Identifying low risk insecticides to address both food shortages and the biocontrol of human schistosomiasis

Running head: Insecticides and schistosomiasis biocontrol

Christopher J E Haggerty*[a], Bryan Delius*[b], Nicolas Jouanard[c,d], Pape D Ndao[d,e], Giulio A De Leo[f], Andrea J Lund[g], David Lopez-Carr[h], Justin V Remais[i], Gilles Riveau[c,j], Susanne H Sokolow[k], and Jason R Rohr[a]

[a]Department of Biological Sciences, Environmental Change Initiative, Eck Institute of Global Health, University of Notre Dame, Notre Dame, IN, USA

[b]University of South Florida, Department of Integrative Biology, Tampa, FL, USA

[c]Centre de Recherche Biomédicale Espoir pour la Santé, Saint-Louis, Senegal

[d]Station d'Innovation Aquacole, Saint-Louis, Senegal

[e]Université Gaston Berger (UGB), route de Ngallèle, BP 234, Saint-Louis, Senegal

[f]Department of Biology, Hopkins Marine Station, Stanford University, Pacific Grove, CA, USA

[g]Emmett Interdisciplinary Program in Environment and Resources, Stanford University, Stanford, CA, USA

[h]Human-Environment Dynamics Lab, Department of Environmental Studies, UCSB, Santa Barbara, CA, USA

[i]Division of Environmental Health Sciences, School of Public Health, University of California, Berkeley, CA, United States of America

[j]Institut Pasteur de Lille - CIIL, France

[k]Woods Institute for the Environment, Stanford University, Stanford, CA, USA

*Corresponding authors: chaggert@nd.edu (CJEH) and bdelius@mail.usf.edu (BD)

Keywords: synthetic chemical, contamination, *Macrobrachium*, prawns, pyrethroid, organophosphate, pesticide

Article type: Primary research.

Abstract

Synthetic chemicals, such as pesticides, have increased faster than other agents of global change have, yet their ecological impacts remain understudied. Additionally, agricultural expansion to address human population growth and food shortages is predicted to increase the use of pesticides, some of which have been linked to increases in infectious diseases of humans, such as schistosomiasis, which infects >250 million people worldwide. Previous work revealed that ecologically relevant concentrations of organophosphate and pyrethroid insecticides are highly toxic to crayfish. Whether these same insecticides are also highly toxic to *Macrobrachium rosenbergii* and *M. vollenhovenii* prawns, which are closely related to crayfish and are important predators on snails that transmit schistosomiasis in Asia and Africa, respectively, is unknown. We performed laboratory dose-response studies for *M. rosenbergii* using three pyrethroid (esfenvalerate, λ-cyhalothrin, and permethrin) and three organophosphate (chlorpyrifos, malathion, and terbufos) insecticides. Pyrethroid LC_{50} values were consistently several orders of magnitude lower than for organophosphate insecticides. Pyrethroids also had a greater likelihood of field runoff at levels lethal to prawns. To corroborate these findings in natural settings, we experimentally tracked survival of individually caged *M. vollenhovenii* at 31 waterways in West Africa that varied widely in their insecticide use. Consistent with laboratory results, pyrethroid insecticide use in these villages was positively associated with *Macrobrachium* mortality when controlling for village-level and prawn-level attributes, including levels of organophosphate applications. Villages with the most pyrethroid use had lower prawn survival, despite using on average 20% less total insecticides than villages with high prawn survival. Our findings suggest that pyrethroid insecticides widely used in sub-Saharan Africa have strong non-target effects on *Macrobrachium* spp. prawns, with possible implications for human schistosomiasis. Thus, regulations or incentives to avoid high-risk insecticides, especially near waterways, could have important human health implications in countries undergoing agricultural expansion in schistosomiasis-endemic regions.

1. Introduction

The growing use of synthetic chemicals, including pesticides, meets all criteria for classification as a driver of global change according to the Millennium Ecosystem Assessment (Bernhardt, Rosi, & Gessner, 2017; MA, 2005). Moreover chemical use has outpaced other agents of global change, such as increasing atmospheric CO_2 and the loss of both habitat and biodiversity (Bernhardt et al., 2017). The use of pesticides is expected to increase by 2-5 fold, particularly in developing countries of Africa where the human population will double to > 2 billion by 2050 (Tilman, Balzer, Hill, & Befort, 2011; UN, 2020). Use of pesticides increased harvest value by approximately one-third across several sub-Saharan African countries (Sheahan, Barrett, & Goldvale, 2017). Most food production is by smallholder farmers (Salami, Kamara, & Brixiova, 2010), and spraying organophosphate or pyrethroid insecticides is the most common method of controlling insect damage to crops in this region (Atwood & Paisley-Jones, 2017). Organophosphates are a class of insecticides that act by inhibiting the enzyme acetylcholinesterase (Newman & Unger, 2003), whereas pyrethroids interfere with voltage-gated sodium channels (Soderlund & Bloomquist, 1989). Although application of both insecticide classes can help fight malnutrition by improving food production, pesticide pollution can have non-target ecological effects that can negatively impact public health in these same locations (Bertrand, 2019). Unfortunately, the ecological impacts of pesticides are far less studied than other agents of global change, and adverse effects of pesticides found under laboratory conditions remain largely unverified in the environment (Bernhardt et al., 2017). Importantly, pesticide use has recently been positively associated with infectious diseases of humans (Rohr et al., 2019), including human schistosomiasis that is transmitted by parasites released from freshwater snails.

Two genera of freshwater snails in Africa, *Bulinus* and *Biomphalaria*, are responsible for transmitting human schistosomiasis, a disease that infects more than 250 million people worldwide, nearly 200 million in sub-Saharan Africa, and causes approximately 200,000 deaths in Africa annually (Adenowo, Oyinloye, Ogunyinka, & Kappo, 2015; Vos et al., 2016). While many countries are making progress towards elimination of schistosomiasis, disease control in Africa has been hampered by limited access to clean water and sanitation (Grimes et al., 2014). In many African countries, collecting water for drinking and washing household items at local, and sometimes polluted, lakes and rivers is a daily part of life. Excreta (urine or feces) of humans with schistosome eggs enter freshwaters by hygienic washing or urination when swimming (Coulibaly et al., 2013). Such contamination infects intermediate host snails that subsequently produce thousands of free swimming parasites into the water each day for up to a year (Mutuku et al., 2014). Free swimming parasites penetrate human skin when people enter the water, and thus disease control is very difficult when intermediate snail hosts occur in contaminated waters (King & Bertsch, 2015). Schistosomiasis is especially prevalent in rural areas where agricultural expansion has occurred (Rohr et al., 2019), suggesting that agrochemical pollution of waterways might be one important factor contributing to disease risk. Recent experimental evidence suggests that insecticide runoff into aquatic systems can foster snails, and schistosomiasis transmission risk, by killing important snail predators (Halstead et al., 2018).

Widespread loss of wild prawns that eat snails is associated with increased schistosomiasis prevalence, which is problematic given that half the global human population at risk of schistosomiasis live in regions where prawns are native (Sokolow et al., 2017). The river prawns *M. vollenhovenii* and *M. rosenbergii*, native to Africa and the Indo-Pacific region, respectively, are both key invertebrate predators of snails that transmit schistosomiasis (Sokolow, Lafferty, & Kuris, 2014), and may represent an important nexus between insecticide use and human schistosomiasis prevalence (Hoover et al., 2019). While *M. rosenbergii* is not native to Africa, it is physiologically very similar to native *M. vollenhovenii* (FAO, 2012) and has already been successfully introduced to parts of Africa within aquaculture facilities (New & Valenti, 2000). Monosex *M. rosenbergii* that are unable to interbreed with *M. vollenhovenii* (Savaya-Alkalay et al., 2018) are being considered as biological control agents for schistosomiasis in Africa (Levy et al., 2019). Top-down effects of invertebrate snail predators have also been negatively associated with snail parasite production (Haggerty et al., 2020).

