Recommendations for Estimating Pesticide Effects on Nontarget Organisms during Mosquito Eradication Programmes in New Zealand

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1. Summary

- The potential introduction of mosquitoes that vector human and animal disease poses a threat to New Zealand. Pesticides are required for control and/or eradication of these pest species. However, long-term and wide-scale use of pesticides may cause negative impacts on nontarget organisms. The potential impact of the pesticides used to eradicate mosquitoes on nontarget organisms in New Zealand should be considered.
- S-methoprene and *Bacillus thuringiensis israelensis* have been or are being applied as part of a programme to eradicate the southern saltmarsh mosquito, *Ochlerotatus camptorhynchus* in the North and South Island. Presently, applications of these products are being made in two areas, Kaipara Harbour, North Island and the Wairau Lagoons/Lake Grassmere area in the South Island.
- The two areas are very different from one another and provide habitat for different types of nontarget organisms. Furthermore, mosquito detections in Kaipara Harbour are often in hoof prints, drains and other small temporary pools while detections in Wairau Lagoons/Lake Grassmere have been in runnels, more permanent drains and streams and ponds.
- S-methoprene and *Bacillus thuringiensis israelensis* have been shown to be two of the least environmentally damaging pesticides available for use in mosquito control and eradication. However, several long-term field studies conducted in the United States have indicated that repeated applications of both pesticides greatly reduce benthic insect biomass, particularly the chironomid midges.
- Midges make up a large part of the diet of many organisms that inhabit wetlands. However, in the above-mentioned field studies, no loss of ecosystem function was found because organisms that fed on chironomids were able to switch to other food sources.
- Several other studies have shown that S-methoprene and *Bacillus thuringiensis israelensis* have little effect on other species including birds, fish and non-insect invertebrates. S-methoprene has been implicated as one of the possible causes for limb malformations in frog populations in the United States. However, there is no definitive evidence that S-methoprene or breakdown products of methoprene can cause the types of malformations being observed at the concentrations being applied. New Zealand is home to several indigenous species of frogs, but none of these species live in the saltmarsh habitat.
- No threatened or endangered species live in the marshlands being treated with pesticides for eradication of *Oc. camptorhynchus*.
- Because pesticide applications in the eradication programme are being made to relatively small areas, recolonisation by affected nontarget organisms will be rapid from surrounding untreated areas. Thus, use of S-methoprene and/or *Bacillus thuringiensis israelensis* in an eradication programme should not result in long-term negative effects to ecosystem function.
- The important question to be asked then is: what is the cost-benefit of conducting nontarget studies in New Zealand? Long-term, large scale nontarget monitoring programmes often cost $100,000 per year or more to conduct. Will new information be gained from nontarget studies or will the same answers found in other studies be obtained - namely that chironomid populations will decline temporarily in pesticide-treated areas?
- A nontarget study in New Zealand is considered unnecessary because the likely main nontarget effect will be a reduction in chironomid midge populations in treated areas. These reductions will be temporary and midge populations should remain high in surrounding untreated areas and thus provide food for waterfowl and other species that feed on midges.

- Although large-scale control programmes are often ideal situations to evaluate nontarget effects, eradication programmes are not conducive for nontarget studies. Untreated areas, which are necessary from an experimental perspective and serve as control sites, are unacceptable in eradication programmes because they serve as a reservoir for the pest species. Therefore, traditional sampling of treated and control sites cannot be easily conducted during an eradication programme.
- To get around the problem of not having valid control sites in the field during an eradication programme, a different approach must be taken. One of the easiest and least expensive approaches that can be used to detect effects of pesticides on nontarget organisms during an eradication programme is to use sentinel organisms that are placed in pesticide application areas. Chironomid larvae can be placed in containers (glass jars) in the field. Some of the containers are then left open during pesticide application while others are covered during application and serve as controls. Subsequently these containers are brought to the laboratory where larval mortality and adult emergence are recorded. The advantages of this approach are that exposures are realistic, and the data are amenable to statistical analysis.
- The most likely outcome of the above-mentioned sentinel organism study is that applications of S-methoprene and *Bacillus thuringiensis israelensis* will reduce numbers of chironomid midges. These reductions will be temporary and midge populations are likely to recover quickly. This outcome has been seen before in other studies and therefore no new information will be gained by a nontarget study of *Bacillus thuringiensis israelensis* and methoprene. It is thus recommended that a nontarget study is not necessary when S-methoprene and *Bacillus thuringiesis israelensis* are the pesticides being used for eradication.

2. Introduction

Exotic mosquitoes with the potential to vector serious mammalian diseases have entered New Zealand in the past and may be occasionally introduced in the future (Frampton 2004). The potential for future introductions highlights the need for control technologies for use in mosquito control and/or eradication programmes. Following the recommendations of Glare and O'Callaghan (1998, 1999), S-methoprene (methoprene) and to a lesser extent, *Bacillus thuringiensis israelensis* (Bti) have been effectively used in an eradication programme for the southern saltmarsh mosquito, *Ochlerotatus camptorhynchus* in the North and South Island. The potential impact that applications of methoprene and Bti might have on nontarget organisms in New Zealand is an important concern due to the unique fauna that exists in the country. This document was prepared to evaluate the need to monitor nontarget effects of mosquito control agents and to present a plan to actually monitor effects on nontarget organisms inhabiting areas to be treated with mosquito control agents if a nontarget study is deemed necessary. A summary and review of the various types of nontarget research studies that have been untaken in the past is presented. Additionally, specific methodologies are outlined, and using previous examples of nontarget studies associated with mosquito control in brackish/saltwater, an alignment of these approaches with the situations in Kaipara Harbour and Wairau Lagoons/Lake Grassmere areas is made.

3. Selective pesticides and nontarget effects

Pesticides, by definition, are designed to kill living things. As such, whenever pesticides are applied to kill a particular pest, there is the potential to cause negative consequences to nontarget organisms. The latest generation of insecticides, including the neonicitinoids, natural products such as spinosad and neem and products with unique modes of action such as pymetrozine and fipronil are supposed to be much more selective than previous generations of insecticides including the organochlorines, organophosphates, carbamates and pyrethroids (Stark and Banks 2001). A pesticide is considered selective when it is more toxic to a pest species than to a nontarget or beneficial species. Many of these new pesticides have been shown to be more toxic to pest species than to selected nontarget organisms. Furthermore, application rates of the new pesticides are much lower than the products used extensively in the past and therefore, hazard, which is a function of toxicity and exposure (see section 7.1 for more detail) is reduced (Stark and Vargas 2003, Stark and Vargas in press). Even though methoprene and Bti, the two pesticides being used to eradicate mosquitoes in New Zealand, have been available for a long time and are not part of the new generation of insecticides, they have been shown to be selective products that have minimal effects on nontarget organisms, including humans (Palmer and Palmer 1995; EPA 1998a,b; Glare and O'Callaghan 1998, 1999; Jackson et al. 2002; Stark 2005 a,b).

4. Endpoints evaluated in nontarget studies

Exposure to pesticides can result in mortality (lethal effect) or if an individual survives exposure, sublethal effects may be manifested. Sublethal effects include reduction in the number of offspring produced over a lifetime, sterility, loss of weight or the inability to gain weight, reduction in lifespan, cancer, mutations, behavioural changes etc. More than one sublethal effect may occur after exposure to a pesticide. Most toxicological studies are designed to evaluate one or perhaps two toxicity endpoints. The most commonly estimated effect is mortality followed by effects on reproduction. A significant debate about the value of various toxicity endpoints and the best way to measure the total effect (lethal and multiple sublethal effects) that pesticide exposure may cause has been ongoing for a number of years among various scientists (e.g. Forbes and Calow 1999, Stark et al. 2004, Stark 2005c). However, this debate has more to do with laboratory evaluations and the ecological risk assessment process (see section 7.2) that utilises laboratory toxicity data (LC50) for estimation of risk than with data collected during field studies. The problem is that there is an abundance of laboratory toxicity data but a dearth of field data dealing with the effects of pesticides on nontarget organisms (Croft 1990). The reasons for the lack of field studies are many, but for the most part, field studies are much more difficult and expensive to conduct compared to laboratory studies. However, whenever possible, well-designed field studies are preferable to laboratory studies when investigating effects of pesticides on nontarget organisms. Measures of abundance of pesticide-exposed and unexposed populations over time take into account all possible effects of the toxicant.

