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Changes in Zebrafish (Danio rerio) Startle Responses due to Stressors Related to Marine Bycatch

Margot Wilsterman
Connecticut College, margotwilsterman@yahoo.com

Changes in Zebrafish (*Danio rerio*) Startle Response Due to Stressors Related to Marine Bycatch.

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Margot Wilsterman '15

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Correspondence: mwilster@conncoll.edu
Adviser: Robert Askins, Department of Biology, Connecticut College
Readers: Stephen Loomis, Department of Biology, Connecticut College
Karina Mrakovcich, Department of Marine Science, United States Coast Guard Academy

Abstract

Marine bycatch is one of the most pressing issues that result from modern industrialized fishing. Scientists do not have good estimates of rates of cryptic mortality, which is the mortality of individuals that are not landed but die from interactions with fishing gear below the water surface. One of the main causes of cryptic mortality is depredation, which occurs after individuals have escaped through fishing equipment and are eaten by predators waiting close behind for an effortless meal. In this study the effects of scale loss, slime loss, and exhaustion on the startle response of zebrafish were examined. These stressors may lead to the depredation of escapees from trawls and other fishing equipment. To test this hypothesis, zebrafish in four treatment groups were first exposed to scale loss, slime loss, exhaustion or no stressor and then exposed to a model predator. There were two sets of experiments, one with individual fish, and the other with fish in schools. Descaled zebrafish travelled a significantly shorter distance than zebrafish in the control group. The distance to nearest neighbor was also significantly greater in the descaling group when compared to the control in the schooling trial. In the schooling trials, fish in the exhaustion and slime loss trials travelled significantly shorter distances when compared to the control, but this was not seen in the individual trials, suggesting that schooling behaviors are an important consideration when analyzing startle responses. By understanding how interactions with fishing gear influence fish behavior and physiology, fisheries managers can learn the true impact that bycatch is having on stocks, facilitating more educated management decisions.

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Introduction

Until recently scientists have struggled to quantify the effects of bycatch, because it is for the most part invisible, and in the past had a smaller impact than it has now (Swartz et al., 2010). Much as humans are able to ignore the somewhat hidden issue of climate change, they are also able to overlook millions of pounds of wasted marine resources per year because what is out of sight is also out of mind. In fishing events that involve nets, many individuals are able to escape through the mesh. These escapees compose an invisible and understudied group of fish that may be heavily impacted in their physiology and behavior by interactions with fishing gear. In this study I examined how three important stressors associated with escape from fishing gear - scale loss, slime loss, and exhaustion - affect the startle response of zebrafish were examined. These stressors may reduce escape responses, leading to depredation of escapees from trawls and other fishing equipment

The past tendency of conservationists to focus on single species management has also created a situation where bycatch of one species may not be an issue, but the entire ecosystem and many of its inhabitants are still impacted. One classic example of this focus on a single species is the “dolphin safe” canned tuna fish label. In 1990, the Earth Island Institute and the International Marine Mammal Project teamed up to develop a campaign that included a consumer boycott to decrease dolphin bycatch levels in the multi-million tuna fishing industry, which used harmful drift nets and purse seines to capture these valuable fish. This consumer pressure caused the three largest tuna companies in the world to change their practices, and now about 90% of canned tuna is “dolphin safe” (Earth Island Institute, 2009). While this was definitely a success in terms

of dolphin conservation, it displays the flaws of a single species conservation approach. The issues of the sustainability of this type of fishing in terms of the bycatch of other species is largely ignored and so are the tuna, many of which (including Albacore Tuna (*Thunnus alalunga*), Yellowfin Tuna (*Thunnus albacares*), and Bluefin Tuna (*Thunnus thynnus*)) are labeled as vulnerable, endangered, or critically endangered by the IUCN (IUCN, 2011). The general public's understanding of which marine animals are worth saving is largely based on the "cuddly" factor. Humans feel a connection with dolphins, whales, and sea turtles that they simply do not feel with the harsher looking tuna (*Thunnus thynnus*) or a simple scup (*Stenotomus chrysops*). Many of these less charismatic organisms then become "trash fish" that are so valueless to humans that it seems appropriate to throw them overboard from a fishing boat after they are dead or dying.

With modern technology, bycatch has become too large of an environmental issue to ignore. This unnecessary waste carries with it moral, economical, and biological issues. Morally, it is difficult for policy makers and marine resource managers to justify this level of waste. In what other type of harvesting is there this much waste of perfectly good resources? With about ten to fifteen kilograms of discards for every one-kilogram of shrimp landed (Alverson et al., 1994), fishers and legislators will soon be forced to reduce such waste of an internationally important source of protein. Economically, most bycatch is a nuisance to fishers. Either the fish caught are too small to be retained or they are undesirable species that will not sell well in the market. Keeping these unwanted fish would require increased freezer space on vessels and would fill quotas more quickly (Alverson et al., 1994). Biologically, fishing gear can drastically change the structure of

the ocean floor and affect trophic structure by changing abundances of many marine species (Collie et al., 1997). The waste produced through bycatch could potentially cause ecosystems to collapse and many may never be the same again. The biological impacts make the reduction of bycatch worthwhile for fishers and managers alike.

With this much waste there is huge mortality for fishes in all trophic levels. Not only are the fishes themselves being harvested, but also their reproductive potential is ruined. The young of the harvested species can also be overfished, creating a population that is no longer able to grow or sustain a stable population (Davis, 2002). The target species are usually landed and the non-target species are thrown back into the water, most likely dead or dying. Even target species are victims of bycatch, however, because there are size restrictions that require fishers to throw individuals back that are under a certain length. These fish are likely injured, if not killed in the process of being caught and then discarded. Often targeted fish will be damaged in the process of landing and will be discarded, as the fishers know they will gain nothing from these individuals at market. This decrease in abundance of target species causes fishers to change their practices to continue catching the remaining fish. For example a fishing vessel without a fish finder would have much less success than those with this technology. This race to catch the few remaining target fish from stocks that were once plentiful causes a “natural selection” of fishers (Hall et al., 2000). They can either keep up with new and expensive technology to find fishes in deeper, darker places, or they can lose their jobs. The ecological damage that bycatch creates is often catastrophic, leading to massive population declines and changes in trophic structure. Bycatch is a threat to most pelagic and benthic species and

the unnecessary deaths of these individuals creates a situation where the general public must make the "distinction between wants and needs" (FAO, 2001).

There are many problems with the current policy regarding marine bycatch. These stem from a lack of communication and trust between policy makers, scientists, and fishers. Cardinale and Svedang (2008) claimed "it is the practice of ignoring the scientific advice more than the advice itself that is to be blamed for the waste of former large marine resources." Most of the current legislation is economically and politically focused, and the lobby from the commercial fishing industry has tremendous power to sway management decisions (Froese, 2011). For example, despite the known decline in bluefin tuna abundance, lobbyists were able to keep this species off the endangered species list for years because of its economic importance and the Pacific stock is still labeled as only "vulnerable" (Collette et al., 2014). Many policies also promote research on the topic of bycatch as a placeholder but don't offer any real solutions, leaving fishers able to use many loopholes. Another major issue in the current policy is the single-species focus, which tends to be inadequate for protecting marine resources.

