three in which the presence of berried *M. rosenbergii* females has been reported (Silva-Oliveira et al. 2011). The invasion potential of this prawn is high due to its high reproductive rate, its adaptive behavior, and its omnivorous diet. It is therefore an urgent challenge to halt the prawn invasions before they spread further, and one way that this could be done would be by introducing neo-females of this species to skew the sex ratio towards all male populations.

## 11.4 Epilogue

The rapid development of genomic and transcriptomic sequencing techniques has the potential for the discovery of thousands of new crustacean genes; from these it would be possible to select candidates to be used as conservation tools to fight invasive species by applying RNAi-based or similar biotechnologies. One major drawback of current RNAi biotechnologies is that non-GMO delivery mechanisms are currently based on microinjection methods that are labor intensive and require the professional training and special equipment that are not available in low-tech facilities. Another disadvantage of RNAi is the high price of the gene silencing

agents. However, applying RNAi treatments at the brood stock level (Ventura and Sagi 2012; Aflalo and Sagi 2014), where each treated animal produces thousands of offspring, makes the price of dsRNA more acceptable. In contrast, when RNAi is considered as a treatment to be utilized on an entire population of an invasive or problematic species (for vaccination, environmental manipulations, or as a growth promoter), the price might indeed be a limiting factor. A global research effort is thus needed to address the above challenges by focusing on a more efficient delivery method (preferably a non-invasive technique to be delivered orally as a food supplement) and on a more cost effective dsRNA production method. Such developments would enable the establishment of more elegant solutions to addressing the problems associated with invasive species; these solutions could then be applied by conservationists in their fight against the ever-increasing loss of global biodiversity.

## References

- Aflalo, E. D., & Sagi, A. (2014). Sustainable aquaculture biotechnology using temporal RNA interference in crustaceans: The case of the insulin-like androgenic gland hormone and prawn monosex culture. In J. N. Govil (Ed.), *Animal biotechnology* (pp. 319–331). USA: Studium Press LLC.
- Aflalo, E. D., Hoang, T. T. T., Nguyen, V. H., Lam, Q., Nguyen, D. M., Trinh, Q. S., et al. (2006). A novel two-step procedure for mass production of all-male populations of the giant freshwater prawn *Macrobrachium rosenbergii*. *Aquaculture*, 256, 468–478. doi:10.1016/j.aquaculture.2006.01.035.
- Agrawal, N., Dasaradhi, P. V., Mohammed, A., Malhotra, P., Bhatnagar, R. K., & Mukherjee, S. K. (2003). RNA interference: Biology, mechanism, and applications. *Microbiology and Molecular Biology Reviews*, 67, 657–685. doi:10.1128/MMBR.67.4.657-685.2003.
- Anastacio, P. M., Correia, A. M., & Menino, J. P. (2005a). Processes and patterns of plant destruction by crayfish: Effects of crayfish size and developmental stages of rice. *Archiv für Hydrobiologie*, 162, 37–51. doi:10.1127/0003-9136/2005/0162-0037.
- Anastacio, P. M., Correia, A. M., Menino, J. P., & da Silva, L. M. (2005b). Are rice seedlings affected by changes in water quality caused by crayfish? *Annales de Limnologie-International Journal of Limnology*, 41, 1–6. doi:10.1051/Limn/2005002.
- Anastacio, P. M., Parente, V. S., & Correia, A. M. (2005c). Crayfish effects on seeds and seedlings: Identification and quantification of damage. *Freshwater Biology*, 50, 697–704. doi:10.1111/j.1365-2427.2005.01343.x.
- Anastacio, P. M., Soares, M., & Correia, A. M. (2005d). Crayfish (*Procambarus clarkii*) consumption of wet-seeded rice plants (*Oryza sativa*): Modifications throughout the rice growing period. *Internationale Vereinigung für Theoretische und Angewandte Limnologie Verhandlungen*, 29, 849–851.
- Barker, G. M. (2002). *Molluscs as crop pests*. New York: CABI Publishing.
- Basilio, R. (1991). Problems of golden snail infestation in rice farming. In R. S. Pullen (Ed.), *Acosta BO*. Philippines: ICLARM conference proceedings Central Luzon State University.
- Bauer, R. T. (2011). Amphidromy and migrations of freshwater shrimps. II. Delivery of hatching larvae to the sea, return juvenile upstream migration, and human impacts. In A. Asakura (Ed.), *New frontiers in crustacean biology* (pp. 157–168). Koninklijke Brill NV: Leiden.