Although large-scale pest control programmes are often ideal situations to evaluate nontarget effects, eradication programmes are not conducive for nontarget studies. For a

nontarget study to be valid, untreated areas that serve as control sites are necessary. Results obtained from untreated areas are compared to pesticide-treated areas. Untreated areas are unacceptable in eradication programmes because they serve as a reservoir for the pest species. This makes sampling for nontarget effects in an eradication programme very difficult. In fact, traditional field sampling for nontarget effects cannot be done in eradication programmes. Because pesticides are being applied as relatively localised applications in Kaipara Harbour and the Wairau Lagoons/Lake Grassmere area, control sites might be established. For example, an area that has had no detections of *Oc. camptorhynchus* for quite some time might be compared to treated areas. But there is no guarantee that the site being used as a control will remain free of *Oc. camptorhynchus* throughout the course of the nontarget study.

4.1 Measuring effects of pesticides on nontarget organisms in the field

Several endpoints can be measured to estimate the impact that pesticide applications might have on nontarget organisms in the field.

4.1.1 Acute mortality

In some studies, acute mortality can be measured in the field. This is particularly true where acute neurotoxins are being used such as organophosphorous insecticides. Because many organisms find hiding places to die, it is very difficult to measure death in field populations. However, *in situ* studies (see section 6.3 below for more detail) where caged animals are exposed in the field to pesticide applications are particularly amenable to this measurement of effect. Pierce et al. (2000) present an example of a field study where mortality was the measured endpoint of effect. In this study, the effect of temephos on two saltmarsh crab species, the marsh fiddler crab, *Uca rapax* and the mangrove tree crab, *Aratus pesonii* was evaluated by placing crab larvae in floating field exposure trays in dug-out depressions in the lower and middle areas of a marsh in Florida, USA prior to pesticide application. Survival was recorded in the field six, 24 and 48 hours after pesticide application or the crab larvae were left in the field for five hours after application and then transported to the laboratory and monitored daily through the first larval moult.

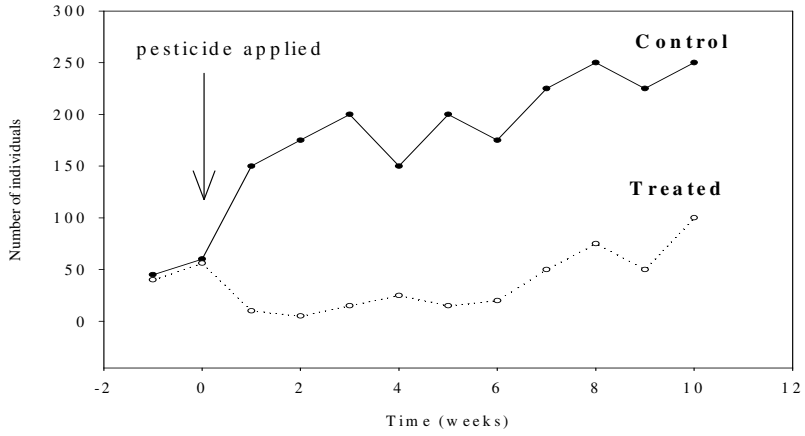
However, the validity of comparing LC50 estimates among nontarget species has been called into question (Stark et al. 2004). Because the LC50 is only a measure of mortality and, as mentioned is section 4, exposure to pesticides can result in multiple sublethal effects, LC50 estimates may be grossly inaccurate in terms of predicting population-level effects. Additionally, recent studies have shown that LC50s cannot be compared among species to predict population-level outcomes because of differences in life history variables. For some species, 50% mortality will result in significant population declines, while for others, 50% mortality will only slow population growth slightly. In other words, 50% mortality does not mean the same thing for all species and thus comparisons of LC50 estimates among nontarget species may not be valid.

4.1.2 Measures of abundance over time

One of simplest and most widely used approaches to estimate the impact of pesticides on nontarget organisms in the field is to measure abundance of a particular species over time comparing populations in treated and untreated areas (Stark 1992; Hanowski et al. 1997a,b;

Hershey et al. 1998; Niemi et al. 1999). Abundance is measured prior to pesticide application to determine whether there are differences in abundance among the different "plots" being evaluated. If initial differences are found, then abundance can still be compared as a percent reduction from the initial population levels. In abundance studies, one species can be studied as a surrogate for the entire community (Figure 1) or a community of organisms can be measured. Individual organisms are collected with nets, traps, dredges, sediment/soil cores or other means in studies of this type.

Fig. 1. Abundance of a hypothetical organism over time after exposure to a pesticide application.



4.2 Surrogate measures of abundance

In some studies, measuring actual organisms is difficult if not impossible. This is particularly true with cryptic organisms. Surrogate measures of abundance can be used where it is difficult to actually measure individuals. For example, Pinkney et al. (1999) evaluated the effects of temephos on two fiddler crab species inhabiting a saltmarsh in Delaware, USA by measuring the number of burrow holes in tidal areas. Burrow holes had previously been found to be highly correlated with the number of crabs present in an area.

4.3 Species diversity studies

In studies of this type, as many species as possible are studied or the focus is on a particular group of organisms such as the arthropod community of an ecosystem (Stark 1992).

4.4 Measures of community impact

Several measures of species richness and diversity have been developed that can be used to compare communities that have been exposed to pesticides to those that have not been exposed. The Shannon-Weaver diversity index is the most commonly used index of diversity.

5. Statistical considerations

One of the major problems associated with sampling populations in the field is that there can be great variation in the data. High levels of variation may result in a failure to detect statistically significant differences among treatments. Trial and error must often be used to determine the number of samples necessary for adequate statistical analysis. A widely accepted approach for statistical evaluation of abundance data taken at different times after treatment is repeated measures analysis of variance (RMANOVA). However, this method of analysis is only valid for more than two treatments and cannot be used when comparing a control to one treatment. RMANOVA works best when several pesticides are being evaluated. Other statistical methods that can be used for comparison of two treatments (control versus pesticide-exposed) are analysis of covariance, correlation and regression analysis. Additionally, for short-term effects, t-tests can be used to compare before and after treatment data.

6. Methods for estimating impacts of pesticides on nontarget organisms

Several different approaches have been used to estimate the impact that pesticide applications might have on nontarget organisms after field application. However, the two most common approaches are: 1) sampling of wild populations of specified organisms before and after pesticide application; and 2) placing sentinel organisms (usually laboratory-raised) in the field in containers or small replicated ecosystems (micro- or mesocosms), prior to application and monitoring survival, growth and/or reproduction for some designated time interval after pesticide application. A third, less commonly used approach involves taking pesticide-treated media (water, soil, plant foliage) from the field to the laboratory and exposing organisms to treated and untreated media for a specified time interval. Mortality, growth, and development are then recorded.

6.1 Laboratory studies with field-collected organisms and treated media

One approach that has been used to obtain an estimate of possible effects of pesticide applications on nontarget organisms is to collect the organisms or a representative species from the field prior to pesticide application and establish them in the laboratory for a day or so before pesticide application. This can be done with organisms that exist on plants (biological control agents) (Banken and Stark 1998) or organisms that live in water or soil. With studies in surface water systems, samples of the water body to be treated with pesticide are collected from the

treatment site prior to application and used in the control treatment group. Just after pesticides are applied, water samples are taken again, transported to the laboratory and bioassays with field-collected organisms are conducted whereby some groups are exposed to untreated field-collected water and other groups are exposed to pesticide-treated water. Mortality and/or sublethal effects are recorded. The advantage of this approach is that it is more controlled than an actual field study and less expensive to conduct. The disadvantage is that this type of study does not take into account degradation of pesticide over time and/or the effects of sunlight and other conditions in the field.