Thus, interventions that release prawns at local water points might benefit public health (Hoover et al., 2019). Recent experimental work has demonstrated that environmentally common insecticide concentrations reduce survival of invertebrate snail predators, including the crayfish *Procambarus alleni* (Halstead, Civitello, & Rohr, 2015) and *M. lar* from the Philippines (Bajet, Kumar, Calingacion, & Narvacan, 2012). *P. alleni* is a crayfish that is ecologically and morphologically similar to *Macrobrachium*, having two chelae or claws used for foraging on plants or animals, and that was previously used in several mesocosm experiments examining the effects of insecticides on snails and *Schistosoma* parasites (Halstead et al., 2015; Halstead et al., 2018). However, it is unclear whether insecticides used by rural communities in developing countries are reducing the survival of the two *Macrobrachium* species that are among the most important biological control agents of human schistosomiasis.

This study aimed to address the above knowledge gaps by using a laboratory LC_{50} study to determine the relationship between concentrations of six insecticides, three organophosphates and three pyrethroids, and survival of *M. rosenbergii*. We then performed a field study using caged *M. vollenhovenii* placed into 31 waterways in Senegal, Africa that varied in organophosphate and pyrethroid applications in their surrounding landscape. Based on previous work using similar invertebrate snail predators and insecticides (Bajet et al., 2012; Halstead et al., 2015), we hypothesized that both insecticide classes would lower *Macrobrachium* survival relative to controls, but that pyrethroids would be associated with greater mortality than organophosphates.

2. Material and Methods

2.1 Lab Study

2.1.1. Experimental Design

To perform a dose-response study of the six insecticides, we procured juvenile *M. rosenbergii* (25 – 40 mm) from a commercial supplier (Aquaculture of Texas, Inc., Weatherford, TX, USA). During the experiment, prawns were maintained individually in artificial spring water (ASW) (Table S1; (Cohen & Neimark, 1980) in the laboratory at a pH of 7.7, temperature of 23.5 °C, and 3.3 mg/L oxygen.

Three organophosphate (chlorpyrifos, malathion, and terbufos) and three pyrethroid insecticides (esfenvalerate, λ-cyhalothrin, and permethrin) were selected for this study. Technical grade insecticides were used for all trials (purity >98%; Chemservice, West Chester, PA, USA). To select experimental concentrations, we determined the likely concentration of runoff generated from applying these insecticides on crops in accordance with instructions on the product label (Table S2). We used corn exposure scenarios on insecticide labels as inputs to generate 150 simulated annual peak estimated environmental concentrations (EECs) for these insecticides using United States Environmental Protection Agency (US EPA) software, all as described in Halstead et al. (2015). Corn application rates were used to reduce variation in application that would exist had we used application rates of different crops and because label rates for corn were available for all six insecticides. A range of experimental concentrations for each insecticide was selected that spanned the EECs as well as known LC_{50} values of related species for these or related insecticides (Table S3).

Our LC50 experiment used a static, nonrenewal (no water changes) dose-response design for this study with five concentrations of each insecticide, in addition to a solvent control (12 mL/L acetone). We used five replicates of each insecticide concentration and 10 replicates of the control and conducted trials in March 2017. Each replicate consisted of a single *M. rosenbergii* in a 500 mL glass jar, filled with 400 mL of ASW, and capped with a screen. Each individual was fed 0.16 g of shrimp pellets (Cobalt International, South Carolina, USA) *ad libitum*. Survival was assessed 3, 12, and 24 hours after insecticide application, and daily thereafter for 10 days.

2.1.2. Data Analysis

We used the *drc* package (Ritz, Baty, Streibig, & Gerhard, 2015) in *R* statistical software (RCoreTeam, 2018) to generate dose-response curves and estimate LC_{50} values. Two-parameter logistic models were used to estimate 96-h and 10-d LC_{50} values, and we approximated 95% confidence intervals around each LC_{50} value using the variance of the estimate and then back-transforming from the log scale used for concentrations (Ritz et al., 2015). We then determined the proportion of the simulated EEC values that exceeded the US EPA's level of concern (0.5 x LC_{50}) for each LC_{50} estimate (USEPA, 2020).

To determine if differences in prawn survival was most associated with either individual chemicals or chemical class, we performed a Cox mixed effects model using package s*urvival* (Therneau, 2020). We converted all concentrations to toxic units (TUs) using SPEAR Calculator software (v0.8.1, Department System Ecotoxicology – Helmoltz Center for Environmental Research, 2014) to account for variation in absolute toxicity among chemicals. Standardized chemical concentration was used as a continuous fixed effect in the model, and random intercepts for each chemical were nested within their respective chemical classes (pyrethroid or organophosphate). Coefficients of the random effects were used to determine the contribution of each chemical and chemical class to overall mortality risk.

2.2. Field Study

2.2.1. Study area and village selection

Our field study took place at 31 water points across 16 villages in Northern Senegal, a schistosomiasis hyper-endemic region experiencing rapid agricultural expansion. All of our sites were located along the Senegal and Lampsar Rivers and the shore of Lac de Guiers (16°15′N 15°50′W). Our study region was once populated by *M. vollenhovenii* before the construction of the Diama dam that prevented prawn breeding migrations to estuaries, which led to the loss of prawns upstream of the dam (Savaya Alkalay et al., 2014). Shortly after the dam was constructed, and its associated environmental changes materialized, schistosomiasis infection increased and lead to perennially high infection levels (Steinmann, Keiser, Bos, Tanner, & Utzinger, 2006; I. Talla, Kongs, & Verlé, 1992; Idrissa Talla et al., 1990).

2.2.2. Insecticide use

We conducted a survey of 663 households at the 16 study villages in 2016 to collect data on the area of crop land where different types of insecticides were used. A respondent from each household was asked to report the area of cultivated land they controlled as well as their use of insecticide on their land. We then calculated the total area on which each class of insecticide was applied in each village. Household surveys were approved by Internal Review Board of the University of California, Santa Barbara (Protocol # 19-170676) and in Senegal by the National Committee of Ethics for Health Research from the Republic of Senegal (Protocol #SEN14/33).

2.2.3. Prawn survival

To investigate prawn survival, we captured wild *M. vollenhovenii* downstream of the Diama dam and temporarily housed them in an outdoor freshwater pond located nearby in St. Louis, Senegal. A subset of mature prawns were collected from the holding pond and transported to experimental cage enclosures at village water points. The average weight of prawns used in the experiment (± 1 SD) was 23.3 g (±9.2). We released adult *M. vollenhovenii* individually into small known-fate enclosures made of a fishing-net overlaid upon a metal frame that was approximately 30 x 30 x 60 cm in size. The cages were not baited and thus prawns were allowed to forage upon prey that naturally entered the cage over time. We deployed one cage for each water access point of each village (n = 31 water access points). The status of prawns in each cage was checked daily by a local villager who was compensated for their effort. Prawns that died were replaced as needed at the end of each month from the start of the study in March until the end in October 2019 (8 months). Before each prawn was placed in a cage, we recorded its mass (g), sex, and number of claws. We also recorded the water temperature (°C) at the cage during the prawn release.