- Beisel, J. N. (2001). The elusive model of a biological invasion process: Time to take differences among aquatic and terrestrial ecosystems into account? *Ethology Ecology Evolutionary*, *13*, 193–195. doi:10.1080/08927014.2001.9522785.
- Burky, A. J. (1974). Growth and biomass production of an amphibious snail, *Pomacea urceus* (Müller), from the Venezuelan savannah. *Journal of Molluscan Studies*, *41*, 127–143.
- Burky, A. J., Pacheco, J., & Pereyra, E. (1972). Temperature, water, and respiratory regimes of an amphibious snail, *Pomacea urceus* (Müller), from the Venezuelan savannah. *The Biological Bulletin*, *143*, 304–316.
- Charniaux-Cotton, H. (1954). Discovery in, an amphipod crustacean (*Orchestia gammarella*) of an endocrine gland responsible for the differentiation of primary and secondary male sex characteristics. *Comptes rendus hebdomadaires des séances de l'Académie des sciences*, *239*, 780–782.
- Chung, J. S., Manor, R., & Sagi, A. (2011). Cloning of an insulin-like androgenic gland factor (IAG) from the blue crab, *Callinectes sapidus*: Implications for eyestalk regulation of IAG expression. *General and Comparative Endocrinology*, *173*, 4–10. doi:10.1016/j.ygcen.2011.04.017.
- Clark, E. A., Sterritt, R. M., & Lester, J. N. (1988). The fate of tributyltin in the aquatic environment. *Environmental Science and Technology*, *22*, 600–604. doi:10.1021/es00171a001.
- Covich, A. P., Palmer, M. A., & Crowl, T. A. (1999). The role of benthic invertebrate species in freshwater ecosystems—Zooenthic species influence energy flows and nutrient cycling. *Bio Science*, *49*, 119–127. doi:10.2307/1313537.
- Degerman, E., Hammar, J., Nyberg, P., & Svardson, G. (2001). Human impact on the fish diversity in the four largest lakes of Sweden. *A Journal of the Human Environment*, *30*, 522–528.
- Dieguez-Uribeondo, J., & Söderhäll, K. (1993). *Procambarus clarkii* Girard as a vector for the crayfish plague fungus, *Aphanomyces astaci* Schikora. *Aquaculture Research*, *24*, 761–765. doi:10.1111/j.1365-2109.1993.tb00655.x.
- Dumbauld, B. R., Booth, S., Cheney, D., Suhrbier, A., & Beltran, H. (2006). An integrated pest management program for burrowing shrimp control in oyster aquaculture. *Aquaculture*, *261*, 976–992. doi:10.1016/j.aquaculture.2006.08.030.
- Dyck, V. A., Hendrichs, J., & Robinson, A. S. (2005). *Sterile insect technique*. Netherland: Springer.
- Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E., & Mello, C. C. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature*, *391*, 806–811. doi:10.1038/35888.
- Gherardi, F. (2000). Are non-indigenous species “ecological malignancies”? *Ethology Ecology Evolutionary*, *12*, 323–325. doi:10.1080/08927014.2000.9522805.
- Gherardi, F. (2006). Crayfish invading Europe: The case study of *Procambarus clarkii*. *Marine and Freshwater Behaviour and Physiology*, *39*, 175–191. doi:10.1080/10236240600869702.
- Gherardi, F., Raddi, A., Barbaresi, S., & Salvi, G. (2000). Life history patterns of the red swamp crayfish, *Procambarus clarkii*, in an irrigation ditch in Tuscany, Italy. In F. R. Schram (Ed.), *von Vaupel Klein JC* (pp. 99–108). Rotterdam: Crustacean Issues. A.A. Balkema.
- Gowans, A., Armstrong, J., & Priede, I. (1999). Movements of adult Atlantic salmon in relation to a hydroelectric dam and fish ladder. *Journal of Fish Biology*, *54*, 713–726. doi:10.1111/j.1095-8649.1999.tb02028.x.
- Guan, R. Z., & Wiles, P. R. (1997). Ecological impact of introduced crayfish on benthic fishes in a British lowland river. *Conservation Biology*, *11*, 641–647. doi:10.1046/j.1523-1739.1997.96073.x.
- Hirai, Y. (1988). Apple snail in Japan. *Japan Agricultural Research Quarterly*, *22*, 161–165.