In spite of these setbacks, there has been some progress. Policies such as the Marine Mammal Protection Act (1972) and the Endangered Species Act (1973) helped to put pressure on fishers to use more sustainable methods. Policies focused on the threat of bycatch have also been created. For example, the United States played a large part in the implementation of the United Nations moratorium on large-scale pelagic driftnets (1992), a very destructive type of fishing gear (Bellido et al., 2011). The Magnuson-Stevens Act of 1995 was a very important amendment to the Magnuson Act of 1976. One of the three main requirements of this act was to "reduce bycatch or reduce mortality of bycatch"

(The Magnuson-Stevens Act, 1995). Though this has not happened on a large scale, many steps have been taken to improve the situation. Some policies such as those in Iceland and Norway ban the discard of some or all species (CFP; European Commission, 2011). The Common Fisheries Policy of the EU even flirted with this idea (Bellido et al., 2011). The 1999 FAO International Plan of Action for Reducing Incidental Catch of Seabirds in Longline Fisheries and the 2009 FAO Guidelines to Reduce Sea Turtle Mortality in Fishing Operations have also helped to decrease bycatch of certain species but ignore the bulk of the less charismatic organisms that are also being affected by bycatch. In response to the ineffectiveness of these policies, more and more countries are beginning to use "ecosystem-based management," which takes all aspects of the marine environment into consideration rather than focusing on a single species or ecosystem (Bellido et al., 2011).

Creating a Definition of Bycatch

There are many different definitions of bycatch, which causes further confusion for implementing policy. Some believe that bycatch refers to non-target fish species whether they are discarded or landed (kept for sale). Others believe that bycatch consists of all fishes, both target and non-target species, that are returned to the sea (Alverson et al., 1994). The Magnuson-Stevens Fishery Conservation and Management Act defines bycatch as, "fish which are harvested in a fishery, but which are not sold or kept for personal use, and includes economic discards and regulatory discards. Such term does not include fishes released alive under a recreational catch and release fishery management program" (The Magnuson-Stevens Act, 1995). This definition however, does not

incorporate marine mammals, elasmobranchs, sea birds, or sea turtles, which are common victims of bycatch. Because of this the National Oceanic and Atmospheric Administration (NOAA) has started defining bycatch as: "discarded catch of any living marine resource, plus unobserved mortality due to a direct encounter with fishing gear" (NOAA). Other organizations and fisheries researchers are trying to create even more comprehensive definitions to make sure all organisms are covered. For example, Davies et al. (2008) put forth a new definition: "bycatch is catch that is either unused or unmanaged." This broad definition attempts to create a consistent definition that includes all marine animals from small invertebrates to megafauna. However the term "unmanaged" is so vague that it makes the definition confusing. This confusion with the precise meaning of bycatch leaves marine managers and policy makers in an even more difficult situation.

Types of Bycatch

There are many different subcategories of marine bycatch. The first distinction that must be made is between economic and regulatory discards. Regulatory discards are defined as "catch that is required by regulation to be discarded" (Kelleher, 2005). These regulations are set by governments in an effort to maintain healthy fish stock sizes. Economic discards or discretionary discards on the other hand, are defined as "catch that is discarded because of undesirable species, size, sex or quality, or for other non-regulatory reasons" (Kelleher, 2005). Another type of economic discard, which has become popular with the rise of quota restrictions, is highgrading or the "discarding of lower value commercial catch to maximize the value of quota" (Kelleher, 2005). In other

words, fishers will discard small but good quality fish in an effort to catch larger more profitable fish. Highgrading can be economically beneficial “at the firm level” (Squires et al. 1998), but it is an ecologically wasteful practice because there are high mortality levels associated with the fish discarded in this practice.

Once a fish is caught there is a chain of events that decides the fate of the animal. The individual fish can either be landed, contributing to the total fishing mortality, or discarded. If a fish is discarded, it can either be discarded dead, again contributing to the total fishing mortality, or it can be discarded alive. Both of these distinctions are fairly easy to make. However, when it comes to fish that are discarded alive there are those that live and those that die. It is this discard mortality rate that has stumped most scientists over the years. There are many events that occur after releasing a live fish that can lead to an untimely death. Post-release mortality due to stress, injury, or infection is one of the main causes of death for a fish after a fishing event. This problem has been heavily studied recently in an effort to predict what percentages of discarded individuals survive post-release. Post-release predation is another factor that must be taken into consideration. After a fish has been discarded it has already been through many stressors including long handling times and air exposure, physical trauma from interactions with the net, and being crowded in an area with many other fish. When these weakened fish are released back into the ocean, their anti-predation behaviors are often less efficient leaving them an easy target for nearby predators (Gilman et al., 2013).

There is another group of fish that are the focus of this study. These fish are never landed but are still affected by fishing gear in one way or another. Escapee mortality refers to fishes escaping from the fishing gear in question yet dying from physical trauma

inflicted by the gear. For example, a fish that fights its way out of a net will often lose some of its scales and its slime layer in the process, which can lead to infection (Olsen et al., 2012). Avoidance mortality refers to individuals who are killed in their effort to avoid fishing gear, which often occurs with industrial fishing. Many fish try to avoid gear but they are fatally injured in the process. For example, a fish trying to avoid a trawl net may be crushed by the metal plates holding it to the ocean floor. Another byproduct of large-scale industrial fishing is habitat degradation mortality, which refers to the death of individuals whose habitats were ruined by the passing of fishing gear such as dredges or bottom trawls (FAO, 2001). Lastly, depredation occurs when an individual is caught in fishing gear that has not been brought up yet and they are preyed upon while they are still caught and defenseless. For example, many fish caught on long lines will be eaten off the hook by sharks or other larger species (Tixier et al., 2014).

Fishing Gear and Their Impact

Fishers have a wide range of gear at their disposal and some types of equipment are much more harmful to the ocean environment than others. There are two types of trawling both of which are very efficient at catching target and non-target species that they come in contact with. Bottom trawling disrupts the natural architecture of the sea floor, creating a homogeneous area with fewer habitats needed by many species. Jennings et al. (2001) report that these changes could lead to collapses in trophic structure and function of entire benthic communities. They found that the biomass of infauna and epifauna was drastically reduced, with significant decreases in the abundance of bivalves and sea urchins. There are also high levels of bycatch associated with bottom trawling,

especially various shrimp trawls which can have up to 98% of the catch discarded (Alverson et al., 1994). Dredging involves dragging a heavy metal frame with an attached mesh bag to collect shellfish living on or below the sea floor. Much like bottom trawling, this method of fishing is very destructive to the marine environment as it can smooth out complex benthic areas, destroying habitats and crushing individuals. Jenkins et al., (2001) found that not only were non-target species damaged through direct bycatch, but also by being crushed. In this case, post-release mortality, escapee mortality, and depredation all have to be taken into consideration.