We flew a drone with a 12.4 MP camera above each water access point in July 2019 to estimate aquatic vegetation cover because it is very dense in our study region and might intercept insecticide runoff or change abiotic conditions near prawn cages. The drone flew at an altitude of approximately 150 m above the prawn cages, and travelled in all four cardinal directions from those locations, capturing images every 5 seconds to a distance of 300 m from the water access point. All images for each village were aligned using Agisoft Photoscan Professional to create both an orthomosaic and a digital elevation model (DEM). We used the orthomosaic (16 cm/pix resolution) in QGIS 3.2 to estimate the amount of emergent vegetation within a 100-m buffer around each prawn cage. To characterize site topography, we used the DEM (6 m/pix resolution) in QGIS 3.2 to estimate percent slope from each prawn cage to the nearest planted field. Finally, we took the average values of each predictor per village because insecticide use was only available at the village-level. To compare abiotic conditions of sites descriptively in terms of their suitability for prawns, we also visited each waterway in July 2019 to record average salinity and dissolved oxygen values at each village using a YSI Pro multimeter.

2.2.4. Data analyses

All statistical analyses were conducted with *R* statistical software (RCoreTeam, 2018). To predict prawn survival, we performed a Cox proportional hazards regression analysis using the *survival* package in *R* and including a single survival time value for each prawn (n = 225 total prawns). One assumption of the Cox model is that risk is consistent throughout the study. However, that assumption is unlikely to be true in this system because insecticide exposure will temporally vary with rainfall or application times. In addition, exploratory data analyses showed that prawn survival was dependent upon month. Thus, a stratified Cox model was fit by including a strata term for month, which fits separate baseline hazard functions for each month. Beta coefficients (and associated hazard ratios) optimized for all strata are then fitted. In a stratified Cox model, it is not possible to test for differences among levels of the strata term (here month). The term *+cluster(village)* was included to account for clusters of correlated observations at the village-level and produce robust estimates (standard errors adjusted for the non-independence) using the grouped jackknife method. We fit an initial global using all village and prawn-level predictors mentioned above, and present parameter estimates for both this model and a a final model were reached by sequentially dropping the least significant predictor until all terms were significant.

3. Results

3.1. Lab Study

We found that LC_{50} values of pyrethroid insecticides were generally an order of magnitude lower (greater toxicity) than LC_{50} values of organophosphate insecticides (Table 1). Overall, the LC_{50} (95% CI) of the most toxic pyrethroid and organophosphate were 0.25 µg/L (0.07 - 0.43) for esfenvalerate and 16.73 µg/L (7.86 - 25.60) for chlorpyrifos, respectively (Table 1). Greater toxicity of pyrethroid than organophosphate insecticides for *M. rosenbergii* in our laboratory experiment is consistent with data from the US EPA's Ecotox database for other *Macrobrachium* species (Table 1, Table S3).

We found that EEC values of each pyrethroid tested commonly exceeded the EPA's level of concern, defined as half the LC_{50} value (Fig. 1). In contrast, we found that organophosphates rarely exceeded the EPA's level of concern for *M. rosenbergii* (Table 1). Among organophosphates, only chlorpyrifos generated EEC simulations that exceeded the EPA's level of concern, which spanned only three percent of the simulations (Table 1). For the three pyrethroids, 17-81% of the exposure simulations exceeded the EPA's level of concern (Table 1). Thus, pyrethroids had a consistently greater chance of exceeding levels of concern than organophosphates (Fig1; Table 1).

A Cox mixed-effects survival model indicated that insecticide class accounted for 70.7% of the variance in prawn mortality. After 96 hours of exposure, the three most deadly insecticides (i.e., highest hazard ratios or risk per μg/l) were the two pyrethroids esfenvalerate and λ-cyhalothrin, and the organophosphate chlorpyrifos (Table 2). Converting all concentrations to toxic units (TUs) using the SPEAR Calculator software revealed that, on average, pyrethroid insecticides led to 275% more mortality than organophosphates (Table S4). The coefficients of random effects suggest that variation among individual insecticides within the organophosphate class was largely driven by the lack of risk presented by malathion (Table 1 and 2).

Table 1. LC_{50} (μg/L) values for *M. rosenbergii* after 96-h or 10-d exposure to multiple concentrations of three pyrethroid (esfenvalerate, λ-cyhalothrin, and permethrin) and three organophosphate (chlorpyrifos, malathion, and terbufos) insecticides. The second column for each endpoint reports the proportion out of 150 annual peak estimated environmental

concentrations (EEC) calculated from the US EPA Pesticide in Water Calculator (v.1.52) that exceeded the US EPA level of concern defined as one-half the estimated LC_{50}.

Chemical class	Chemical	96-h endpoint		10-d endpoint	
		LC_{50} (95% C.I.)	EEC > 0.5 x LC_{50}	LC_{50} (95% C.I.)	EEC > 0.5 x LC_{50}
Pyrethroid	Esfenvalerate	0.49 (0.12 - 0.86)	0.71	0.49 (0.12 - 0.86)	0.71
Pyrethroid	λ-cyhalothrin	0.97 (0.55 - 1.39)	0.81	0.97 (0.55 - 1.39)	0.81
Pyrethroid	Permethrin	5.21 (1.31 - 9.12)	0.17	5.21 (1.30 - 9.12)	0.17
Organophosphate	Chlorpyrifos	132.70 (18.12 - 47.28)	0.06	22.40 (11.50 - 33.31)	0.03
Organophosphate	Malathion	4238.6 (1999.60 – 6477.60)	0.00	4238.60 (1999.6 - 6477.6)	0.00
Organophosphate	Terbufos	197.40 (61.52 - 333.28)	0.00	114.07 (51.28 - 176.85)	0.00

Figure 1. Dose-response curves for *Macrobrachium rosenbergii* after 96-h of exposure to three pyrethroid (a-c) and three organophosphate (d-f) insecticides. The horizontal bar represents the 95% confidence interval around the LC_{50} estimate, with the estimate itself at the point where the confidence interval intersects the curve. The shaded areas represent concentrations above the US EPA's level of concern of 0.5 x LC_{50} (medium gray) for acute high risk to aquatic organisms. The light gray and dark gray regions represent the area of concern calculated from the lower and upper 95% confidence limits of the LC_{50} estimate, respectively. The dashed curves give the kernel density estimates from 150 simulated annual peak environmental concentrations (EECs) in ponds determined from the US EPA Surface Water Calculator (SWCC) for each insecticide. Thus, those portions of the curve within the shaded areas of each plot indicate simulated peak EECs above the US EPA's level of concern. The open and black triangles along the x-axes indicate the median and maximum EECs, respectively, from the SWCC simulations.