- Hobbs, H. H., Jass, J. P., & Huner, J. V. (1989). A review of global crayfish introductions with particular emphasis on 2 North-American species (Decapoda, Cambaridae). *Crustaceana*, *56*, 299–316. doi:10.1163/156854089x00275.
- Hoch, M. (2001). Organotin compounds in the environment—an overview. *Applied Geochemistry*, *16*, 719–743. doi:10.1016/S0883-2927(00)00067-6.

- Hofkin, B. V., Koech, D. K., Ouma, J., & Loker, E. S. (1991a). The North American crayfish *Procambarus clarkii* and the biological control of schistosome-transmitting snails in Kenya: Laboratory and field investigations. *Biological Control*, 1, 183–187. doi:10.1016/1049-9644(91)90065-8.
- Hofkin, B. V., Mkoji, G. M., Koech, D. K., & Loker, E. S. (1991b). Control of schistosome-transmitting snails in Kenya by the North-American crayfish *Procambarus clarkii*. *The American Journal of Tropical Medicine and Hygiene*, 45, 339–344.
- Hofkin, B. V., Hofinger, D. M., Koech, D. K., & Loker, E. S. (1992). Predation of *Biomphalaria* and nontarget Mollusks by the Crayfish *Procambarus clarkii*—implications for the biological-control of Schistosomiasis. *Annals of Tropical Medicine and Parasitology*, 86, 663–670.
- Horgan, F. G., Stuart, A. M., & Kudavidanage, E. P. (2014). Impact of invasive apple snails on the functioning and services of natural and managed wetlands. *Acta Oecologica-International Journal Of Ecology*, 54, 90–100. doi:10.1016/j.actao.2012.10.002.
- Huner, J. (2002). *Procambarus*. In D. M. Holdich (Ed.), *Biology of freshwater crayfish*. Oxford: Blackwell Science.
- Ibrahim, A., Khalil, M., & Mobarak, F. (1995). On the feeding behavior of the exotic crayfish *Procambarus clarkii* in Egypt and its prospects in the biocontrol of local vector snails. *Journal Union Arabian Biology Cairo*, 4, 321–340.
- Jiang, X. H., & Qiu, G. F. (2013). Female-only sex-linked amplified fragment length polymorphism markers support ZW/ZZ sex determination in the giant freshwater prawn *Macrobrachium rosenbergii*. *Animal Genetics*, 44, 782–785. doi:10.1111/Age.12067.
- Katakura, Y. (1989). Endocrine and genetic-control of sex-differentiation in the malacostracan crustacea. *Invertebrate Reproduction & Development*, 16, 177–182. doi:10.1080/07924259.1989.9672075.
- Kolar, C. S., & Lodge, D. M. (2001). Progress in invasion biology: Predicting invaders. *Trends in Ecology & Evolution*, 16, 199–204. doi:10.1016/S0169-5347(01)02101-2.
- Kwong, K.-L., Chan, R. K., & Qiu, J.-W. (2009). The potential of the invasive snail *Pomacea canaliculata* as a predator of various life-stages of five species of freshwater snails. *Malacologia*, 51, 343–356. doi:10.4002/040.051.0208.
- Laughlin, R. B., Jr., & Lindén, O. (1985). *Fate and effects of organotin compounds* (pp. 88–94). North America: Ambio.
- Lee, P. G., Rodrick, G. E., Sodeman, W. A., Jr., & Blake, N. J. (1982). The giant Malaysian prawn, *Macrobrachium rosenbergii*, a potential predator for controlling the spread of schistosome vector snails in fish ponds. *Aquaculture*, 28, 293–301. doi:10.1016/0044-8486(82)90071-0.
- Lezer, Y., Aflalo, E. D., Manor, R., Sharabi, O., Abilevich, L. K., & Sagi, A. (2015). On the safety of RNAi usage in aquaculture: the case of all-male prawn stocks generated through manipulation of the insulin-like androgenic gland hormone. *Aquaculture*, 435, 157–166. doi:10.1016/j.aquaculture.2014.09.040.
- Lindqvist, O. V., & Huner, J. V. (1999). Life history characteristics of crayfish: What makes some of them good colonizers? *Crayfish European Alien Species*, 11, 23–30.
- Lodge, D. M., & Lorman, J. G. (1987). Reductions in submersed macrophyte biomass and species richness by the crayfish *Orconectes rusticus*. *Canadian Journal of Fisheries and Aquatic Sciences*, 44, 591–597. doi:10.1139/f87-072.