6.2 Semi-field studies

Semi-field studies may involve the use of cages (Tucker and Burton 1999), enclosures (Solomon et al. 1989), containers (Shaw et al. 1995), replicated man-made ponds (Lawler et al. 2000, Pinkney et al. 2000) and streams (Clements et al. 1989). There are several ways in which these studies are designed. The first approach involves placing sediment and water in containers and stocking them with the organisms that are to be studied. The second approach is to place enclosures in ponds, lakes, streams, or rivers (Solomon et al. 1989, Wayland 1991, Lozano et al. 1992, Kreutzweiser et al. 1999, 2000). Naturally occurring organisms are either captured in the enclosures as they are placed in the system or they are added to the enclosures once they are set in place. This approach enables the researcher to use natural water and pesticides can be applied at different concentrations to the enclosures. Some of the containers/enclosures are treated with pesticide while others are untreated and serve as controls. Replication is easily accomplished with these approaches. Organisms are periodically monitored recording abundance over time. Both of these approaches are also called micro- or mesocosm studies depending upon the size of the containers/enclosures.

Sometimes enclosures are placed in the field in an area to be treated with a pesticide and are exposed directly to the spray application (Pierce et al. 2000). In other studies, micro- or mesocosms can be located almost anywhere and treatments are applied to each experimental unit individually (Shaw et al. 1995). The advantage of this approach is that you gain more environmental realism than with a laboratory study because the studies are conducted outside. Furthermore, community interactions occur if enough species are added to the containers. Additionally, valid controls are obtained with this approach which can be difficult to achieve with field studies. The disadvantage to this approach is that you ignore immigration/emigration and some of the interactions that naturally occur among various organisms when an entire community is present do not take place because it is rare in these studies to include all of the species found in a community.

6.2.1 Examples of enclosure studies

Kreutzweiser et al. (1999) evaluated the effects of an insecticide from the neem tree, Neemix® on aquatic organisms using replicated enclosures in a river in Canada. They focused on the effects on aquatic arthropods and found that Neemix®, caused significant changes to an aquatic community, but only at concentrations much higher than the expected environmental concentration.

6.2.2 Examples of microcosm studies

Shaw and Manning (1996) evaluated the effects of copper on macroinvertebrates with 17 m^3 microcosms. Macroinvertebrates that occurred naturally in the microcosms were monitored. Survival and growth of several macroinvertebrates were assessed after three days of exposure. Exposure to copper negatively affected Notonectidae and *Caenis* sp. (Ephemeroptera) but *Caenis* bioassays indicated that the potential for recovery and survival was ≥95%.

A somewhat different approach to estimate the effects of pesticides in the field was used by Brown et al. (1999). In this study, the pesticides, temephos, pirimiphos-methyl, S-methoprene and *Bti* were applied to small (≈3-5 m^2, 5-18 cm deep) saltmarsh pools near a marina in Australia. Field-collected shrimp, *Leander tenuicornis* were enclosed in cages in these pools prior to pesticide application. The cages consisted of open-topped, 280µm mesh cylinders (20 cm deep by 100 cm diameter). Mortality was recorded 24 hours after pesticide application.

The micro-/mesocosm approach has fallen out of favour with the U.S. Environmental Protection Agency because of the difficulty surrounding evaluation of the results. Subtle changes in one species could result in major food chain effects and there was no consensus as to how to analyse and interpret the data.

6.3 In situ studies

Use of sentinel organisms (*in situ* method) to estimate the impact of pesticide applications on nontarget organisms in the field is another method that has been successfully used in the past (Burton 1991). The *in situ* method involves the use of species that are usually common to the ecosystem being studied. Various aquatic organisms have been used for *in situ* testing including amphipods (Chappie and Burton 1997, Lawler et al. 1999, Tucker and Burton 1999), chironomid midges (Chappie and Burton 1997, Tucker and Burton 1999), cladocerans (*Daphnia*) (Ireland et al. 1996, Sasson-Brickson and Burton 1991), crabs (Pierce et al. 2000), insects (Lawler 2000) and fish (Wilde and Parrott 1984, Ziegenfuss et al. 1990, Burton et al. 1992, Hall et al. 1988, Jones and Sloan 1989, Simonin, et al. 1993).

There are at least two ways to conduct *in situ* studies. The first approach is to transport a laboratory-raised indicator species to field sites and place the organisms in either flow-through cages (Figure 2) beneath the water surface or in open containers that float on the water surface (Pierce et al. 2000). Details of the flow-through cage pictured in Figure 2 can be found in Chappie and Burton (1997). Replicated *in situ* cages are placed in areas where pesticides or other chemicals are either applied or are present, such as industrial effluents in rivers. Cages are also placed in a reference site (control) that should be free of the contaminants of interest. The caged organisms are evaluated at a predetermined time interval after being placed *in situ*. Endpoints of evaluation can be mortality of the original individuals placed in the containers (Tucker and Burton 1999) or if the study is conducted for a long enough time period, population size can be measured after reproduction has occurred.

Figure 2 A flow-through cage designed for *in situ* studies.

The second approach is to place water-filled containers (container method) on the ground near a water system prior to pesticide application. Lids are placed on control containers to prevent pesticide exposure. After application, containers are either left in the field for a predetermined time or immediately taken to the laboratory where selected toxicity endpoints such as mortality, growth, and development are recorded.

The advantage of the *in situ* method is that organisms are exposed to field-applied pesticides or other chemicals under natural conditions. The disadvantage to the *in situ* method is that control sites are often questionable. Either upstream areas or completely different systems (ponds, lakes, streams or rivers) are the only available areas for the control sites in many *in situ* studies. For example, in a study by Ireland et al. (1996), controls were 8 km upstream from the test site. The problem with this approach is that depending upon how far upstream the samples are taken, conditions can differ dramatically from the downstream sampling area. This is turn can lead to erroneous conclusions. Another approach is to evaluate a treated water system and use a nearby untreated water system as a control (Tucker and Burton 1999). The problem is the same with this approach. Differences in physical parameters and in pre-existing community structure among different sites can be substantial thus making data interpretation difficult. However, this problem is alleviated when the container method is used and containers are placed on land, not in the water system being evaluated. With this approach some containers are treated and others are not treated and serve as controls.

It is difficult to ascribe effects observed in organisms that have been placed in natural systems such as rivers and streams to a particular toxicant. Many toxicants both natural and man-made may be present in the water at any given time. Additionally, pesticide residues are often present even in untreated water bodies or upstream from areas that are treated with pesticides. This is due to drift and/or volatilisation of active ingredient with the subsequent deposition of the pesticide in other areas.

Another issue with *in situ* studies is that because animals are caged, they are not exposed to predators and no immigration or emigration occurs and thus there is a lack of realism compared to sampling actual field populations. Furthermore, the duration of some of these studies is too short to allow for reproduction to occur and thus only mortality is recorded (Ireland et al. 1996).

6.3.1 Examples of in situ studies

An *in situ* study using flow-through cages was conducted by Tucker and Burton (1999) where nonpoint source runoff in a stream was evaluated. The indicator species used in the study were the amphipod, *Hyalella azteca* and the chironomid midge, *Chironomus tentans*. Both species were cultured in the laboratory, transported to the field and placed separately in flow-through chambers in a reference site (control) located in Little Sugar Creek, and two test sites, Beaver Creek, which is surrounded by an agricultural area and Little Beaver Creek, which is situated within an urban area. All sites were located in the Little Miami River watershed in Greene County, Ohio, USA. Organisms were exposed for 2-18 days in the field and mortality was the endpoint evaluated. Amphipod survival in the control site was high (>80%) while survival in the site receiving agricultural runoff (Beaver Creek) ranged from 20-63%. Survival in the urban area (Little Beaver Creek) ranged from 0-90%. Chironomids also had high survival in the control site (>80%). Survival in the site receiving agricultural runoff (Beaver Creek) ranged from 30-76%.