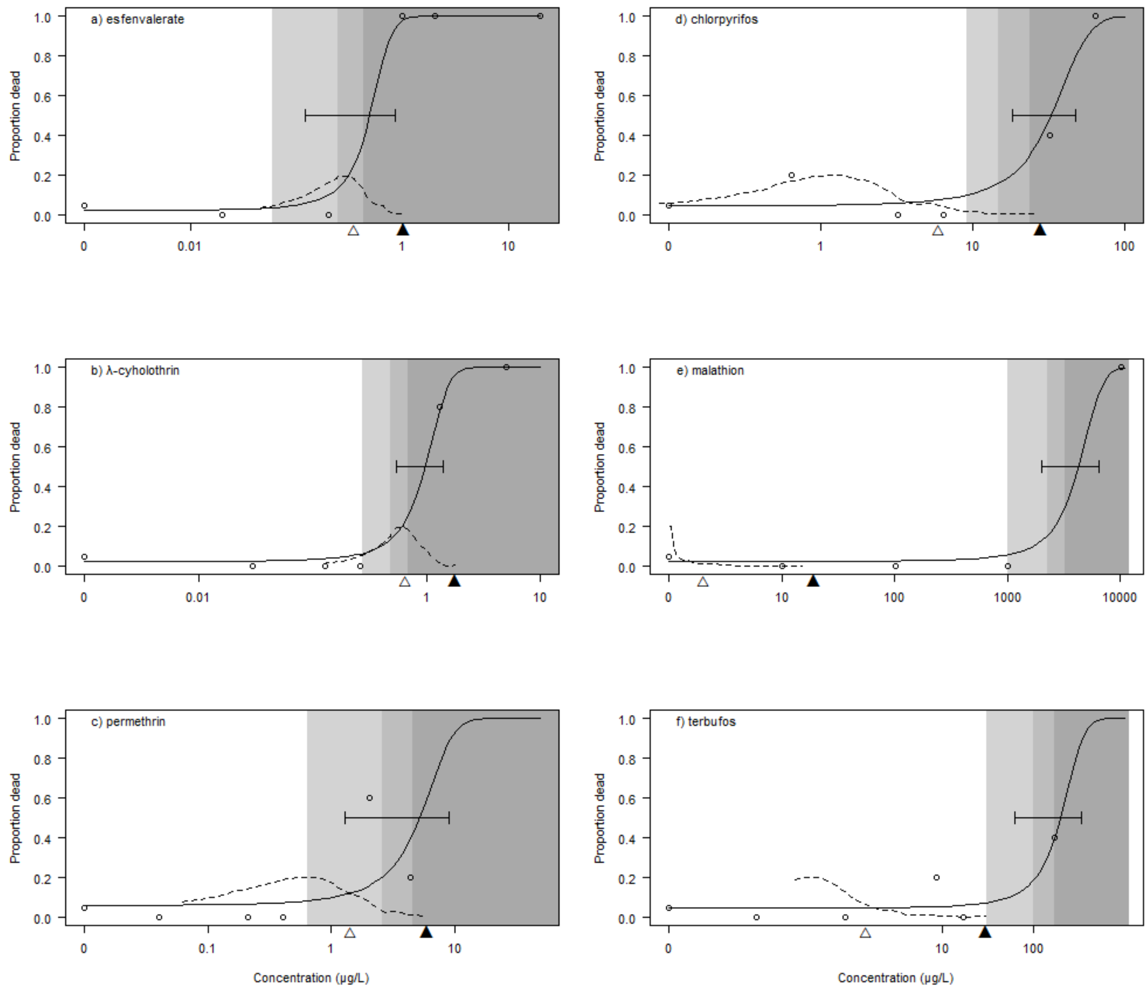

Table 2. Cox survival analysis for *Macrobrachium rosenbergii* exposed to multiple concentrations of three pyrethroid (esfenvalerate, λ-cyhalothrin, and permethrin) and three organophosphate (chlorpyrifos, malathion, and terbufos) insecticides for 10 days. Positive coefficients (coef) indicate that the probability of prawn mortality during the study increased with chemical exposure. The hazard ratio is the exponent of the coefficient and indicates the probability of an increase in mortality for every 1 µg/L increase in concentration. For example, the hazard ratio of 1.051 for esfenvalerate indicates that every 1 µg/L increase in esfenvalerate

increases the probability of mortality during the study increases by 5.1%. The 95% confidence intervals are provided for the hazard ratio.

Chemical	Z	*p*	coef	SE	Hazard Ratio	95% CI
Esfenvalerate	6.62	<0.001	4.98E-02	7.09E-03	1.051	1.037 - 1.065
λ-cyhalothrin	6.69	<0.001	6.27E-01	9.37E-02	1.872	1.688 - 2.056
Permethrin	1.80	0.072	2.93E-01	1.63E-01	1.340	1.021 - 1.660
Chlorpyrifos	7.02	<0.001	1.56E-01	2.36E-02	1.169	1.123 - 1.215
Malathion	6.42	<0.001	3.05E-05	4.74E-06	1.000	1.000 - 1.000
Terbufos	4.60	<0.001	1.46E-02	3.18E-03	1.015	1.008 - 1.020

3.2. Field Study

We documented a total of 1,515 ha of agricultural fields using our social survey in the 16 villages we sampled in Senegal (average Table 3). Insecticides were applied to 60% of the total planted field area and there was an average of 47.8 ha of insecticide application per village. The organophosphate dimethoate and the pyrethroid deltamethrin together made up 78% of the total area where insecticides were applied. Each village received an average of 14.1 prawns (± 1.4 SE) during the study, dependent upon number of water access points.

Given that LC_{50} values for pyrethroids were one to two orders of magnitude lower than LC_{50} values for organophosphates and the laboratory hazard ratios suggested that pyrethroids were generally more toxic in nature than organophosphates, we hypothesized that pyrethroid use would be more positively associated with prawn mortality in Senegalese waterbodies than organophosphate use. As predicted, when accounting for significant covariates in the final model (Table S5), *M. vollenhovenii* mortality was positively associated with total pyrethroid applications (ha) (Fig. 2a), but was not significantly related to organophosphate applications (Table 3). In villages with pyrethroid use, prawn survival decreased rapidly in the first few days after prawn release, consistent with the 96-h results of the LC_{50} trials.

Prawn mortality was also associated with several covariates. For example, prawn mortality was positively associated with water point temperature at release (Table 3; Fig. 2b; mean water temperature: 28.2° C, range: 20 - 32.6° C), and the average amount of emergent vegetation within 100 m of cages (Fig. 2d). Male prawns experienced significantly higher mortality than female prawns (Table 3; Fig. 2c). Finally, prawn mortality was negatively associated with dissolved oxygen (Fig. 2e), whereas mortality was positively associated with salinity (Fig. 2f).

Table 3 Average values and cox model results for prawn field study prior to model selection (Wald tests substituted for LR tests to provide robust variances).

Experimental level	Predictor	*Average (± 1 SE)*	*z-statistic*	*Robust SE*	*p-val*
Village	Total organophosphate use (ha)	32.3 (7.5)	1.174	0.010	0.
Village	**Total pyrethroid use (ha)**	5.2 (1.3)	**2.724**	**0.033**	**0.**
Prawn	**Water point temperature (°C)**	28.2 (0.5)	**2.479**	**0.103**	**0.**
Prawn	**Sex of the prawn (male)**		**2.017**	**0.206**	**0.**
Prawn	Weight of the prawn (g)	23.3 (0.6)	0.145	0.006	0.
Prawn	Number of prawn claws	1.4 (0.1)	-0.513	0.080	0.
Village	Average percent slope cage to nearest field	4.1 (0.6)	-0.442	4.844	0.
Village	Average distance nearest field to water (m)	18.1 (2.2)	-0.432	0.033	0.
Village	Average distance cage to nearest field (m)	4.1 (0.6)	-0.317	0.004	0.
Village	**Average emergent vegetation in 100m**	11,150.6 (898.3)	**3.161**	**0.000**	**0.**
Village	**Average dissolved oxygen (ppm)**	0.5 (0.5)	**-2.130**	**0.151**	**0.**
Village	Average salinity (ppt)	0.1 (0.0)	1.404	7.455	0.