- Lodge, D. M., Stein, R. A., Brown, K. M., Covich, A. P., Bronmark, C., Garvey, J. E., et al. (1998). Predicting impact of freshwater exotic species on native biodiversity: Challenges in spatial scaling. *Australian Journal of Ecology*, 23, 53–67. doi:10.1111/j.1442-9993.1998.tb00705.x.
- Lodge, D. M., Rosenthal, S. K., Mavuti, K. M., Muohi, W., Ochieng, P., Stevens, S. S., et al. (2005). Louisiana crayfish (*Procambarus clarkii*) (Crustacea: Cambaridae) in Kenyan ponds: Non-target effects of a potential biological control agent for schistosomiasis. *African Journal of Aquatic Science*, 30, 119–124. doi:10.2989/16085910509503845.

- López, M. A., Altaba, C. R., Andree, K. B., & López, V. (2010). *First invasion of the apple snail pomacea insularum in Europe*, *The Newsletter of the IUCN/SSC Mollusc Specialist Group*. International Union for Conservation of Nature: Species Survival Commission.
- Lowe, S., Browne, M., Boudjelas, S., & De Poorter, M. (2000). *100 of the world's worst invasive alien species: A selection from the global invasive species database*. World Conservation Union (IUCN), Auckland: Invasive Species Specialist Group Species Survival Commission.
- Lv, S., Zhang, Y., Liu, H.-X., Hu, L., Yang, K., Steinmann, P., et al. (2009). Invasive snails and an emerging infectious disease: Results from the first national survey on *Angiostrongylus cantonensis* in China. *PLoS Neglected Tropical Diseases*, 3, e368. doi:10.1371/journal.pntd.0000368.
- Manor, R., Aflalo, E. D., Segall, C., Weil, S., Azulay, D., Ventura, T., et al. (2004). Androgenic gland implantation promotes growth and inhibits vitellogenesis in *Cherax quadricarinatus* females held in individual compartments. *Invertebrate Reproduction & Development*, 45, 151–159. doi:10.1080/07924259.2004.9652584.
- Manor, R., Weil, S., Oren, S., Glazer, L., Aflalo, E. D., Ventura, T., et al. (2007). Insulin and gender: An insulin-like gene expressed exclusively in the androgenic gland of the male crayfish. *General and Comparative Endocrinology*, 150, 326–336. doi:10.1016/j.ygcen.2006.09.006.
- Mareddy, V. R., Rosen, O., Thaggard, H. B., Manor, R., Kuballa, A. V., Aflalo, E. D., et al. (2011). Isolation and characterization of the complete cDNA sequence encoding a putative insulin-like peptide from the androgenic gland of *Penaeus monodon*. *Aquaculture*, 318, 364–370. doi:10.1016/j.aquaculture.2011.05.027.
- Mkoji, G. M., Hofkin, B. V., Kuris, A. M., Stewart-Oaten, A., Mungai, B. N., Kihara, J. H., et al. (1999). Impact of the crayfish *Procambarus clarkii* on *Schistosoma haematobium* transmission in Kenya. *The American Journal of Tropical Medicine and Hygiene*, 61, 751–759.
- Mochida, O. (1991). Spread of freshwater *Pomacea* snails (Pilidae, Mollusca) from Argentina to Asia. (51–62). Guam: Micronesica
- Moyle, P. B., & Light, T. (1996). Biological invasions of fresh water: Empirical rules and assembly theory. *Biological Conservation*, 78, 149–161. doi:10.1016/0006-3207(96)00024-9.
- Nagamine, C., Knight, A. W., Maggenti, A., & Paxman, G. (1980). Effects of androgenic gland ablation on male primary and secondary sexual characteristics in the Malaysian prawn, *Macrobrachium rosenbergii* (de Man) (Decapoda, Palaemonidae), with first evidence of induced feminization in a nonhermaphroditic decapod. *General and Comparative Endocrinology*, 41, 423–441. doi:10.1016/0016-6480(80)90048-9.
- Nair, C. M., Salin, K. R., Raju, M. S., & Sebastian, M. (2006). Economic analysis of monosex culture of giant freshwater prawn (*Macrobrachium rosenbergii* De Man): A case study. *Aquaculture Research*, 37, 949–954. doi:10.1111/j.1365-2109.2006.01521.x.
- Naylor, R. (1996). Invasions in agriculture: Assessing the cost of the golden apple snail in Asia. *A Journal of the Human Environment*, 25, 443–448.