Though mid-water trawls are not detrimental to the ocean floor and benthic communities, they still produce a large amount of bycatch and are harmful to the stocks of many species. The largest trawling nets are large enough to hold 13 Boeing 747s (The End of the Line, 2009). With nets of this scale there will inevitably be high levels of bycatch. Long lining involves putting out a line that can be up to 30 miles long (Lewison et al., 2004). On this line hooks are placed at intervals to maximize catch of large species such as tuna and swordfish. This type of gear is very destructive, as it lacks any selectivity. Sea turtles, marine mammals, sharks, and sea birds are just a sampling of the animals that end up on these hooks. Because there is a soak time of up to 20 hours involved (Ward et al., 2004), individuals that are hooked have a slim chance of survival. Beach and purse seining, when used on a small scale, are usually not very destructive. However, on an industrial level purse seining can be hugely impactful and can create large amounts of bycatch. Purse seines function by lowering long walls of nets that are cinched closed underneath a group of fish. These nets mostly target schools of tuna, but they are large enough to entrap marine megafauna and other possibly endangered species

along with the target catch (Lewison et al., 2004). Gillnetting is a more passive method of fishing that involves a wall of netting suspended in the water by floats at the surface and weights at the bottom. These nets have mesh that allows for only the head of the animal to get through the net, causing the gills to get caught and leaving the fish helpless. These long, cost effective, and versatile nets are not very selective and they result in the bycatch of many species. They also play a role in the "ghost fishing" phenomenon, which occurs when lost fishing gear continues to catch and kill animals with no benefit to humans (Breen, 1989). Fishers also often use pots and traps to catch lobsters, crabs, and other bottom-dwelling species. This type of fishing gear has very low selectivity, allowing anything of a certain size to fit into the trap. Common in coral reefs, these traps can catch ecologically important herbivorous species such as parrotfish and surgeonfish (Johnson, 2010). Similar to gill nets, these traps and pots can become detached from their floats on the surface and forgotten about, becoming "ghost traps" which continue to kill and waste individuals.

Species Most Susceptible

There are many species that face bycatch, but certain characteristics and life history traits make some animals more susceptible than others. Harrington et al. (2005) concluded that 51% of discards in the United States are made up of crustaceans. This number is likely similar globally because so many varieties of fishing gear are in contact with the benthic environment where most crustaceans live. When it comes to bony fishes, the bycatch is so diverse it is hard to determine which species are most vulnerable. Depending on the fishing gear used, both benthic and pelagic fishes of all sizes are at

risk. Long-lived species such as the orange roughy (*Hoplostethus atlanticus*), which reaches sexual maturity late in life, would be less likely to rebound from declines in abundance. Physiologically, species that are "heartier" and larger have thicker skin, and tougher scales. These fishes may be able to more successfully withstand the injuries caused by fishing nets. However, they are still often victims of bycatch and can be harmed by gear. Dogfish, skates, monkfish, hakes, flatfishes, butterfish and cod are the major discarded fish species in the Northeast fishery region. There are also highly migratory species, such as sharks, tunas, and billfishes that are especially prone to bycatch because their long-distance movements translate into a challenging global management (Harrington et al, 2005). This is a highly diverse group of fishes; some very ancestral like the dogfish or skate, and some very derived like the flatfishes. For the purposes of this review, I have focused mainly on species most susceptible to bycatch in the Northeast. In other regions (Southeast, West Coast, Alaskan, and Western Pacific), different species are more commonly found as bycatch, and researchers at NOAA have attempted to categorize bycaught species for these regions (NOAA, 2011).

One group of species that are subject to bycatch in all regions is the cartilaginous species. Sharks, skates and rays are commonly discarded and also commonly harvested in virtually all marine regions (Camhi et al., 1998), and declining trends in their populations reflect this. Many shark species have declined by over 75% (Baum et al., 2003). Cartilaginous fishes are less resilient and have a lower fecundity than bony fishes (Camhi et al., 1998). This reproductive difference, along with the fact that they are slower growing makes it harder for sharks to combat the threat of marine bycatch. It is difficult to estimate discard mortality for any of these species because of the lack of observer

coverage and documentation, especially for the largely ignored batoid (or ray and skate) species affected by this issue. However, the impacts of bycatch on cartilaginous species may be significant. For example, out of 21 pelagic shark and ray species examined, three quarters (16) were classified as threatened or near threatened (Dulvy et al., 2008).

Many types of seabirds including albatrosses and petrels have been affected by bycatch, especially because of long lining. These birds are mainly injured while hooks are being set. Once they become hooked or entangled on the longline the birds are dragged underwater and drown as the gear sinks to the bottom. This is happening on such a large scale that the populations of many seabirds are in danger (Gilman et al., 2005). The International Union for the Conservation of Nature (IUCN) reports that 61 species of seabirds are impacted by longline fisheries. Of these 61 species, 26 are listed as threatened. Seabirds can also be harmed in trawl nets and gillnets by flying or swimming into them and becoming tangled (Bull, 2007).

Marine mammals are often victims of bycatch. We hear about these cases more often than others because these animals are so often anthropomorphized. Most marine mammal bycatch occurs in gillnets, but they can also be caught and entangled in virtually any fishing gear. The Marine Mammal Protection Act in the United States prohibits the use and sale of these animals, so by law they must be released and wasted as bycatch (Read et al., 2006). Because these animals are large they are more likely to come in contact with fishing gear. Marine mammals have life history characteristics that also make them more vulnerable than some other species. They are K strategists that have long lifespans, low reproductive output, and reach sexual maturity rather late in life

(Lewison et al., 2004). These characteristics combined with the low selectivity of our fishing gear make for a deadly combination.

All species of sea turtles are listed as either threatened or endangered by the IUCN. The laws in place sparked by their declining abundance, however, cannot protect them from marine bycatch. Sea turtles are mostly affected by longlines, gillnets, and trawls. Lewison et al. (2004) estimate that “more than 200,000 loggerheads and 50,000 leatherbacks were likely taken as pelagic longline bycatch in 2000” and that “thousands of these turtles die each year from longline gear in the Pacific alone.” The species mentioned above are most commonly taken as bycatch but the olive ridley, green, hawksbill, and Kemp’s ridley turtles can also be victims of bycatch. Like marine mammals, sea turtles are K strategists with long life spans and low reproductive output. These life history traits combined with bycatch and illegal fishing are bringing these animals to the brink of extinction (Gilman et al., 2006).