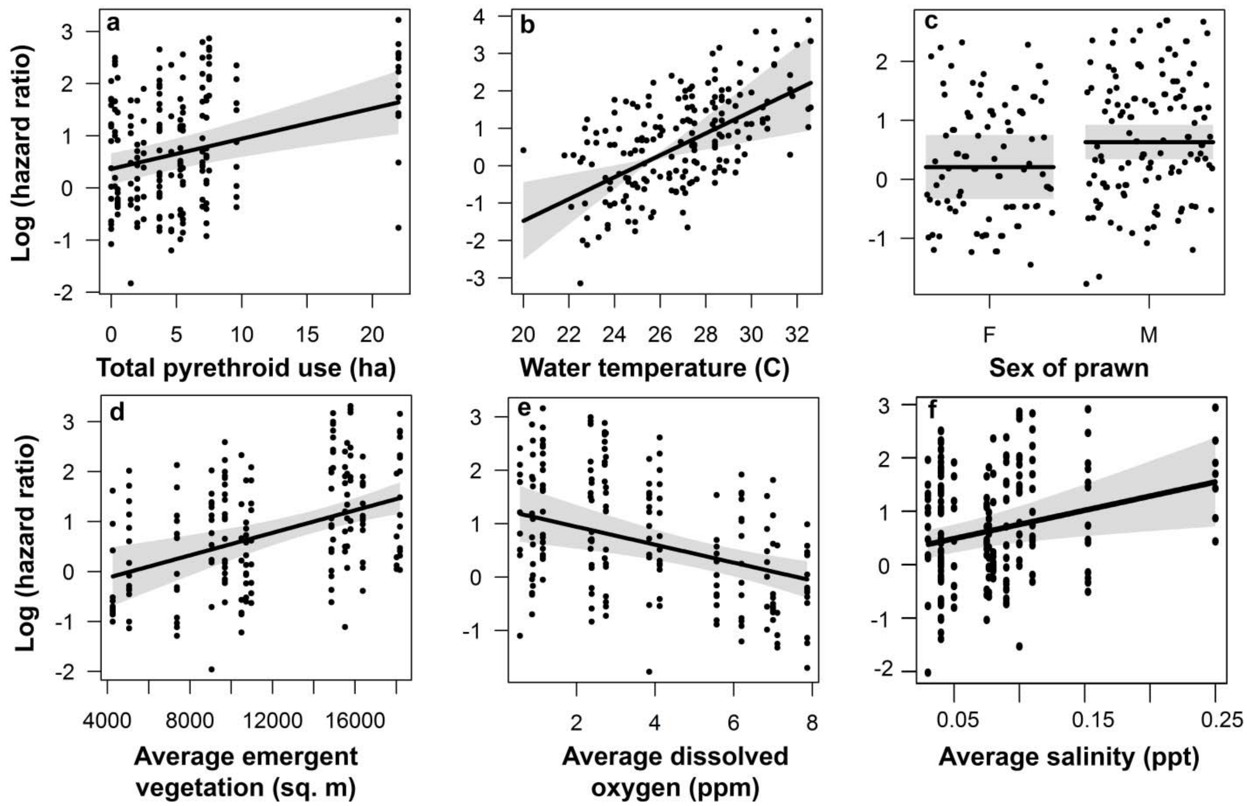

Fig. 2. Partial residual plot of the final Cox model (after model selection) showing the association between prawn mortality (log(hazard ratio)) and pyrethroid use (a), water temperature (b), sex of the prawn (c), average emergent vegetation with 100m of the prawn cage (d), average dissolved oxygen (e), and average salinity (f). Plots were generated in the R package *visreg*.

4. Discussion

Synthetic chemicals are important agents of global change, but adverse effects of pesticides observed in laboratory studies are rarely verified in the wild (Bernhardt et al., 2017). Our laboratory and field experiments support the hypothesis that pyrethroid insecticides pose a high mortality risk for two *Macrobrachium* species, which might increase their snail prey, and ultimately, global schistosomiasis infections because millions of people at risk of schistosomiasis live in areas with native prawns (Sokolow et al., 2017). We found that *M. rosenbergii* LC_{50} values for three pyrethroids were consistently lower by one or more orders of magnitude than organophosphate insecticides, in agreement with previous laboratory studies of other invertebrate snail predators (Bajet et al., 2012; Halstead et al., 2015). Importantly, expected concentrations of pyrethroid insecticides in waterbodies are more likely to exceed LC_{50} values of *Macrobrachium* prawns than expected field concentrations of organophosphate insecticides. Thus, in nature, pyrethroid use may be more deadly to prawns than organophosphate use. We corroborated these laboratory findings in a natural setting by documenting that caged *M. vollenhovenii* survival at water points was best predicted by reported pyrethroid rather than organophosphate applications on crop fields near these water points. Thus, prawn survival was negatively associated with pyrethroid and not organophosphate use, despite our study sites actually averaging more total organophosphate than pyrethroid insecticide applications reported by households living nearby. Our findings suggest that the impact of the predicted rise in insecticide use associated with human population growth (Tilman et al., 2011), could depend upon which insecticides are adopted. If pyrethroids are heavily used, this might affect schistosomiasis by reducing survival of an important native predator of the snail intermediate hosts in coastal Africa, where the vast majority of global schistosomiasis cases occur (Steinmann et al., 2006).

Introducing *Macrobrachium* prawns into waterways has recently been proposed as a public health intervention in our study region (Sokolow et al., 2017), and identifying abiotic factors that affect prawn survival in the wild will be very important to the success of such interventions. Consistent with previous studies (Cheng, Liu, & Kuo, 2003), we found that oxygen was a strong positive determinant of *Macrobrachium* survival. Prawn mortality increases quickly below 2 mg/L oxygen (Ferreira, Bonetti, & Seiffert, 2011), and approximately 25% of our field measurements were below this threshold. Temperature increases the metabolic rate of ectotherms, with optimal temperatures being approximately 30°C for both *M. rosenbergii* and *M. vollenhovenii* (Akinwunmi, Bello Olusoji, & Sodamola, 2014; New, 1995). Prawns in our experiment were likely acclimated to ambient temperatures in our outdoor growing ponds but could not migrate to more favorable temperatures in waterways once placed in experimental cages. Summer water temperatures in the Senegal River Basin can reach 32°C (Sane, Ngansoumana, Arfi, Samb, & Noba, 2017), with air temperatures reaching 40°C (Cheikh, Moctar, & Raymond, 2013). Given that each degree Celsius rise in temperatures increases oxygen demand by increasing prawn metabolism (Manush, Pal, Chatterjee, Das, & Mukherjee, 2004; Xi-lin et al., 1999), water temperatures at release sites may have increased prawn mortality by raising oxygen demands.