- Nyström, P. (1999). Ecological impact of introduced and native crayfish on freshwater communities: European perspectives. *Crustacean issues*, 11, 63–86.
- Nyström, P., Br Önmark, C., & Graneli, W. (1996). Patterns in benthic food webs: A role for omnivorous crayfish? *Freshwater Biology*, 36, 631–646.
- Oluoch, A. (1990). Breeding biology of the Louisiana red swamp crayfish *Procambarus clarkii* Girard in Lake Naivasha, Kenya. *Hydrobiologia*, 208, 85–92.
- Paglianti, A., & Gherardi, F. (2004). Combined effects of temperature and diet on growth and survival of young-of-year crayfish: A comparison between indigenous and invasive species. *Journal of Crustacean Biology*, 24, 140–148. doi:10.1651/C-2374.
- Pain, T. (1960). *Pomacea* (Ampullariidae) of the Amazon river system. *Journal of Conchology*, 24, 421–432.
- Perera, G. (1996). *Apple snails in the aquarium: Ampullariids-their identification, care, and breeding*. USA: TFH Publications.
- Prashad, B. (1925). *Anatomy of the common Indian apple-snail*. Zoological Survey of India: Pila globosa.

- Qi, Y., & Hannon, G. J. (2005). Uncovering RNAi mechanisms in plants: Biochemistry enters the foray. *Federation of European Biochemical Societies letters*, 579, 5899–5903. doi:[10.1016/j.febslet.2005.08.035](https://doi.org/10.1016/j.febslet.2005.08.035).
- Roberts, J. K., & Kuris, A. M. (1990). Predation and control of laboratory populations of the snail *Biomphalaria glabrata* by the freshwater prawn *Macrobrachium rosenbergii*. *Annals of Tropical Medicine and Parasitology*, 84, 401–412.
- Roll, U., Dayan, T., Simberloff, D., & Mienis, H. K. (2009). Non-indigenous land and freshwater gastropods in Israel. *Biological Invasions*, 11, 1963–1972. doi:[10.1007/s10530-008-9373-4](https://doi.org/10.1007/s10530-008-9373-4).
- Sagi, A., & Cohen, D. (1990). Growth, maturation and progeny of sex-reversed *Macrobrachium rosenbergii* males. *World Aquaculture*, 21, 87–90.
- Sagi, A., Ra'anani, Z., Cohen, D., & Wax, Y. (1986). Production of *Macrobrachium rosenbergii* in monosex populations: Yield characteristics under intensive monoculture conditions in cages. *Aquaculture*, 51, 265–275.
- Sagi, A., Snir, E., & Khalaila, I. (1997). Sexual differentiation in decapod crustaceans: Role of the androgenic gland. *Invertebrate Reproduction & Development*, 31, 55–61. doi:[10.1080/07924259.1997.9672563](https://doi.org/10.1080/07924259.1997.9672563).
- Sagi, A., Manor, R., & Ventura, T. (2013). Gene silencing in crustaceans: From basic research to biotechnologies. *Genes*, 4, 620–645. doi:[10.3390/genes4040620](https://doi.org/10.3390/genes4040620).
- Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., et al. (2000). Biodiversity—global biodiversity scenarios for the year 2100. *Science*, 287, 1770–1774. doi:[10.1126/science.287.5459.1770](https://doi.org/10.1126/science.287.5459.1770).
- Savaya-Alkalay, A., Rosen, O., Sokolow, S. H., Faye, Y. P., Faye, D. S., Aflalo, E. D., et al. (2014). The prawn *Macrobrachium vollohovenii* in the Senegal River Basin: towards sustainable restocking of all-male populations for biological control of schistosomiasis. *PLoS Neglected Tropical Diseases*, 8, e3060. doi:[10.1371/journal.pntd.0003060](https://doi.org/10.1371/journal.pntd.0003060).
- Silva-Oliveira, G. C., Ready, J. S., Iketani, G., Bastos, S., Gomes, G., Sampaio, I., et al. (2011). The invasive status of *Macrobrachium rosenbergii* (De Man, 1879) in Northern Brazil, with an estimation of areas at risk globally. *Aquatic Invasions*, 6, 319–328. doi:[10.3391/ai.2011.6.3.08](https://doi.org/10.3391/ai.2011.6.3.08).
- Sokolow, S. H., Lafferty, K. D., & Kuris, A. M. (2013). Regulation of laboratory populations of snails (*Biomphalaria* and *Bulinus* spp.) by river prawns, *Macrobrachium* spp. (Decapoda, Palaemonidae): Implications for control of schistosomiasis. *Acta Tropica*, 132C, 64–67. doi:[10.1016/j.actatropica.2013.12.013](https://doi.org/10.1016/j.actatropica.2013.12.013).