An *in situ* study using replicated ponds was conducted by Lawler et al. (2000). The effects of Duplex, a formulation containing both Bti and methoprene, on the water boatman, *Trichocorixa reticulata* were evaluated by placing the organism in cages in replicated saltmarsh ponds. No negative effect on survival or maturation of *T. reticulata* was detected.

6.4 Field studies

There are many examples in the literature of field studies designed to study the effects of pesticides on nontarget organisms. The endpoints of interest in field studies are abundance as well as community health (Campbell and Denno 1976). It is usually impossible to collect all organisms living in an aquatic system and actually not advisable because removal of large numbers of organisms can affect future sampling especially when populations of a species of interest are low to begin with. Estimations of abundance are made from samples taken from a population. Thus the goal is to sample the population without causing it to go to extinction. The most thorough published field studies on the effects of pesticides used for mosquito control on nontarget organisms were presented in four papers dealing with different aspects of the same research programme (Hanowski et al. 1997a,b, Hershey et al. 1998, Niemi et al. 1999). These studies are discussed in detail in sections 6.4.3 and 6.4.4. The studies were conducted in a freshwater wetland area of Minnesota, USA. This project began in 1988 and was terminated in 1993. A grant of $1.5 million dollars was obtained to conduct these studies (Niemi personal communication). This dollar value did not include overhead to the university and thus was the actual cost to carry out the study. However, Dr. Niemi also indicated that the true cost of the project was double the value of the grant because his university subsidizes the salaries and benefits of its employees.

6.4.1 Monitoring of one species (an indicator) versus monitoring a community

In some nontarget pesticide studies, entire communities are monitored, while in others only one species is evaluated. The difference in cost can be enormous. Monitoring of communities is not only difficult and expensive, interpretation of the data can be problematic. Subtle changes in numbers of one species can cause a cascade of changes in other species.

6.4.2 Limitations of field studies

There are major limitations to actual sampling in the field. The first is that a valid control site is often difficult if not impossible to find (Hurlbert 1984). Another disadvantage with conducting field studies is that different results can be obtained among various field studies even if they are conducted in the exact same manner. Environmental variables change so rapidly that it could be said that many ecological field studies are one-time events (Stark and Banks 2000). Furthermore, a field study conducted in one geographic region may have little or no bearing on a field study conducted in another geographical region. It is also difficult to establish statistical significance in field studies because spatial and population variation can be very large (Hurlbert 1984).

6.4.3 Example of a macroinvertebrate field study

One of the most thorough and well-designed studies to detect effects of mosquito control agents on nontarget organisms was conducted by Hershey et al. (1998). This long-term study on the effects of Bti and methoprene on nontarget benthic macroinvertebrates was conducted in wetlands in Minnesota, USA. A total of 27 sites were investigated; nine were treated with Bti, nine were treated with methoprene and nine served as controls. Pesticides were applied six times at three-week intervals per year during the treatment years from spring until mid to late summer. Pretreatment sampling was carried out in 1988, 1989 and 1990. Pesticide treatments were applied in 1991, 1992 and 1993. Sampling of benthic macroinvertebrates was accomplished by taking eight sediment cores from each site on each sampling date. The eight samples consisted of four randomly taken cores collected from each of two randomly drawn transects perpendicular to a 50 metre transect along the site perimeter. The sampling device was a hand-held plastic core tube (19.6 cm^2 area). Each sample consisted of two pooled cores (total are of 35.4 cm^2) taken from the same location. The eight samples were mixed together and a subsample was taken equal to 3/8 of the total volume (total area = 106 cm^2). The subsamples were preserved in 95% ethanol until processed. In this sampling scheme, each site was the unit of replication (eight replications) per sampling date. Cores were taken five times from April through July each year. Invertebrates were counted in the core samples by pouring the contents into a pan, adding sufficient water and sorting through the samples to remove all invertebrates. Additionally, this part of the study was a "blind design", sorters had no knowledge of which treatment was represented by the samples sorted. For each taxonomic group, data from this study were log transformed prior to statistical analysis using the following transformation: $\log_e (x + 94)$, where x is the number of animals/m^2 and where 94 individuals/ m^2 was equivalent to one individual per pooled sample. In addition to evaluating the number of individuals over time, species richness and dominance analyses were conducted. The experimental design was a randomised block design using repeated measures analysis. During this study, at least 179 genera of aquatic insects representing seven orders were collected. Of these genera, 101 were Diptera, the majority of which (51 genera) were Chironomidae. Collembola, Bivalvia, Isopoda, Annelida and Gastropoda were also collected. Statistical analyses revealed that methoprene and Bti treatments

had minimal effects on nontarget organism during the first year of treatment but by the second year (1992) of treatment, highly significant reductions in several insect groups were seen in the Bti- and methoprene-treated areas. Predatory insects were reduced in methoprene but not Bti treated sites in 1992. By 1993, wetland communities were depauperate of most benthic insects. Diptera and in particular the chironomids were most affected. Minimal effects were seen in non-insect invertebrates. Bti and methoprene treatments also reduced species richness and resulted in an increased tendency of communities to be dominated by one or a few genera.

6.4.4 Sampling of bird populations

Two companion studies to that of Hershey et al. (1998) were published by Hanowski et al. (1997a,b). In these studies, the effects of Bti and methoprene applied to the wetlands described above on a wetland breeding bird community were determined. Bird species were monitored by taking a census twice during the breeding season and one again in early June during 1988, 1990-1993. No effect due to pesticide treatments was found on the bird community or on 19 individual bird species.

6.4.5 Importance of level of taxonomic identification

In many studies on nontarget effects, organisms are grouped into broad categories such as class or family. However, the results of several studies have indicated that species level identification, if possible, is very important because negative impacts can be missed when coarser identifications are made (Stark 1992, Stark in press). Pesticides may be much more toxic to certain species than to others within a community and one species may be greatly reduced in number while others increase. Certain species may disappear altogether while others now become more abundant. These changes will not be noticed if only family level identifications are made. Thus a shift in species composition may occur that goes undetected. However, there is a trade-off here in terms of time and money. Species-level identification is much more expensive than genus level identification which is much more expensive than family identification etc.

7. Alternatives to sampling nontarget organisms in the field

There are a few alternatives to sampling for nontarget effects in the field. These are usually restricted to modeling exercises that try to predict impacts with limited data or hazard/risk assessments that involve expert judgment based on the available scientific data.

7.1 Hazard and Risk Assessment

Hazard and risk assessment are processes that attempt to predict effects of toxicants on ecosystems using laboratory toxicity data and either a measure of environmental concentration of the chemical in a question or a prediction of the amount that may end up in an ecosystem.

Hazard assessment is based on the quotient method whereby an environmental concentration is divided by a toxicity endpoint. This process is presented in Stark and Banks (2001) for several insecticides and *Daphnia pulex*. For example, if the *D. pulex* LC50 for spinosad is 0.129 mg/l and the expected environmental concentration (EEC) for spinosad is 0.068 mg/l, then you would divide the EEC by the LC50 as follows:

Hazard assessment for spinosad and *D. pulex* Hazard = 0.068/0.129 = 0.53

Numbers less than 1 indicate no hazard while numbers greater than 1 indicate that the toxicant poses a hazard. Clearly in this example, spinosad does not pose a hazard to *D. pulex*.