Biodiversity Issues Associated with Marine Bycatch

There are clearly many impacts associated with bycatch. Perhaps the most glaring problem is the loss of biodiversity across the world’s oceans. As people continue to demand larger pelagic species coming from fisheries that create high levels of bycatch, the oceans may become more homogeneous; creating ecological and economical problems that cannot be reversed. With about 200 million livelihoods depending on the fishing industry, the decline in the abundance of commercially important species will cause many fishers to lose their jobs. Bycatch is also an issue of food security. It is estimated that 1 billion people rely on fish as their primary protein source and for 4.3

billion people seafood makes up 15% of the protein in their diets. With human population growing at a steady rate, our oceans will not be able to keep up (UNEP, 2013). Fishing gear associated with bycatch also leads to the destruction of many marine habitats. As habitats are destroyed, benthic species are less able to thrive. Fishers are for the most part unable to avoid certain species with the gear they possess. Because of this, it is difficult to fully protect endangered species that come into contact with industrial fishers. When fishing is occurring on such a large scale, many endangered species are becoming more vulnerable and there is not an easy way to reverse this problem without drastically changing our fishing practices.

Ecologically, marine bycatch is devastating. The most economically important finfishes are large top predators that define ecosystem function. In our effort to catch these fishes, other large non-target keystone species are caught as bycatch. For example, the insatiable demand for fishes like tuna, swordfish, and cod has created an ocean with depleted abundances of these species and the bycatch associated with their fisheries. With fewer of these apex predators, trophic cascades and phase shifts occur. As we "fish down the food web" (Pauly, 1998), the decimation of the target species leads to an overabundance of their prey. With the populations of the larger species' prey growing, they eat more smaller, ecologically important fishes, reducing their abundance. This shift in ecological checks and balances can create drastic changes to the marine ecosystems we see today. For example, vast coral reefs are replaced with macroalgae, the abundance of kelp is reduced off the coast of California, and algal blooms occur in lakes and rivers that were once clear.

Possible Solutions to Reduce Marine Bycatch

There have been efforts to reduce marine bycatch and some have been successful. The reduction of dolphin bycatch in the 1990s was an important first step, as was the creation of many turtle excluder devices (TEDs), which allow sea turtles to escape from trawl nets. Though these strides in gear engineering and marine policy are promising, there is still a focus on particular species that are victims of bycatch. Many studies have shown that ecosystem-based management is a much more productive method to reduce marine bycatch and conserve marine environments (Hall et al., 2011, Kennelly & Broadhurst, 2002, Pikitch et al., 2004). Ecology and fisheries management have evolved over the decades and scientists have known for years that single species approach to conservation is not effective. However, the general public has shown the desire to conserve animals such as sea turtles rather than monkfish or skates, and conservationists tend to take enthusiastic support whenever they can get it. This is why it is important to educate the public on the importance of entire ecosystems rather than exclusively on the individual charismatic species that we disproportionately place value on.

There are many proposed solutions to bycatch that have the potential to reduce discard levels. These ideas can be broken down into gear modifications and policy changes. Many bycatch reduction devices have been created in an effort to continue current fishing practices while avoiding non-target species. These devices would be especially helpful in some of the most destructive fisheries, such as shrimp fisheries worldwide. By creating bycatch reduction devices the engineer is faced with the seemingly impossible task of not only excluding juvenile target species but also all non-target species. Broadhurst (2000) explains the two ways of creating more selective gear:

(1) separating species by differences in behavior, or (2) excluding unwanted organisms according to their size. Using these two approaches, researchers and fishers have created different gear modifications that have many different labels: fish escape devices (FEDs), turtle excluder devices (TEDs), and bycatch excluder devices (BEDs) have all been used in an effort to make gear more selective (Broadhurst, 2000). The bottom line with all these devices is that they can only do so much. Commercial fishing practices are still hugely destructive even with these modifications. Researchers have also studied the possibilities of using acoustic, chemical, or electrical deterrents to dissuade some species of fishes, and especially elasmobranches, which are very sensitive to electrical currents because of their ampullae of Lorenzini (which are sensitive to electrical charge). Some of these deterrents have been shown to be effective. Robbins et al. (2011) found that the use of rare earth metal magnet discs showed potential to prevent sharks from coming too close to nets. But other experiments using very similar rare earth metals have shown that the sharks were not affected. Godin et al. (2013) concluded that electropositive metals did not reduce the catch of sharks. The lack of agreement in the scientific community on whether electromagnetic deterrents are useful mitigation strategies makes it difficult for fishers to easily adopt these practices. Visual deterrents, such as LED lights and glow sticks (Wang et al, 2010), audible deterrents such as pingers, and natural olfactory deterrents have been slightly more effective, with the scientific community agreeing that these methods may deter marine mammals, sea turtles, and seabirds from nets (Carlstrom et al., 2002, Pierre, 2006, Dietrich et al., 2008). All of these methods are mostly directed at non-fish species and they do not usually allow the escape of fish. Again, this method can only go so far in reducing bycatch

Perhaps the most promising method to reduce bycatch that has been utilized is changing current policies to force fishers to be more selective and sustainable in their practices. MPAs, which are areas of ocean where human activities are limited, and area closures have been shown to reduce levels of bycatch of all species by restricting fishers (both commercial and recreational) from entering the areas (Bartholomew & Bohnsack, 2005, Lombard et al., 2007). These no-take areas have been shown to increase productivity and allow stocks and habitats to rebound after intense commercial fishing (Walters et al., 1999). Quotas and size restrictions have also been changed over the years in an effort to maximize population growth. These restrictions change based on a whole list of criteria, including new scientific findings about the biology, stock size, and the survival capacity of fish that are thrown back in the water after being landed. In order for these restrictions to be followed and respected there needs to be a high level of enforcement. One of the most important aspects of marine bycatch management is the fisheries observer program (NOAA, 2014). These programs station biologists and volunteers onboard fishing vessels, where they collect data and monitor the practices of fishers. Depending on the size of the vessel and the fishery in question, operations must be required to have observers on board for part of the trip or for the entire duration. However, these observer programs emphasize collecting data rather than enforcing laws. A more effective enforcement technique may be to place cameras on fishing vessels to hold fishers accountable for their practices.

Goals of this Study

In this study, I will explore escapee predation, which is one of the largely unobserved effects of marine bycatch. Specifically, I plan to look at the immediate effects of slime layer loss, scale loss, and exhaustion on the response of zebrafish (*Danio rerio*) to a model predator. Zebrafish are a widely used teleost species known for their hardiness and ease of care in the laboratory. They are a small species that will model the type of bait-sized fish that may experience this type of scale loss, exhaustion, and slime loss by escaping through nets. I am not focusing on a specific method of fishing, as I will not attempt to replicate an entire fishing event. During an interaction with fishing gear these three stressors are likely to occur and they may affect the behaviors that follow shortly after the event. Main and Sangster (1990) found that haddock and whiting going through demersal nets lost an average of 20% of their scales. In another study by Sangster et al., (1996) it was found that individuals with moderate scale loss "showed distress" and badly descaled fish "remained apart from the shoaling groups and repeatedly sank to the sea floor." Based on this research, it is clear that the loss of scales represents a large portion of the injuries sustained by fish during an interaction with gear.