- Sokolow, S. H., Huttinger, E., Jouanard, N., Hsieh, M. H., Lafferty, K. D., Kuris, A. M., et al. (2015). Reduced transmission of human schistosomiasis after restoration of a native river prawn that preys on the snail intermediate host. *Proceedings of the National Academy of Sciences*, 112, 9650–9655.
- Southgate, V. R. (1997). Schistosomiasis in the Senegal River Basin: Before and after the construction of the dams at Diama, Senegal and Manantali, Mali and future prospects. *Journal of Helminthology*, 71, 125–132.
- Southgate, V., Tchuem Tchuente, L. A., Sene, M., De Clercq, D., Theron, A., Jourdane, J., et al. (2001). Studies on the biology of schistosomiasis with emphasis on the Senegal river basin. *Memórias do Instituto Oswaldo Cruz*, 96(Suppl), 75–78. doi:[10.1590/S0074-02762001000900010](https://doi.org/10.1590/S0074-02762001000900010).
- Souty-Grosset, C. (2006). *Atlas of crayfish in Europe*. The Netherlands: Backhuys Publishers.
- Sow, S., de Vlas, S. J., Engels, D., & Gryseels, B. (2002). Water-related disease patterns before and after the construction of the Diama dam in northern Senegal. *Annals of Tropical Medicine and Parasitology*, 96, 575–586. doi:[10.1179/000349802125001636](https://doi.org/10.1179/000349802125001636).
- Taketomi, Y., Murata, M., & Miyawaki, M. (1990). Androgenic gland and secondary sexual characters in the crayfish *Procambarus clarkii*. *Journal of Crustacean Biology*, 10, 492–497.
- Touir, A. (1977). New data concerning sexual endocrinology of hermaphroditic and gonochoristic crustacea decapoda natantia. 2. Maintenance of gonidia and evolution of gametogenesis in vivo and in vitro. *Comptes Rendus Hebdomadaires Des Seances De L Academie Des Sciences Serie D*, 284, 2515–2518.

- Vázquez-Islas, G., Garza-Torres, R., Guerrero-Tortolero, D. A., & Campos-Ramos, R. (2014). Histology of the androgenic gland and expression of the insulin-like androgenic gland hormone precursor gene in the genital organ of Pacific white shrimp *Litopenaeus vannamei*. *Journal of Crustacean Biology*, *34*, 293–299. doi:10.1163/1937240X-00002232.
- Ventura, T., & Sagi, A. (2012). The insulin-like androgenic gland hormone in crustaceans: From a single gene silencing to a wide array of sexual manipulation-based biotechnologies. *Biotechnology Advances*, *30*, 1543–1550. doi:10.1016/j.biotechadv.2012.04.008.
- Ventura, T., Manor, R., Aflalo, E. D., Weil, S., Raviv, S., Glazer, L., et al. (2009). Temporal silencing of an androgenic gland-specific insulin-like gene affecting phenotypical gender differences and spermatogenesis. *Endocrinology*, *150*, 1278–1286. doi:10.1210/en.2008-0906.
- Ventura, T., Manor, R., Aflalo, E. D., Weil, S., Rosen, O., & Sagi, A. (2012). Timing sexual differentiation: Full functional sex reversal achieved through silencing of a single insulin-like gene in the prawn. *Macrobrachium rosenbergii*. *Biology of Reproduction*, *86*, 6. doi:10.1095/biolreprod.111.097261.
- Vitousek, P. M., DAntonio, C. M., Loope, L. L., & Westbrooks, R. (1996). Biological invasions as global environmental change. *American Science*, *84*, 468–478.
- WHO. (2013a). *Schistosomiasis—facts sheet*. World health organization. Publ. Internet. <http://www.who.int/mediacentre/factsheets/fs115/en/>. Accessed Dec 30, 2015
- WHO. (2013b). Schistosomiasis: Progress report 2001–2011, Strategic Plan 2012–2020.
- Yusa, Y., Sugiura, N., & Wada, T. (2006). Predatory potential of freshwater animals on an invasive agricultural pest, the apple snail *Pomacea canaliculata* (Gastropoda: Ampullariidae), in southern Japan. *Biological Invasions*, *8*, 137–147. doi:10.1007/s10530-004-1790-4.