Prawn- and village-level characteristics may also influence prawn responses to abiotic conditions in Senegalese waterways. Male *M. vollenhovenii* could be more sensitive to oxygen because they reach a larger size (Olele & Kalayolo, 2012), and body size in crayfish determines oxygen consumption (Armitage & Wall, 1982). Unlike temperature, salinity does not impact oxygen stress in *M. rosenbergii* (Ern, Huong, Nguyen, Wang, & Bayley, 2013). All salinity values that we observed were freshwater (<0.5 ppt) and suitable for adult prawns (New, 1995). However, salinity is strongly associated with dissolved ions or conductivity, the latter of which is an indicator of agricultural runoff (Harwell, Surratt, Barone, & Aumen, 2008). Eutrophication associated with nutrients in runoff can increase the chances of hypoxia at sites (Dodds & Whiles, 2019) and eutrophication from nutrients has been observed in our study system (Cogels, Frabouiet-Jussiia, & Varis, 2001). Fertilizers, which are used at all 16 villages, are also positively associated with invasive macrophytes, such as *Typha* spp., that were the most common emergent aquatic plant in our study and are distributed worldwide (Bansal et al., 2019). Emergent plants such as *Typha* spp. can shade waterways, limiting photosynthesis by algae or submerged plants that produce oxygen. *Typha* spp. can also create leaf litter that lowers dissolved oxygen as it decays (Bunch, Allen, & Gwinn, 2010, 2015). Although no previous study has, to our knowledge, examined prawns in relation to emergent plants, *Typha* spp. invasion can lead to aquatic communities dominated by hypoxic-tolerant species (Schrank & Lishawa, 2019). Crayfish, which are phylogenetically and functionally similar to prawns, will feed on a variety of aquatic plants but do not readily consume *Typha* (Bolser, Hay, Lindquist, Fenical, & Wilson, 1998). This might suggest that *Typha* may also provide little direct benefit to *Macrobrachium*. Together, these findings suggest that prawn- and site-level factors can influence prawn mortality that, in turn, can have important impacts on population densities of intermediate host snails of human schistosomiasis.

Extrapolating hazards among insecticide classes from laboratory to field settings is key to understanding the effects that different insecticide classes might have on *Macrobrachium* biocontrol of schistosomiasis. Our laboratory *M. rosenbergii* LC_{50} values for three organophosphates were generally within the 95% CIs of the LC_{50}'s reported for *P. alleni* from Halstead et al. (2015). Similar to Halstead et al. (2015), we found that the two insecticides with the lowest hazard ratios were the organophosphates malathion and terbufos, whereas chlorpyrifos posed a higher risk among the organophosphates. Additionally, previous laboratory studies support our finding of greater toxicity of pyrethroid than organophosphate insecticides to crayfish and *Macrobrachium* prawns (Bajet et al., 2012; Halstead et al., 2015; Halstead et al., 2018). Pyrethroids, including deltamethrine, the most common pyrethroid reported in our household surveys in Senegal, had such a high toxicity to *M. lar* in the Philippines that pyrethroid environmental concentrations actually exceeded LC_{50} values in the laboratory (Bajet et al., 2012). Environmental exposure simulated in our study, using EPA software showed patterns consistent within insecticide class and with previous studies (Halstead et al., 2015). However, we are the first to provide evidence from nature supporting all of these laboratory findings.

The loss of river prawns in the Senegal River Delta following agricultural projects that coincided with disease outbreaks (Sokolow et al., 2017; Steinmann et al., 2006) emphasizes the need to identify low-risk insecticides for increasing crop yields without harming native prawns. Successfully re-introducing *Macrobrachium* prawns for biocontrol of schistosomiasis will require identifying low-risk insecticides in endemic and developing regions undergoing agricultural expansion. Among organophosphates, we found that malathion has a particularly low toxicity to prawns, consistent with experiments using the prawn species *M. lar* (Bajet et al., 2012) and the crayfish *P. alleni* (Halstead et al., 2015). As the *M. rosenbergii* used in our laboratory study were commercially bred for human consumption in a hatchery, they had no known previous exposure to insecticides in their familial history, which strongly suggests that the displayed resistance by *Macrobrachium* prawns to malathion is innate. Thus, our results suggest that malathion may be a particularly useful insecticide to protect crops from pests without increasing the spread of human schistosomiasis. Although the pyrethroids we tested were generally more toxic than organophosphates, the pyrethroid permethrin had a lower chance of reaching EPA levels of concern ($EEC > 0.5 \times LC_{50}$) than λ-cyhalothrin or esfenvalerate pyrethroids. Permethrin also has the lowest desorption rate among pyrethroids we examined

(Fojut & Young, 2011), which could be important for lowering its bioavailability in agricultural regions where organic carbon levels are low (Fojut & Young, 2011).

Our study has several limitations that could influence our understanding of insecticide effects on prawn biocontrol agents in field settings. We did not have spatial information on the agricultural area reported in our social surveys. Thus, we assumed that villages with more fields also have more fields near their water access points. However, some fields reported in our surveys were likely too distant generate runoff into waterways. In this case, village-level survey data might not capture the agricultural runoff that occurs at prawn cages as accurately as had we known where each field location in relation to the cages. An additional caveat to our field study is that we did not have insecticide application rate data, and, thus, we assumed that chemical application rates were comparable among the study villages. Quantifying chemical concentrations in waterways each month could have potentially addressed both of the above limitations but is also logically challenging and costly. Future studies that can address the limitations of our field study may be able to further improve our understanding of insecticide risks.

In conclusion, our findings suggest that levels of different insecticide classes used by rural subsistence farmers near waterways may adversely affect the biocontrol of schistosomiasis. Importantly, previous mesocosm and modeling studies demonstrated that loss of snail predators arising from insecticide toxicity can increase snail densities and the risk of snail-transmitted disease (Halstead et al., 2018; Rohr et al., 2008). Thus, our findings offer one potential explanation for the positive links between schistosomiasis transmission and agricultural expansion and can help inform future *Macrobrachium* prawn introductions to control snails. Our findings further suggest that prawn natural recolonization in aquatic systems may be hampered by insecticide runoff. While insecticides will remain essential in developing countries (Snyder, Smart, Goeb, & Tschirley, 2015), educating farmers about the risks of particular insecticides (particularly pyrethroids) for native fauna may be warranted. Future studies are needed to examine the effects of farmers' switching from pyrethroids to alternative insecticides with fewer impacts on crustacean predators of snails, such as malathion. Careful choice of insecticides may be needed to reduce crop pests without increasing the risk of disease in areas endemic for schistosomiasis.

Author contributions

CJEH, BD, NJ, and JRR conceived and designed the experiment. PDN, NJ, and GR lead prawn cultivation in Senegal and organized the field project. AJL and DLC conducted village surveys of insecticide use. CJEH and BD wrote the initial manuscript draft of the field and lab experiment, respectively, and all authors contributed to the preparation of the manuscript.

Acknowledgements

We are grateful for the staff of Espoir La santé for their cooperation in the field aspects of the project, and to Amit Savaia of Ben-Gurion University for assistance with prawn transport and cage methodological advise.

Funding

The household survey data were collected with support from the National Science Foundation Coupled Natural Human Systems program (grant # 1414102). AJL was supported by a Davis Family E-IPER Fellowship and a James and Nancy Kelso Fellowship from the Stanford Interdisciplinary Graduate Fellowship program.

Declaration of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Adenowo, A. F., Oyinloye, B. E., Ogunyinka, B. I., & Kappo, A. P. (2015). Impact of human schistosomiasis in sub-Saharan Africa. Braz J Infect Dis, 19(2), 196-205. doi:10.1016/j.bjid.2014.11.004

Akinwunmi, M. F., Bello Olusoji, O. A., & Sodamola, M. Y. (2014). The rearing of African river prawn, Macrobrachium vollenhovenii in concrete tank using locally formulated diet. International Journal of Fisheries and Aquatic Studies, 2(2), 265-270.