Slime layer loss is also an issue associated with bycatch. Vander Haegen et al., (2004) found that adult salmon caught in gill nets were subject to slime layer loss, which damages their protective coating, making them not only more susceptible to disease, but also less streamlined in the water. Both of these direct effects may make victims of bycatch more susceptible to predators. Exhaustion can also result from being caught in a trawl net. When fish are exhausted, they can no longer outswim the trawl net, and they are forced through square-meshes. During this process they exhibit high levels of scale

loss (Broadhurst et al., 1997). Their senses and rates of movement are also dampened by the exhaustion and muscle fatigue, and this may affect the rapidity of their response to predators. Based on this previous research, it is hypothesized that scale loss will significantly affect the behavior of zebrafish, and will be more harmful slime layer loss or exhaustion.

Despite the high frequency of these stressors in bycatch, there is little information on escapee mortality rates because affected individuals are hidden under water. In this controlled study of simulated gear interactions, I hope to quantify how zebrafish anti-predator behaviors are affected in an effort to improve our understanding of how fishing affects populations of non-targeted species. This study will not simulate the entirety of a fishing event due to all the complex variables, but it will focus on scale loss, slime layer loss, and exhaustion, all of which have been shown to be part of the overall experience for captured fish.

If this experiment contributes to our understanding of the potential negative effects of fishing gear on fish, it could ultimately be important in saving the lives of millions of fish through gear modifications or policy changes. By understanding how fish react to these stressors, this research could contribute to sustainable management of fisheries.

Materials and Methods

Acclimation of zebrafish

In November 2014 and in February 2015, 90, and 210 zebrafish (*Danio rerio*) were ordered from liveaquaria.com respectively. Fish were housed in the New London Hall Aquarium Room in five 20-gallon tanks (Figure 1). A temperature of 27˚C and a pH

of about 7 were maintained, and water levels were recorded daily. Water was also aerated and filtered. Because zebrafish are usually kept on a 14-hour light/ 10-hour dark schedule, this cycle was used in this study. Zebrafish were fed a diet of standard commercial tropical freshwater fish flakes. The fish were kept in the holding tanks for one week prior to the start of the experiment. All fish used in the trials were apparently healthy.

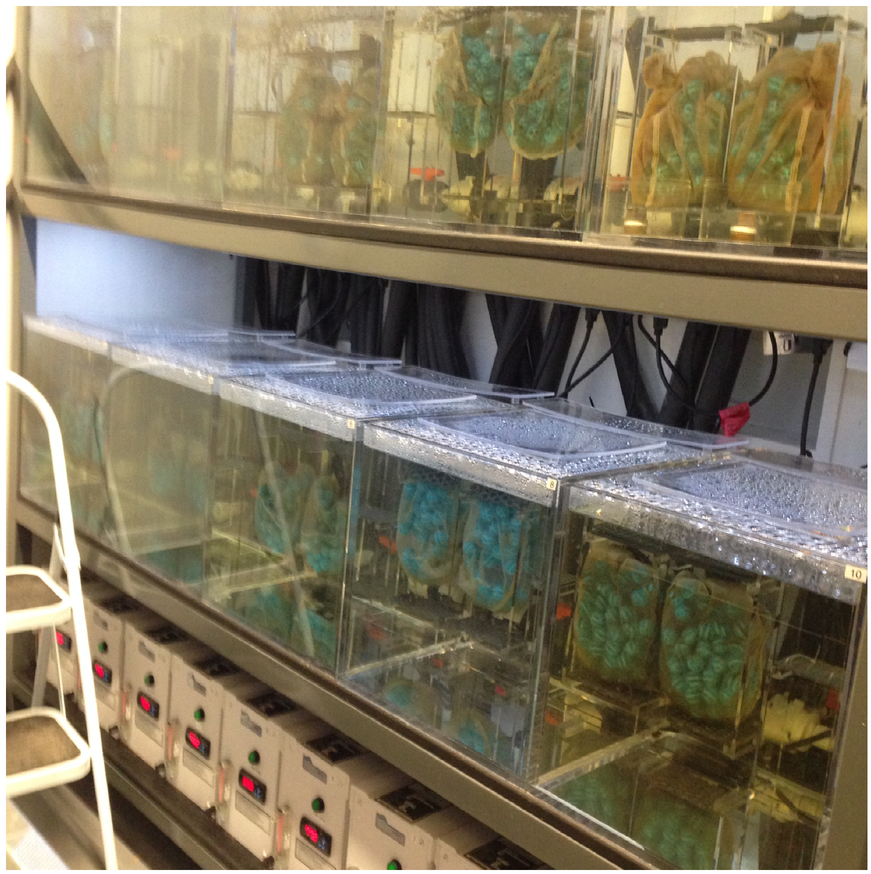

Figure 1. Housing tanks for experimental zebrafish at Connecticut College, New London Hall, Room 114.

Model predator testing

This experiment was a pilot study conducted in the fall of 2014 in order to determine whether the model predators were effective in eliciting an escape response and could be used in experimental trials instead of a live predator that would prey on the

zebrafish. There were 3 experimental groups subjected to different treatments: a 6-inch largemouth bass replica as a model predator, a shadow to simulate the approach of a potential predator, and a control with no simulated predator (Figure 3). The model predator was a 6-inch reproduction of a largemouth bass (*Micropterus salmoides*) attached to a wire and weighed down with two stir bars. Each treatment was tested with 15 zebrafish in separate trials. For each experiment, a zebrafish was placed in the 5-gallon experimental tank, which was filled with water from their housing tank (Figure 2). It was allowed 3 minutes to acclimate. In the predator replica trial, the experiment began by lowering the model predator into the water behind a partition (Figure 2). In the shadow trial, I waved my hand over the tank 3 times from left to right. This was done in a controlled manner, with the same movement replicated each time. In the control trial, the individuals were not exposed to a stimulus. Previous studies have shown that zebrafish startle responses consist of an increased velocity and distance travelled (Cachat et al., 2011, Piato et al., 2011, Chanin et al. 2012), so these parameters were measured by using Anymaze (San Diego Instruments) to analyze video recordings collected during trials. This software is designed for behavioral research. It automatically tracks animals and records the distance travelled as well as many other measurements.

Figure 2. Experimental tank set up: a five-gallon fish tank with translucent partition filled with water from the housing tank.