7.2 Ecological risk assessment

Ecological risk assessment is more complicated and involves developing probabilities of risk (Klaine et al. 1996, Solomon et al. 1996a,b, Stark et al. 2004). The ecological risk assessment process involves comparing a plot of the acute lethal concentration estimates (LC50) for all species found in the scientific literature for a given chemical along with a plot of measured environmental concentrations in a given watershed or river for a specific chemical. The goal is to protect 90% of the species in an ecosystem. If there is overlap of the two plots and 10% or more of the LC50 values overlap with observed environmental concentrations, then the chemical is considered to pose a significant ecological risk.

8. What is the best approach for New Zealand?

I visited New Zealand in March 2005 to observe the current *Ochlerotatus camptorhynchus* eradication programme and associated surveillance by making site visits to the two remaining areas being treated, Kaipara Harbour and Wairau Lagoons/Lake Grassmere area. Methoprene, and to a lesser extent Bti, have been or are being used to eradicate the southern saltmarsh mosquito, *Oc. camptorhynchus* from New Zealand. The eradication programme has been conducted in several areas including Napier, Mahia, Porangahau, Gisborne, Kaipara Harbour, Whitford, Mangawhai, Whangaparaoa and Wairau Lagoons/Lake Grassmere, but as mentioned above is now restricted to Kaipara Harbour/Whangaparaoa and the Wairau Lagoons/Lake Grassmere areas. Both of the pesticides used in the programme have been shown to be relatively safe to the environment compared to other types of pesticides, particularly the organophosphate insecticide, temephos that is also used in many areas of the world for mosquito control (EPA 1998a,b; Stark 2005a,b). Much of the area being treated, particularly in the Kaipara Harbour region, is transient in nature. In other words, *Oc. camptorhynchus* larvae are often detected in temporary pools or in hoofs prints. This is in stark contrast to the study conducted by Hershey et al. (1998) where effects of mosquito control agents were monitored in permanent wetlands. The preferred habitat of *Oc. camptorhynchus* (temporary pools) makes it much more difficult to design a study let alone undertake valid nontarget sampling in an eradication programme in Kaipara Harbour and Wairau Lagoons/Lake Grassmere.

8.1 Description of the treatment sites

8.1.1 Kaipara Harbour

Kaipara Harbour lies on the west coast of the North Island. It is New Zealand's largest enclosed harbour and is an important sanctuary for native and migratory birds including the waders, South Island pied oystercatchers, wrybills, curlew, godwits, plovers, snipe and sandpipers, and the shorebirds/seabirds, New Zealand fairy terns, New Zealand dotterels, banded dotterels, black billed gulls, variable oystercatchers, white-fronted terns and Caspian terns

(www.forestandbird.org.nz/Marine/kaipara/ramsar.pdf). Some of these species, particularly the waders feed on amphipods, worms, crabs and shellfish along the shore and in the marshlands.

The area is characterised by a large harbour consisting of 520 km^2. The southern saltmarsh mosquito, *Oc. camptorhynchus* was first discovered in Kaipara Harbour in February 2001. Treatments with Bti were initiated in January 2002. Methoprene treatments (applied at a rate of 6 kg/ha) replaced Bti treatments in October 2002 and have been applied to the present time. The area treated for control of *Oc. camptorhynchus* is approximately 2,700 hectares. The habitat that supports *Oc. camptorhynchus* is along the edge of the harbour and consists primarily of farmland, scrub, mangrove and marshlands that drain upland areas. A combination of fresh and brackish water exists in this area. Fresh water streams, rivers and drains become brackish as they approach the harbour where tides move salt water upstream.

Initially, pesticides were applied every 21 days to the 2,700 hectares. Latterly the area is rigorously monitored and when suitable habitat for *Oc. camptorhynchus* is detected (i.e., recently inundated with water), pesticides are applied to that limited inundated area. Detections of *Oc. camptorhynchus* were in potholes, hoof prints, temporary pools, and drains. The actual areas treated with methoprene every 21 days therefore changes over time during the treatment phase.

8.1.2 Wairau Lagoons/Lake Grassmere area

The Wairau Lagoons area consists of approximately 2000 hectares of saline marshlands on the northeast coast of the South Island near Blenheim. The Wairau River runs through this area. The southern saltmarsh mosquito, *Oc. camptorhynchus* was first discovered in Wairau in May 2004. Treatments with Bti were initiated in June 2004. Methoprene treatments replaced Bti treatments in September 2004 and this pesticide is still being used in the eradication programme. The area treated to eradicate *Oc. camptorhynchus* amounts to about 1000 hectares presently. Detections in Wairau have been in runnels in fields, brackish streams that run through farmland and more or less permanent saline ponds.

During my brief visit, I took samples from various water sources in Kaipara Harbour and the Wairau Lagoons/Lake Grassmere areas. The organisms I found are listed in Table 1.

Table 1. Nontarget species found in Kaipara Harbour and Wairau Lagoon/Grassmere area.

Kaipara Harbour

Various spiders
Daphnia
Amphipods
Snail - an unidentified and extremely small species
Brine shrimp
Saltmarsh crab
Damselflies
Dragonflies

Chironomid larvae
Backswimmers
Waterstriders
Diving beetles
Fish - unidentified minnow
Paradise shelduck
Grey duck
Mallard duck
Grey/Mallard duck crosses
White-faced heron (or reef heron)
Black swan

Wairau Lagoons/Lake Grassmere

Various spiders
Daphnia
Amphipods
Damselflies
Dragonflies
Chironomid larva
Backswimmers
Waterstriders
Diving beetles
Fish - unidentified minnows
Brine shrimp
Tadpoles (*Litoria* sp.)
Paradise shelduck
Grey duck
Mallard duck
Grey/Mallard duck crosses
White-faced heron (or reef heron)

Some of the same species were found in both locations, but there were differences in the types of organisms between the two sites. For example, the tadpoles listed in Table 1 were observed only at Station Creek near Lake Grassmere. This is not a native species because native frog species do not have a tadpole stage.

8.2 Chironomidae of New Zealand

The nontarget species shown to be especially susceptible to Bti and methoprene in the field are the chironomids. I collected very few chironomids from brackish water on my visit. Only three chironomid larvae were found, two in Kaipara Harbour and one in Wairau Lagoons. However, Browne et al. (unpublished) reported that large numbers of a native chironomid species, *Chironomus zealandicus* were found in the fringe areas of the brackish water areas being treated with methoprene during the *Oc. camptorhynchus* eradication campaign in New Zealand. The

low numbers of chironomids that I detected may have to been due to the time of year of my visit (March) or the fact that I was sampling only brackish water. Also, low sample size may have influenced my observations. Most chironomids however prefer fresh water. In New Zealand, chironomid larvae are known to inhabit freshwater and serve as food for native fish, sports fish, riverine birds and other invertebrates (Collier 1993). The ecology of many New Zealand chironomid species is either unknown or published in hidden or "grey literature" (Boothroyd and Cranston 1995). Recent studies have suggested that many new and as yet undescribed chironomid species await description (Boothroyd and Cranston 1995). Species unique to New Zealand have been described as well. For example, a new genus, *Kaniwhaniwhanus* gen. n. has been described and is found in North Island streams and Lake Grassmere (Boothroyd 1999). Because there is such a paucity of information regarding New Zealand Chironomidae, especially with regard to species that may inhabit saltmarsh ecosystems, further studies on these species are justified, notwithstanding that in a laboratory study, Browne et al. (unpublished) found that mortality in *C. zealandicus* exposed to methoprene (Prolink XR-G) applied at the field treatment dosage rate (6 kg/ha) was not significantly higher than control populations. These results indicate that chironomids inhabiting saltmarsh ecosystems in New Zealand may not be susceptible to methoprene at the concentrations being applied. Additional studies, particularly field studies are needed to validate these findings.