Armitage, K. B., & Wall, T. J. (1982). The effects of body size, starvation and temperature acclimation on oxygen consumption of the crayfish Orconectes nais. Comparative Biochemistry and Physiology Part A: Physiology, 73(1), 63-68. doi:https://doi.org/10.1016/0300-9629(82)90092-5

Atwood, D., & Paisley-Jones, C. (2017). Pesticides industry sales and usage: 2008–2012 Market Estimates. Retrieved from Washington, DC:

Bajet, C. M., Kumar, A., Calingacion, M. N., & Narvacan, T. C. (2012). Toxicological assessment of pesticides used in the Pagsanjan-Lumban catchment to selected non-target aquatic organisms in Laguna Lake, Philippines. Agricultural Water Management, 106, 42-49. doi:https://doi.org/10.1016/j.agwat.2012.01.009

Bansal, S., Lishawa, S. C., Newman, S., Tangen, B. A., Wilcox, D., Albert, D., . . . Windham-Myers, L. (2019). Typha (Cattail) Invasion in North American Wetlands: Biology, Regional Problems, Impacts, Ecosystem Services, and Management. Wetlands, 39(4), 645-684. doi:10.1007/s13157-019-01174-7

Bernhardt, E. S., Rosi, E. J., & Gessner, M. O. (2017). Synthetic chemicals as agents of global change. Frontiers in Ecology and the Environment, 15(2), 84-90. doi:https://doi.org/10.1002/fee.1450

Bertrand, P. G. (2019). Uses and Misuses of Agricultural Pesticides in Africa: Neglected Public Health Threats for Workers and Population. In M. L. Larramendy (Ed.), Pesticides use and misuse and their impact in the environment. London, UK: IntechOpen.
Bolser, R. C., Hay, M. E., Lindquist, N., Fenical, W., & Wilson, D. (1998). Chemical defenses of freshwater macropytes against crayfish herbivory. Chemical Ecology, 24(10), 1639-1658.
Bunch, A. J., Allen, M. S., & Gwinn, D. (2010). Spatial and temporal hypoxia dynamics in dense emergent macrophytes in a Florida lake. Wetlands, 30(3), 429-435.
Bunch, A. J., Allen, M. S., & Gwinn, D. (2015). Influence of macrophyte-induced hypoxia on fish communities in lakes with altered hydrology. Lake and reservoir managment, 31, 11-19.
Cheikh, B. G., Moctar, D., & Raymond, M. (2013). Assessing the impacts of climate change on water resources of a West African trans-boundary river basin and its environmental consequences (Senegal River Basin). Sciences in Cold and Arid Regions, 5(1), 140. doi:10.3724/SP.J.1226.2013.00140
Cheng, W., Liu, C.-H., & Kuo, C.-M. (2003). Effects of dissolved oxygen on hemolymph parameters of freshwater giant prawn, Macrobrachium rosenbergii (de Man). Aquaculture, 220(1), 843-856. doi:https://doi.org/10.1016/S0044-8486(02)00534-3
Cogels, F. X., Frabouiet-Jussiia, S., & Varis, O. (2001). Multipurpose use and water quality challenges in Lac de Guiers (Senegal). Water Sci Technol, 44(6), 35-46.
Cohen, L., & Neimark, H. (1980). Schistosoma mansoni:response of cercariae to a thermal gradient. Parasitology, 66, 362-364.
Coulibaly, J. T., N'Gbesso, Y. K., N'Guessan, N. A., Winkler, M. S., Utzinger, J., & N'Goran, E. K. (2013). Epidemiology of schistosomiasis in two high-risk communities of south Cote d'Ivoire with particular emphasis on pre-school-aged children. Am J Trop Med Hyg, 89(1), 32-41. doi:10.4269/ajtmh.12-0346
Dodds, W., & Whiles, M. (2019). Freshwater Ecology (Vol. 3). Cambridge, MA: Academic Press.
Ern, R., Huong, D. T. T., Nguyen, V. C., Wang, T., & Bayley, M. (2013). Effects of salinity on standard metabolic rate and critical oxygen tension in the giant freshwater prawn (Macrobrachium rosenbergii). Aquaculture Research, 44(8), 1259-1265. doi:10.1111/j.1365-2109.2012.03129.x
FAO. (2012). FAO Specifications and Evaluations for Agricultural Pesticides: Deltamethrin. Retrieved from Rome, Italy:
Ferreira, N. C., Bonetti, C., & Seiffert, W. Q. (2011). Hydrological and Water Quality Indices as management tools in marine shrimp culture. Aquaculture, 318(3), 425-433. doi:https://doi.org/10.1016/j.aquaculture.2011.05.045
Fojut, T. L., & Young, T. M. (2011). Desorption of pyrethroids from suspended solids. Environ Toxicol Chem, 30(8), 1760-1766. doi:10.1002/etc.566
Grimes, J. E., Croll, D., Harrison, W. E., Utzinger, J., Freeman, M. C., & Templeton, M. R. (2014). The relationship between water, sanitation and schistosomiasis: a systematic review and meta-analysis. PLoS Negl Trop Dis, 8(12), e3296. doi:10.1371/journal.pntd.0003296
Haggerty, C. J. E., Bakhoum, S., Civitello, D. J., De Leo, G. A., Jouanard, N., Ndione, R. A., . . . Rohr, J. R. (2020). Aquatic macrophytes and macroinvertebrate predators affect densities of snail hosts and local production of schistosome cercariae that cause human

schistosomiasis. PLOS Neglected Tropical Diseases, 14(7), e0008417. doi:10.1371/journal.pntd.0008417

Halstead, N. T., Civitello, D. J., & Rohr, J. R. (2015). Comparative toxicities of organophosphate and pyrethroid insecticides to aquatic macroarthropods. Chemosphere, 135, 265-271.

Halstead, N. T., Hoover, C. M., Arakala, A., Civitello, D. J., De Leo, G. A., Gambhir, M., . . . Rohr, J. R. (2018). Agrochemicals increase risk of human schistosomiasis by supporting higher densities of intermediate hosts. Nat Commun, 9(1), 837. doi:10.1038/s41467-018-03189-w

Harwell, M. C., Surratt, D. D., Barone, D. M., & Aumen, N. G. (2008). Conductivity as a tracer of agricultural and urban runoff to delineate water quality impacts in the northern Everglades. Environ Monit Assess, 147(1-3), 445-462. doi:10.1007/s10661-007-0131-3

Hoover, C. M., Sokolow, S. H., Kemp, J., Sanchirico, J. N., Lund, A. J., Jones, I. J., . . . De Leo, G. A. (2019). Modelled effects of prawn aquaculture on poverty alleviation and schistosomiasis control. Nature Sustainability, 2(7), 611-620. doi:10.1038/s41893-019-0301-7

King, C. H., & Bertsch, D. (2015). Historical perspective: snail control to prevent schistosomiasis. PLoS Negl Trop Dis, 9(4), e0003657. doi:10.1371/journal.pntd.0003657

Levy, T., Rosen, O., Manor, R., Dotan, S., Azulay, D., Abramov, A., . . . Sagi, A. (2019). Production of WW males lacking the masculine Z chromosome and mining the Macrobrachium rosenbergii genome for sex-chromosomes. Scientific Reports, 9(1), 12408. doi:10.1038/s41598-019-47509-6

MA. (2005). Ecosystems and human well-being: synthesis. Retrieved from Washington DC:

Manush, S. M., Pal, A. K., Chatterjee, N., Das, T., & Mukherjee, S. C. (2004). Thermal tolerance and oxygen consumption of Macrobrachium rosenbergii acclimated to three temperatures. Journal of Thermal Biology, 29(1), 15-19. doi:https://doi.org/10.1016/j.jtherbio.2003.11.005

Mutuku, M. W., Dweni, C. K., Mwangi, M., Kinuthia, J. M., Mwangi, I. N., Maina, G. M., . . . Mkoji, G. M. (2014). Field-derived Schistosoma mansoni and Biomphalaria pfeifferi in Kenya: a compatible association characterized by lack of strong local adaptation, and presence of some snails able to persistently produce cercariae for over a year. Parasit Vectors, 7, 533. doi:10.1186/s13071-014-0533-3

New, M. B. (1995). Status of freshwater prawn farming. Aquacul Res, 26, 1-54.