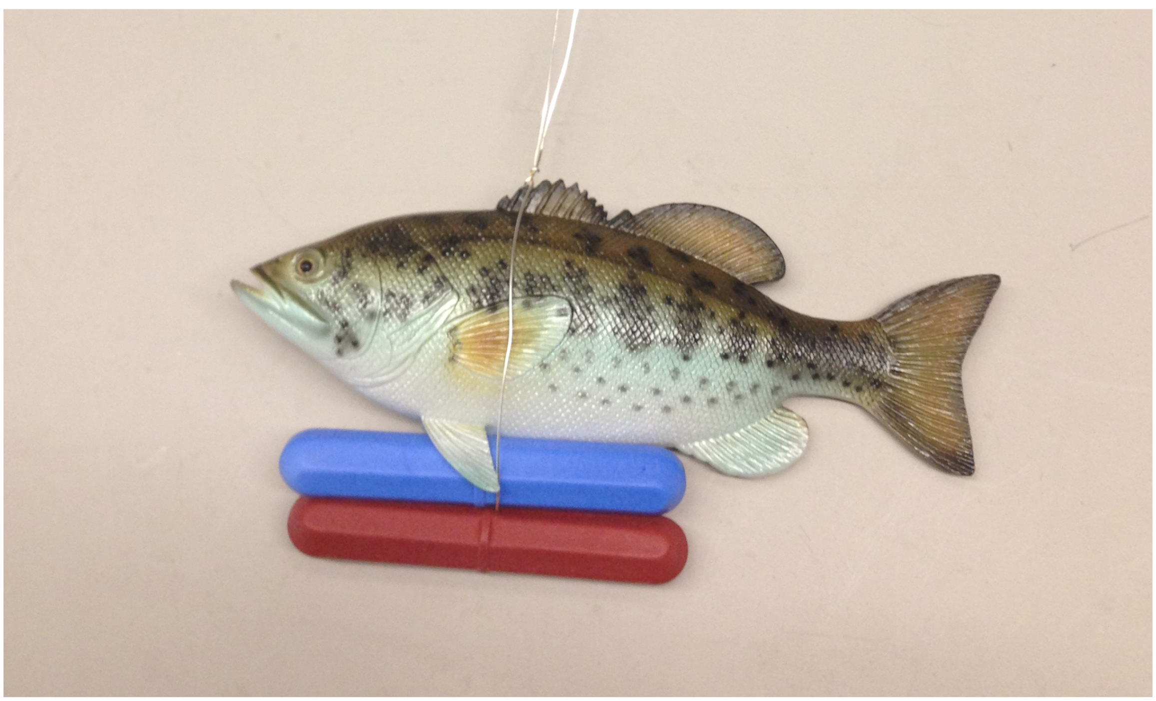
Figure 3. The model predator: a 6-inch reproduction of a largemouth bass (*Micropterus salmoides*) attached to a wire and weighed down with two stir bars

Individual Experiment

There were 4 experimental groups with each group subjected to different treatment: a scale-loss group, an exhaustion group, a slime-loss group, and a control group with no stressor. The experiments were run over the course of one month. In each trial, one zebrafish was placed into the testing tank with a translucent partition (Figure 2). They were allowed one minute to acclimate before the stressor was applied. In order to monitor individual zebrafish movements and responses, a video camera was placed beside the tank. The zebrafish were recorded for 20 seconds without the model predator to establish a baseline. After 20 seconds, a model predator was introduced behind the partition. Once the model predator was introduced, the zebrafish startle response was recorded for an additional 20 seconds. If a fish appeared moribund in any of the trials, it was euthanized and no trial took place. The procedure was stopped completely if more than four fish suffered severe injury or death due to the stressor.

Descaling trial

The de-scaling procedure is based on methods developed by Olsen et al. (2012). Individual zebrafish were transferred onto a petri dish with water from the housing tank. The petri dish was housed in a large plastic container with water from the housing tank so that any fish that jumped from the petri dish would not fall on the floor. A piece of netting with ¼ in x ¼ in mesh was placed over the zebrafish (Figure 4). De-scaling was performed through the mesh on one side of the fish to minimize stress. Approximately 10% of each zebrafish was descaled along the flank of the body from the tail root. 10% was chosen to minimize stress to the fish while also approximately replicating Main and

Sangster's (1990) estimate of scale loss during trawling. This was done by allowing the same person to pull the blunt side of a scalpel from the tail to the pectoral fins of each experimental individual. Approximately the same amount of pressure was applied for each specimen.

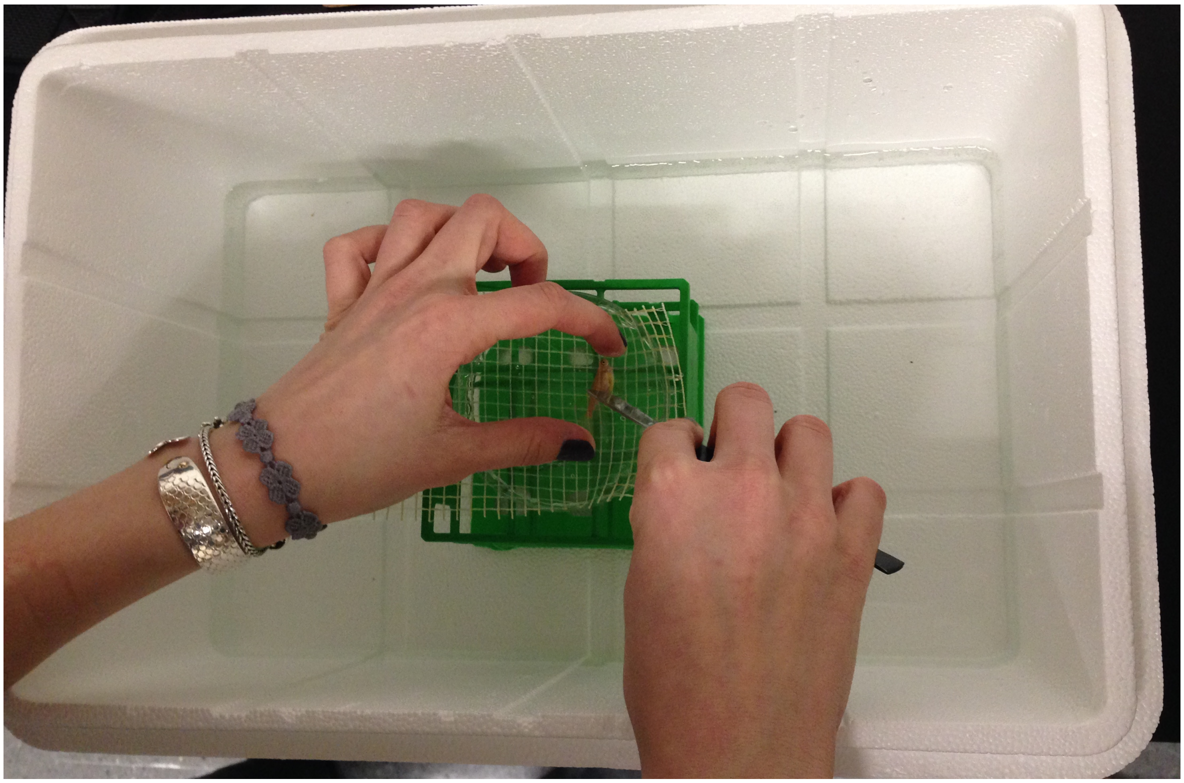

Figure 4. Descaling trial procedure: zebrafish in a petri dish gently held in place by a piece of mesh.