8.3 Nontarget Sampling Plan

As mentioned above in section 4, eradication programmes are not conducive to nontarget studies because to conduct nontarget studies, untreated control sites are necessary. Obviously, untreated sites are unacceptable in an eradication campaign. This makes it difficult to conduct a nontarget study, but not impossible. The first question to ask before undertaking a nontarget study associated with any mosquito eradication programme in New Zealand is: what new knowledge will be gained from a study of this nature? Secondly, what will a nontarget study cost? Large-scale nontarget studies can cost hundreds of thousands of dollars per year to conduct (Niemi personal communication). Previous studies indicate that methoprene and Bti have little if any effect on most nontarget organisms, including birds, fish, mammals, and arthropods with the exception of Chironomidae. Browne et al. (unpublished) exposed several non-target aquatic species from New Zealand to methoprene (Prolink XR-G) and recorded mortality 96 hours later. Organisms were exposed to the field rate being used in the *Oc. camptorhynchus* eradication campaign (6 kg/ha) and to a rate 10 times higher than field rate (60 kg/ha). The species tested were *Salmo gairdneri* (Osteichthyes: Salmonidae), *Tenagomysis novaezealandiae* (Crustacea: Mysidacea), *Herpetocypcis pascheri* (Crustacea: Ostracoda), *Austrolestes colesonis* (Insecta: Lestidae), *Anisops assimilis* (Insecta: Notonectidae), *Ephydrella novaezealandiae* (Insecta: Ephydridae) and *Chironomus zealandicus* (Insecta: Chironomidae). Mortality was not significantly higher after exposure to either rate in any of these species except *C. zealandicus,* which showed increased mortality only after exposure to 10 times the field rate. Additionally, Ali (1991) reported that many chironomid species are less susceptible to pesticides than mosquitoes. There is still the question about whether methoprene causes deformities in frogs (Stark 2005a,b). New Zealand is home to several indigenous species of frogs but fortuitously none of these species live in the saltmarsh habitat and therefore, pesticides applied for control of mosquitoes in saltmarsh habitats should have no effect on native frogs. As a result, any nontarget study should focus on chironomid midges because they are the most likely group of organisms to be negatively affected. Chironomids are an important food source for many

organisms including waterfowl, fish and aquatic invertebrates. However, it is questionable as to whether saltmarsh habitats are frequented by chironomids. Several studies have indicated that chironomids in Australia can tolerate a wide range of salinity (1,900-255,000 mg/l) depending upon the species (Timms 1993, 1998a,b). Although some chironomids have a fairly high salt tolerance, most species prefer freshwater. Browne et al. (unpublished) reported that pilot studies indicated that fringe areas of the brackish water operation zones had large numbers of the native chironomid species, *C. zealandicus.* Even though freshwater streams and rivers drain through both the Kaipara Harbour and Wairau Lagoons, most of the detections of southern saltmarsh mosquito have been in saline water and pesticides have only been applied to brackish water. Thus, if few chironomids inhabit the brackish water in these areas, then it is a waste of time to monitor effects on chironomids in these areas. Therefore, prior to any nontarget study of chironomids, baseline information on the chironomid populations in saltmarshes needs to be collected. Additionally, all evidence to date indicates that any impact on chironomid populations could be expected to be temporary because pesticides applications are being made to relatively small areas. Recolonisation of affected organisms will be rapid from surrounding untreated areas. This outcome has been seen before in other studies and therefore no new information will be gained by a nontarget study of Bti and methoprene. Chironomid populations from nearby untreated areas should remain unaffected and thus continue to provide food for the organisms that readily feed on these species. Consequently, I consider a nontarget study to be unnecessary when Bti and methoprene are the pesticides being used for eradication. However, below, I have outlined an approach to evaluate nontarget effects in an eradication programme, if the Ministry of Health, in the knowledge that chironomids are an important component of saltmarsh ecosystems in New Zealand, decides that it is necessary. The primary focus of such a nontarget study should be chironomids. Surveys of waterfowl that either permanently inhabit treated areas or utilise these areas during migration and feed on chironomids and other invertebrates may also be warranted if large numbers of chironomids are found to inhabit marshlands.

8.4 Chironomid sentinel organism study

Because the present eradication programme is well underway, it may not be possible to sample for chironomids now. After the eradication programme is completed, baseline data could be collected on the chironomids inhabiting saltmarsh regions that might be treated in the future. This can be accomplished by taking biweekly sediment cores from the spring until late summer in selected areas. A PVC pipe corer as described in Hershey et al. (1998) can be used to take the sediment cores. The cores can be taken back to the laboratory and analysed for chironomid larvae.

If a large number of individuals and/or species of chironomids are found in the brackish water of the Kaipara Harbour and Wairau Lagoons/Lake Grassmere areas, then sampling for effects on these species in future eradication programmes might be necessary. However, because all areas may potentially be treated in an eradication programme, it may be difficult, if not impossible, to establish valid control sites. This precludes the use of dip samples to monitor nontarget effects. Larval and pupal mosquitoes are monitored by taking dip samples with a dip cup. Swimming nontarget organisms are also collected when taking dip samples and can thus be monitored during the routine sampling for mosquitoes. Because all saline water in a given area might be treated, nontarget organisms collected in dip samples cannot be compared to nontargets caught in untreated areas. The same thing applies for sampling of sediments for chironomid larvae. This

means that a different approach must be taken. Sentinel organisms can be used successfully in an eradication programme to monitor nontarget effects. First midge larvae must be collected from the field by taking dredge or core samples from various areas. The sediment samples are taken to the laboratory and placed in 10 litre-aquaria. Adults are reared and identified. The most abundant species will be used as the surrogate organism for the *in situ* studies. The most abundant species is raised in the laboratory for future use in the field. Detailed midge rearing techniques are presented in USEPA (1994) and Tucker and Burton (1999). Large glass jars (one litre or larger) can be used as *in situ* containers for the field study. A fine layer of sand is placed in the bottom of the jars and 10 midge larvae are added to each container. Groups of jars are transported to the field placed at random next to water to be treated with pesticides. Half of the jars remain open and the other half is sealed with lids. A minimum of eight jars (four controls and four treated) per test should be used in each field test. After pesticides are applied, lids are placed on the treated jars and all of the jars are transported to the laboratory. Adult midge emergence is monitored in control and treated containers. Emergence data are analysed with a t-test.

The problem with conducting this study is that it may not provide any new information regarding the effects of methoprene and Bti on nontarget organisms. The anticipated outcome of this study is that both pesticides will reduce midge emergence.

8.5 Bird surveys

If in the earlier monitoring phase for chironomids, it is found that large numbers of midges inhabit the saltmarsh areas of Kaipara Harbour and Wairau Lagoons/Lake Grassmere, then a bird survey might be necessary to see if nesting success is affected by a reduction in midge populations. In the studies by Hanowski (1997a,b) waterfowl and other bird species living in wetlands either switched food sources or travelled outside the treatment zone to collect food (Stark 2005 a,b). Thus it was concluded that applications of methoprene and Bti had no negative effect on birds. However, any disruption to nesting birds such as having to travel farther distances than usual to obtain food may have a negative impact on nesting success. For example, vulnerability to predators may increase while adults are away from the nest for long periods of time. As mentioned above though, nearby untreated areas should provide large numbers of chironomids as a food source for birds and other organisms.

If a bird survey is deemed necessary, then waterfowl that either permanently inhabit treated areas or utilise these areas during migration should be monitored. A census can be taken of selected species during the breeding season or when birds move into the area as part of a migration pattern. Methods for surveying birds are presented in Hanowski et al. (1997).