New, M. B., & Valenti, W. C. (2000). History and global status of freshwater prawn farming. In Freshwater prawn culture: the farming of Macrobrachium rosenbergii (pp. 1-10). London, England: Blackwell Science.

Newman, M. C., & Unger, M. A. (2003). Fundamentals of Ecotoxicology. Boca Raton, FL: CRC Press.

Olele, N. F., & Kalayolo, P. E. (2012). Morphometric characteristics of the giant African river prawn, Macrobrachium vollenhovenii (Herklot, 1857) caught from Warri River coast. Journal of Agriculture and Biological Sciences, 3(1), 232-239.

RCoreTeam. (2018). R: A language and environment for statistical computing. Retrieved from https://www.R-project.org/

Ritz, C., Baty, F., Streibig, J. C., & Gerhard, D. (2015). Dose-Response Analysis Using R. PLOS ONE, 10(12).

Rohr, J. R., Barrett, C. B., Civitello, D. J., Craft, M. E., Delius, B., DeLeo, G. A., . . . Tilman, D. (2019). Emerging human infectious diseases and the links to global food production. Nat Sustain, 2(6), 445-456. doi:10.1038/s41893-019-0293-3

Rohr, J. R., Schotthoefer, A. M., Raffel, T. R., Carrick, H. J., Halstead, N., Hoverman, J. T., . . . Beasley, V. R. (2008). Agrochemicals increase trematode infections in a declining amphibian species. Nature, 455(7217), 1235-1239. doi:10.1038/nature07281

Salami, A., Kamara, A. B., & Brixiova, Z. (2010). Smallholder agriculture in East Africa:Trends, Constraints and Opportunities. Retrieved from Tunis, Tunisia:

Sane, S., Ngansoumana, B., Arfi, R., Samb, P. I., & Noba, K. (2017). Environmental conditions and primary production in a Sahelian shallow lake (Lake Guiers, North Senegal). International Journal of Biological and Chemical Sciences, 11(3).

Savaya-Alkalay, A., Ndao, P. D., Jouanard, N., Diane, N., Aflalo, E. D., Barki, A., & Sagi, A. (2018). Exploitation of reproductive barriers between Macrobrachium species for responsible aquaculture and biocontrol of schistosomiasis in West Africa. Aquaculture Environment Interactions, 10, 487-499.

Savaya Alkalay, A., Rosen, O., Sokolow, S. H., Faye, Y. P., Faye, D. S., Aflalo, E. D., . . . Sagi, A. (2014). The prawn Macrobrachium vollenhovenii in the Senegal River basin: towards sustainable restocking of all-male populations for biological control of schistosomiasis. PLoS Negl Trop Dis, 8(8), e3060. doi:10.1371/journal.pntd.0003060

Schrank, A. J., & Lishawa, S. C. (2019). Invasive cattail reduces fish diversity and abundance in the emergent marsh of a Great Lakes coastal wetland. Journal of Great Lakes Research, 45(6), 1251-1259. doi:https://doi.org/10.1016/j.jglr.2019.09.013

Sheahan, M., Barrett, C., & Goldvale, C. (2017). Human health and pesticide use in Sub-Saharan Africa. Agricultural Economics, 48(S1), 27-41.

Snyder, J., Smart, J., Goeb, J., & Tschirley, D. (2015). Pesticide use in Sub-Saharan Africa: Estimates, Projections, and Implications in the Context of Food System Transformation. Retrieved from http://ageconsearch.umn.edu/record/230980/files/IIAM_RP_8E_PesticideUse_EN-11-26-2015.pdf

Soderlund, D. M., & Bloomquist, J. R. (1989). Neurotoxic actions of pyrethroid insecticides. Annu Rev Entomol, 34, 77-96. doi:10.1146/annurev.en.34.010189.000453

Sokolow, S. H., Jones, I. J., Jocque, M., La, D., Cords, O., Knight, A., . . . De Leo, G. A. (2017). Nearly 400 million people are at higher risk of schistosomiasis because dams block the migration of snail-eating river prawns. Philos Trans R Soc Lond B Biol Sci, 372(1722). doi:10.1098/rstb.2016.0127

Sokolow, S. H., Lafferty, K. D., & Kuris, A. M. (2014). Regulation of laboratory populations of snails (Biomphalaria and Bulinus spp.) by river prawns, Macrobrachium spp. (Decapoda, Palaemonidae): implications for control of schistosomiasis. Acta Trop, 132, 64-74. doi:10.1016/j.actatropica.2013.12.013

Steinmann, P., Keiser, J., Bos, R., Tanner, M., & Utzinger, J. (2006). Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. Lancet Infect Dis, 6(7), 411-425. doi:10.1016/S1473-3099(06)70521-7

Talla, I., Kongs, A., & Verlé, P. (1992). Preliminary study of the prevalence of human schistosomiasis in Richard-Toll (the Senegal river basin). Transactions of The Royal Society of Tropical Medicine and Hygiene, 86(2), 182-182. doi:10.1016/0035-9203(92)90562-q

Talla, I., Kongs, A., Verle, P., Belot, J., Sarr, S., & Coll, A. (1990). Outbreak of intestinal schistosomiasis in the Senegal River Basin. Annales de la Société belge de médecine tropicale, 70, 173-180.

Therneau, T. (2020). A package for survival analysis in R. R package version 3.2-3. Retrieved from https://CRAN.R-project.org/package=survival

Tilman, D., Balzer, C., Hill, J., & Befort, B. L. (2011). Global food demand and the sustainable intensification of agriculture. Proceedings of the National Academy of Sciences, 108(50), 20260. doi:10.1073/pnas.1116437108

UN. (2020). Population. Global Issues. Retrieved from https://www.un.org/en/sections/issues-depth/population/

USEPA. (2020). Technical overview of ecological risk assessment: risk characterization. https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/technical-overview-ecological-risk-assessment-risk

Vos, T., Allen, C., Arora, M., Barber, R. M., Bhutta, Z. A., Brown, A., . . . Murray, C. J. L. (2016). Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. The Lancet, 388(10053), 1545-1602. doi:https://doi.org/10.1016/S0140-6736(16)31678-6

Xi-lin, D., Wei-ling, Z., Wei-dong, W., Yong-hai, S., Wen-cui, L., Gui-rong, X., & Shi-hua, L. (1999). Effects of temperature and dissolved oxygen content on oxygen consumption rate of Chinese prawn, giant tiger prawn and giant freshwater prawn. Chinese Journal of Oceanology and Limnology, 17(2), 119-124. doi:10.1007/BF02842709