De-Sliming trial

Many teleosts, including the zebrafish have a slime layer, which acts as a barrier against infection (Harper & Lawrence, 2011). To remove this layer, individuals were transferred onto a petri dish with water from the housing tank. The petri dish was housed in a large plastic container with water from the housing tank to keep jumping fish from falling on the floor. A Kimwipe was gently dabbed along the flank of the zebrafish to remove the slime layer.

Exhaustion Trial

For the exhaustion trial, zebrafish were chased to exhaustion by being constantly followed with a ½" diameter wooden dowel for 5 minutes.

Schooling experiment

Because zebrafish are a shoaling species, it was necessary to examine their behavior while in a school. For this portion of the experiment, there were 6 trials, each with 5 individuals. In order to monitor schooling behavior, only one fish was subjected to a stressor so the individual's behavior could be compared to the other fish in the school. The stressed fish could be identified because it was larger than the other individuals in the school. A ruler was placed at the side of the tank, and the software Kinovea (http://www.kinovea.org/, Kempster et al., 2013, Werth, 2012) was used to track the movement of the animal in question and the distance to it's closest neighbor (Figure 5). Distance to closest neighbor was calculated by taking 5 measurements at 10, 15, 20, 25, and 30 seconds and averaging the values. This method was used because the Anymaze software cannot track more than one animal at a time.

Euthanasia

Fish were euthanized upon completion of testing by prolonged exposure to 200-300mg/L tricaine until opercular movement has stopped. The experimental tank was cleaned and the fish were disposed of after each trial.

Data Analysis

In the pilot study, an analysis of variance with multiple comparisons tests was used with the Tamhane post hoc test to compare the velocity of zebrafish in response to the three different treatments. An analysis of variance with multiple comparison tests was also used to compare the distance travelled for the four different treatments. LSD and Tamhane post hoc tests were used to determine significance. The LSD test was used when the Levene's Test showed that there was no significant difference in variances, and the Tamhane test was used when there were significant differences. A student's t-test was used to compare trials with individual fish and fish in schools.

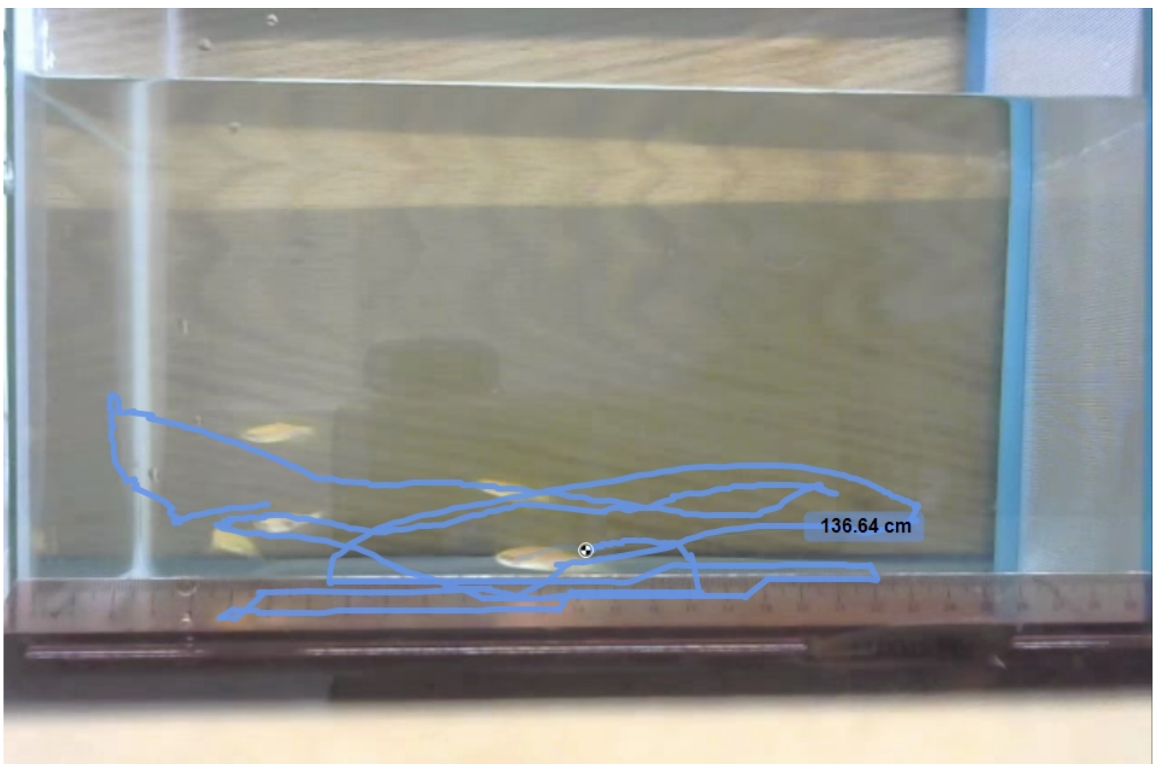

Figure 5. Output from the Kinovea software after tracking fish

Results

Pilot Study

An analysis of variance with multiple comparisons tests was used with the LSD post hoc test to compare the distance travelled by zebrafish (n = 15) in response to the three treatments. There were significant differences in the average distance travelled among treatments ($F = 9.076$, $P = 0.001$). The individuals exposed to a model predator travelled at a significantly higher velocity than those exposed to a shadow ($P = 0.011$), or to no stressor ($P < 0.001$, Figure 6). There were no significant differences in velocity between the shadow group and the control group (exposed to no stressor). The model predator was therefore chosen as an appropriate stressor to mimic a true predator (Figure 6).