9. Conclusions

Methoprene and Bti are being used to eradicate the southern saltmarsh mosquito, *Oc. camptorhynchus* from Kaipara Harbour and the Wairau Lagoons/Lake Grassmere areas of New Zealand. In previous studies both of these pesticides have been found to cause only minor effects on nontarget organisms. Chironomid midges, which are considered pest species in many parts of the world, are susceptible to both products and long-term use can cause significant declines in midge populations. Eradication programmes are not

conducive for nontarget studies because all areas where pests are detected must be treated. This leaves no untreated areas that can serve as control sites. Therefore, few options exist for a valid nontarget monitoring programme. Effects of methoprene and Bti on chironomid midges can be evaluated using a semi-field approach with sentinel midges. Midges can be placed *in situ* in jars prior to pesticide application. Controls consist of midges in jars with lids so that no pesticide exposure occurs. Comparisons of adult midge emergence between treated and untreated jars will determine whether pesticide treatments at the concentrations being applied are having a negative effect on midges. Birds that inhabit salt marshes may also be surveyed for population declines. However, results of both the sentinel midge study and bird survey will probably not provide any new information on the nontarget effects of methoprene and Bti. The likely result of methoprene and Bti applications for eradication of *Oc. camptorhynchus* will be temporary reductions in midge populations. Consequently, I consider that a nontarget study for methoprene and Bti in the *Oc. camptorhynchus* eradication programme is unnecessary.

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11. References

Ali, A. 1991. Perspectives on management of pestiferous Chironomidae (Diptera), an emerging global problem. *Journal of the American Mosquito Control Association* **7:** 260-281.

Banken, J.A.O., Stark, J.D. 1998. Multiple routes of pesticide exposure and the risk of pesticides to biological controls: A study of neem and the seven-spot lady beetle, *Coccinella septempunctata* L. *Journal of Economic Entomology* **91:** 1-6.

Boothroyd, I.K.G. 1999. Description *of Kaniwhaniwhanus* gen. n. (Diptera: Chironomidae: Orthocaldiinae) from New Zealand.

Boothroyd, I.K.G., Cranston, P. 1995. Two Orthocladiinae (Chironomidae) genera common to New Zealand and Australia: *Pirara* n. gen. and *Eukiefferiella* Thienemann. In Cranston, P.S. [ed.] Chironomids: from genes to ecosystems. 1995. CISRO, Melbourne. pp. 389-408.

Brown, M.D., Thomas, D., Mason, P., Greenwood, J.G., Kay, B.H. 1999. Laboratory and field evaluation of the efficacy of four insecticides for *Aedes vigilax* (Diptera: Culicidae) and toxicity to the nontarget shrimp *Leander tenuicornis* (Decapoda: Palaemonidae). *Journal of Economic Entomology* **92:** 1045-1051.

Browne, G.N., Taylor, J.F., Dunford, P.R. (unpublished). Acute Mortality of Non-Target Organisms Exposed to S-Methoprene (Prolink™ XR-G).

Burton, Jr., G.A. 1991. Assessing freshwater sediment toxicity. *Environmental Toxicology and Chemistry* **10:** 1585-1627.

Burton, W.H., Weisberg, S.B., Brindley, Jr. A.G. 1992. Early life stage survival of striped bass in the Delaware River, USA. *Archives of Environmental Contamination and Toxicology* **23:** 333–338.

Campbell, B.C., Denno, R.F. 1976. The effect of temephos and chlorpyrifos on the aquatic insect community of a New Jersey salt marsh. *Environmental Entomology* **5:** 477-483.

Chappie, D.J., Burton, Jr. G.A. 1997. Optimization of in situ bioassays with *Hyalella azteca* and *Chironomus tentans. Environmental Toxicology and Chemistry* **16**: 559-564.

Clements, W.H., Farris, J.L., Cherry, D.S., Cairns, Jr., J. 1989. The influence of water quality on macroinvertebrate community responses to copper in outdoor experimental streams. *Aquatic Toxicology* **14:** 249–262.

Collier, K.J. 1993. Flow preferences of larval Chironomidae (Diptera) in Tongariro River, New Zealand. *New Zealand Journal of Marine and Freshwater Ecology* **27:** 219-26.

Croft, B. 1990. Arthropod Biological Control Agents and Pesticides. John Wiley and Sons, New York. 723 pp.

EPA. 1998a. Reregistration Eligibility Decision (RED) - *Bacillus thuringiensis*. U.S Environmental Protection Agency, Washington DC, U.S.A. 170 pp.

EPA. 1998b. Reregistration Eligibility Decision (RED) - Isopropyl (2E, 4E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate (refered to as Methoprene). U.S Environmental Protection Agency, Washington DC, U.S.A. 47 pp.

Forbes, V.E., Calow, P. 1999. Is the per capita rate of increase a good measure of population-level effects in ecotoxicology? *Environmental Toxicology and Chemistry* **18:**1544-1556.

Frampton, E.R. 2004. Pathways of entry and spread of exotic mosquitoes, with particular reference to southern saltmarsh mosquito, *Ochlerotatus camptorhynchus*. *Report for New Zealand Ministry of Health*.

Glare, T.R., O'Callaghan, M. 1998. Environmental and health impacts of *Bacillus thuringiensis israelensis*. Report for the New Zealand Ministry of Health.

Glare, T.R., O'Callaghan, M. 1999. Environmental and health impacts of the insect juvenile hormone analogue, S-methoprene. Report for the New Zealand Ministry of Health.

Hall, Jr., L.W., Bushong, S.J., Ziegenfuss, M.C., Hall W.S., Herman R.L. 1988. Concurrent mobile on-site and in situ striped bass contaminant and water quality studies in the Choptank River and upper Chesapeake Bay. *Environmental Toxicology and Chemistry* **7:** 815–830.

Hanowski, J.M., Niemi, G.J., Lima, A.R., Regal, R.R. 1997a. Response of breeding birds to mosquito control treatments of wetlands. *Wetlands* **17:** 485-492.

Hanowski, J. M.; Niemi, G. J.; Lima, A. R.; Regal, R. R. 1997b. Do Mosquito control treatments of wetlands affect red-winged blackbird (*Agelaius phoeniceus*) growth, reproduction, or behavior? *Environmental Toxicology and Chemistry* **16:** 1014-1019.

Hershey, A.E., Lima, A.R., Niemi, G.J., Regal, R.R. 1998. Effects of *Bacillus thuringiensis israelensis* (BTI) and methoprene on nontarget macroinvertebrates in Minnesota wetlands. *Ecological Applications* **8:** 41-60.

Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. *Ecological Monographs* **54:** 187-211.

Ireland, D. S., Burton, Jr. G.A., Hess, G.G. 1996. In situ toxicity evaluations of turbidity and photoinduction of polycyclic aromatic hydrocarbons. *Environmental Toxicology and Chemistry* **15**: 574-581.

Jackson, J. K.; Horwitz, R. J.; Sweeney, B. W. 2002. Effects of *Bacillus thuringiensis israelensis* on black flies and nontarget macroinvertebrates and fish in a large river. *Transactions of the American Fisheries Society* **131:** 910-930.

Jones, P.A., Sloan, R.J. 1989. An in situ river exposure vessel for bioaccumulation studies with juvenile fish. *Environmental Toxicology and Chemistry* **8:** 151–155

Klaine, S.J., Cobb, G.P., Dickerson, R.L., Dixon, K.R., Kendall R.J., Smith, EE., Solomon, K.R. 1996. An ecological risk assessment for the use of the biocide, dibromonitrilpropionamide (DBNPA), in industrial cooling systems. *Environmental Toxicology and Chemistry* 15: 21-30.

Kreutzweiser, D.P., Capell, S.S., Scarr, T.A. 1999. Acute lethal and sublethal effects of a neem-based insecticide on nontarget aquatic insects in stream channels. *Bulletin of Environmental Contamination and Toxicology* **63:** 365-371.

Kreutzweiser, D.P., Capell, S.S., Scarr, T.A. 2000. Community-level responses by stream insects to neem products containing azadirachtin. *Environmental Toxicology and Chemistry* **19:** 855-861.

Lawler, S.P., Jensen, T., Dritz, D.A., Wichterman, G. 1999. Field efficacy and nontarget effects of the mosquito larvicides temephos, methoprene, and *Bacillus thuringiensis* var. *israelensis* in Florida mangrove swamps. *Journal of the American Mosquito Control Association* **15:** 446-452.

Lawler, S.P., Dritz, D.A., Jensen, T. 2000. Effects of sustained-release methoprene and a combined formulation of liquid methoprene and *Bacillus thuringiensis israelensis* on insects in salt marshes. *Archives of Environmental Contamination and Toxicology* **39:** 177-182.

Lozano, S.J., Halloran, S.L., Sargent, K.W., Brazner, J.C. 1992. Effects of esfenvalerate on aquatic organisms in littoral enclosures. *Environmental Toxicology and Chemistry* **11:** 35-48.

Niemi, G.J., Hershey, A.E., Shannon, L., Hanowski, J.M., Lima, A., Axler, R.P., Regal, R.R. 1999. Ecological effects of mosquito control on zooplankton, insects, and birds. *Environmental Toxicology and Chemistry* **18:** 549-559.

Palmer, R. W.; Palmer, A. R. 1995. Impacts of repeated applications of *Bacillus thuringiensis var israelensis* de Barjac and Temephos, used in blackfly (Diptera: Simuliidae) control, on macroinvertebrates in the middle Orange River, South Africa. *Southern African Journal of Aquatic Sciences* **21:** 35-55.

Pierce, R., Henry, M.S., Kelly, D., Kozlowski, W. 2000. Hazard assessment of temephos applied to a southwest Florida, USA, salt marsh community. *Environmental Toxicology and Chemistry* **19:** 501-507.

Pinkney, A.E., McGowan, P.C., Murphy, D.R., Lowe, T.P., Sparling, D.W., Meredith, W.H. 1999. Effects of temephos (Abate(R) 4E) on fiddler crabs (*Uca pugnax* and *Uca minax*) on a Delaware salt marsh. *Journal of the American Mosquito Control Association* **15:** 321-329.

Pinkney, A.E., McGowan, P.C., Murphy, D.R., Lowe, T.P., Sparling, D.W., Ferrington, L.C. 2000. Effects of the mosquito larvicides temephos and methoprene on insect populations in experimental ponds. *Environmental Toxicology and Chemistry* **19:** 678-684.

Sasson-Brickson, G., Burton, Jr. G.A. 1991. In situ and laboratory sediment toxicity testing with *Ceriodaphnia dubia. Environmental Toxicology and Chemistry* **10:** 01-207.

Shaw, J.L., Maund, S.J., Hill, I.R. 1995. Fathead minnow reproduction in outdoor microcosms: A comparison to bluegill sunfish reproduction in large mesocosms. *Environmental Toxicology and Chemistry* **14:** 1753–1762.

Shaw, J.L., Manning, J.P. 1996. Evaluating macroinvertebrate population and community level effect in outdoor microcosms: use of in situ bioassays and multivariate analysis. *Environmental Toxicology and Chemistry* **15:** 608-617.

Simonin, H.A., Kretser, W.A., Bath, D.W., Olson, M., Gallagher, J. 1993. In situ bioassays of brook trout (*Salvelinus fontinalis*) and blacknose dace (*Rhinichthys atratulus*) in Adirondack

streams affected by episodic acidification. *Canadian Journal of Fisheries and Aquatic Science* **50:** 902–911.

Solomon, K.R., Stephenson, G.L., Kaushik, N.K. 1989. Effects of methoxychlor on zooplankton in freshwater enclosures: influences of enclosure size and number of applications. *Environmental Toxicology and Chemistry* **8:** 659-669.

Solomon, K.R., Baker, D.B., Richards, R.P., Dixon, K.R., Klaine, S.J,, La Point, T.W., Kendall, R.J., Giddings, J.M., Giesy, J.P., Hall, Jr. L.W., Williams, W.M. 1996a. Ecological risk assessment of atrazine in North American surface waters. *Environmental Toxicology and Chemistry* 15: 31-76.

Solomon, K.R., Baker, D.B., Richards, R.P., Dixon, K.R., Klaine, S.J., La Point, T.W., Kendall, R.J., Weisskopf, C.P., Giddings, J.M., Giesy, J.P., Hall, Jr., L.W., Williams, W.M. 1996b. Ecological risk assessment of atrazine in North American surface waters. *Environmental Toxicology and Chemistry:* Vol. 15, No. 1, pp. 31–76.

Stark, J.D. 1992. Comparison of the impact of Margosan-O and chlorpyrifos on non-target invertebrates inhabiting turf grass. *Pesticide Science* **36:** 293-299.

Stark JD. in press. Short-term effects of the neem insecticide, Margosan-O and chlorpyrifos on a spider (Araneida: Linyphiidae) community inhabiting turf grass. *Biopesticides International.*

Stark, J.D. 2005a. A review and update of the report "Environmental and health impacts of *Bacillus thuringiensis israelensis*" 1998 by Travis R. Glare and Maureen O'Callaghan. http://www.moh.govt.nz/moh.nsf/c7ad5e032528c34c4c2566690076db9b/ff3b628d67e34963cc256ba3000d8476?OpenDocument

Stark, J.D. 2005b. A review and update of the report "Environmental and health impacts of the insect juvenile hormone analogue, S-methoprene" 1999 by Travis R. Glare and Maureen O'Callaghan. http://www.moh.govt.nz/moh.nsf/c7ad5e032528c34c4c2566690076db9b/ff3b628d67e34963cc256ba3000d8476?OpenDocument

Stark, J.D. 2005c. How closely do acute lethal concentration estimates predict effects of toxicants on populations? *Integrated Environmental Assessment and Management* 1: 109-113.

Stark, J.D., Banks, J.E. 2000. The toxicologists' and ecologists' point of view-unification through a demographic approach. Chapter 2, pp. 9-23. In: Eds: Kammenga and Laskowski, Demography in Ecotoxicology. John Wiley & Sons, Ltd. London.

Stark, J.D., Banks, J.E. 2001. "Selective Pesticides": Are They Less Hazardous to the Environment? *BioScience* **51:** 980-982.

Stark, J.D., Vargas, R.I. 2003. Demographic changes in *Daphnia pulex* (leydig) after exposure to the insecticides spinosad and diazinon. *Ecotoxicology and Environmental Safety* **56:** 334-338.

Stark JD, Banks JE, Vargas RI. 2004. How risky is risk assessment? The Role that Life History Strategies Play in Susceptibility of Species to Stress. 2004. *Proceedings of the National Academy of Sciences* 101: 732-736.

Stark J.D., Vargas, R.I. in press. Toxicity and hazard assessment of Fipronil to *Daphnia pulex*. *Ecotoxicology and Environmental Safety.*

Timms, B.V. 1993. Saline lakes of the Paroo inland New South Wales, Australia. *Hydrobiologia* **267:** 269-289.

Timms, B.V. 1998a. Further studies on the saline lakes of the eastern Paroo inland New South Wales, Australia. *Hydrobiologia* **381:** 31-42.

Timms, B.V. 1998b. A study of Lake Wyara, an episodically filled saline lake in southwest Queensland, Australia. *International Journal of Salt Lake Research* **7:** 113-132.

Tucker, K.A., Burton Jr., G.A. 1999. Assessment of nonpoint-source runoff in a stream using *in situ* and laboratory approaches. *Environmental Toxicology and Chemistry* **18:** 2797 – 2803.

United States Environmental Protection Agency 1994. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA 600/R-94/024. Office of Research and Development, Duluth, MN.

Wayland, M. 1991. Effect of carbofuran on selected macroinvertebrates in a prairie parkland pond: an enclosure approach. *Archives of Environmental Contamination and Toxicology* **17:** 165-175.

Wilde, E.W., Parrott, A.B. 1984. A simple, inexpensive in situ method for assessing acute toxicity of effluents to fish. *Water Research* **18:** 783–785.

Ziegenfuss, M.C., Hall, Jr., L.W., Bushong, S.J., Sullivan, J.A., Unger, M.A. 1990. A remote in situ apparatus for ambient toxicity testing of larval and yearling fish in river or estuarine systems. *Environmental Toxicology and Chemistry* **9:** 1311–1315.