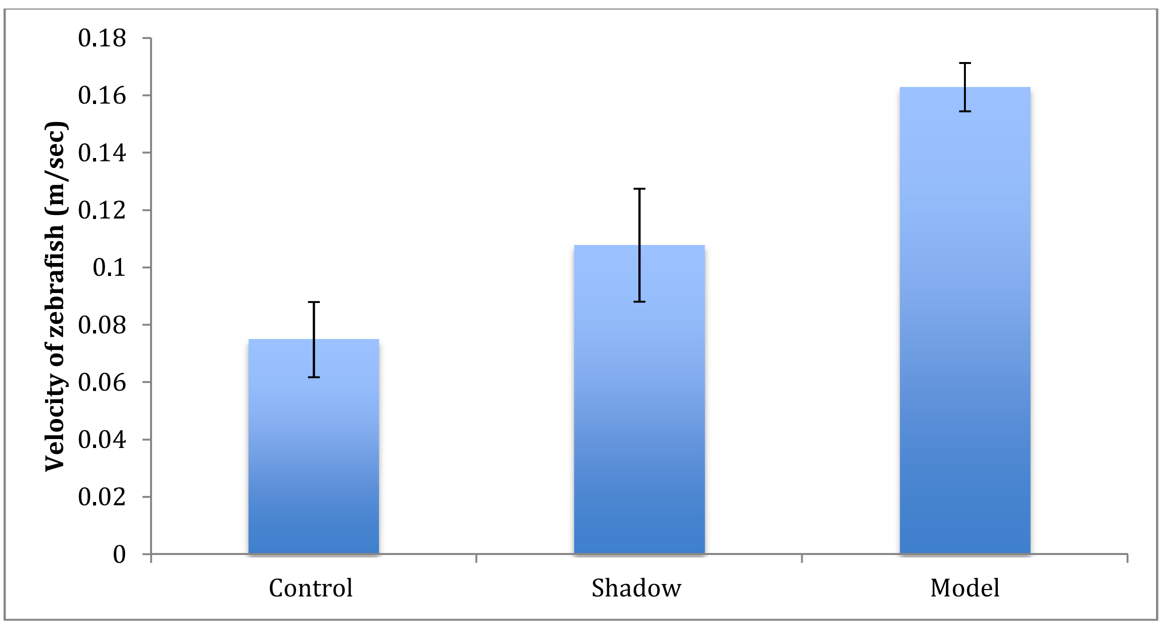

Figure 6. Pilot study results: The average velocity of individual zebrafish (n = 15) exposed to a model predator, a shadow, or no stressor (control). Error bars represent standard error.

Individual Experiment

Individuals in the exhaustion and descaling treatments were most negatively affected. Fish in these groups (n = 15) would often sit at the bottom of the tank for the majority or entirety of the trial. The control group travelled the greatest distance, followed by the de-slimed group, the exhaustion group, and finally, the descaled group (Figure 7.) Analysis of variance was used with the Tamhane post hoc test to compare the distance travelled in response to the four different treatments. There was a significant difference in the average distance travelled among treatments (F = 3.527, P = 0.021). Descaled zebrafish travelled a significantly shorter distance in comparison to the fish in the control treatment (P = 0.001). However, there were no significant differences in distance travelled between zebrafish in the control, de-slime, and exhaustion trials, or in the de-slime, exhaustion, and descale trials. There was also a large standard error value for the de-sliming (SE = 0.29571) and exhaustion (SE = 0.22135) trials (Figure 7).

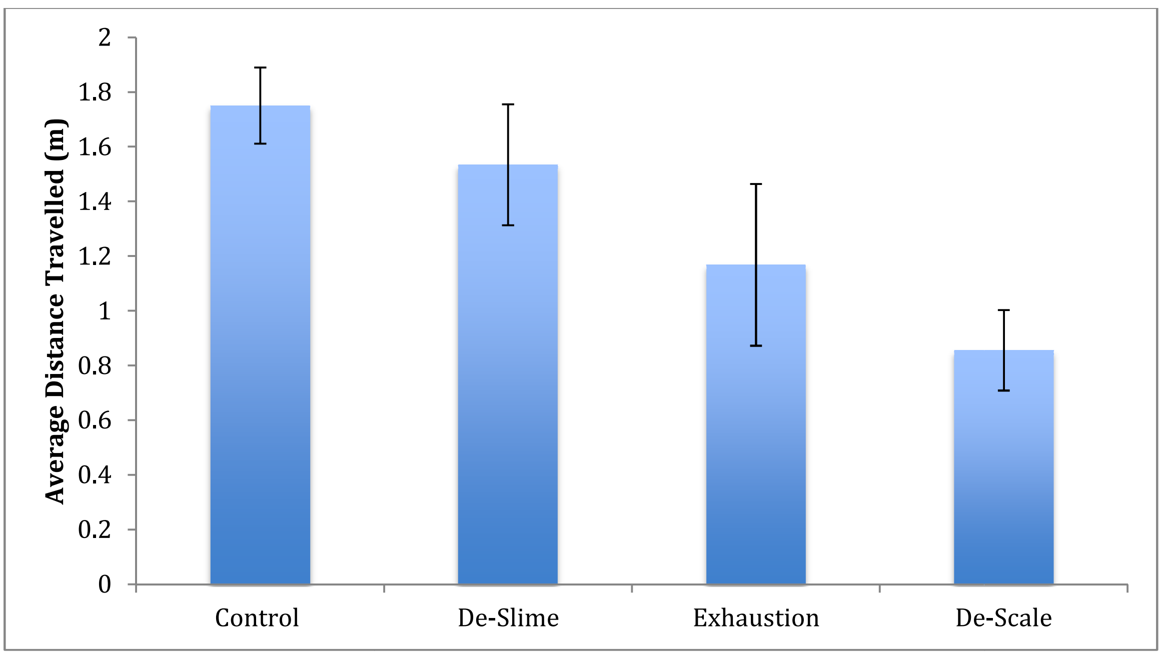

Figure 7. The average distance travelled by individual zebrafish (n=15) stressed by de-sliming, exhaustion, and de-scaling. Error bars represent standard error.

Schooling Experiment

Distance Travelled

During this experiment (n = 6), similarly to the individual experiment, zebrafish in the exhaustion and descaling groups seemed to be most affected by the stressors (Figure 8). An analysis of variance with the LSD post hoc test was used to compare the distance travelled in response to the four different treatments There were significant differences in the average distance travelled among treatments ($F = 7.135$, $P = 0.002$). Similar to the individual experiment, it was found that descaling zebrafish in a school significantly reduced the distance they travelled in comparison to the control treatment ($P < 0.001$, Figure 8) However, significantly lower distances were also found for both the exhausted zebrafish ($P = 0.003$) and the de-slimed zebrafish ($P = 0.14$) when compared to the control. There were no significant differences between the descaling, exhaustion, and de-sliming groups.

An analysis of variance was run to compare the schooling and individual trials. There were no significant differences in the distances travelled by solitary fish and the fish in schools for the same treatment, but there were some interesting trends. The average distance travelled in the control group was very similar (mean = 1.75 m and 1.63 m respectively). In the other three treatments, the zebrafish in the schooling experiment travelled a shorter distance than those in the individual study (Table 1, Figure 9).

Table 1. The mean distances travelled by fish in the four treatments in both the individual and schooling experiments.

Treatment	Individual Experiment: Mean Distance	Schooling Experiment: Mean Distance
Control	1.75 m	1.68 m
Exhaustion	1.17 m	0.61 m
De-Slime	1.53 m	0.98 m
De-Scale	0.85 m	0.52 m

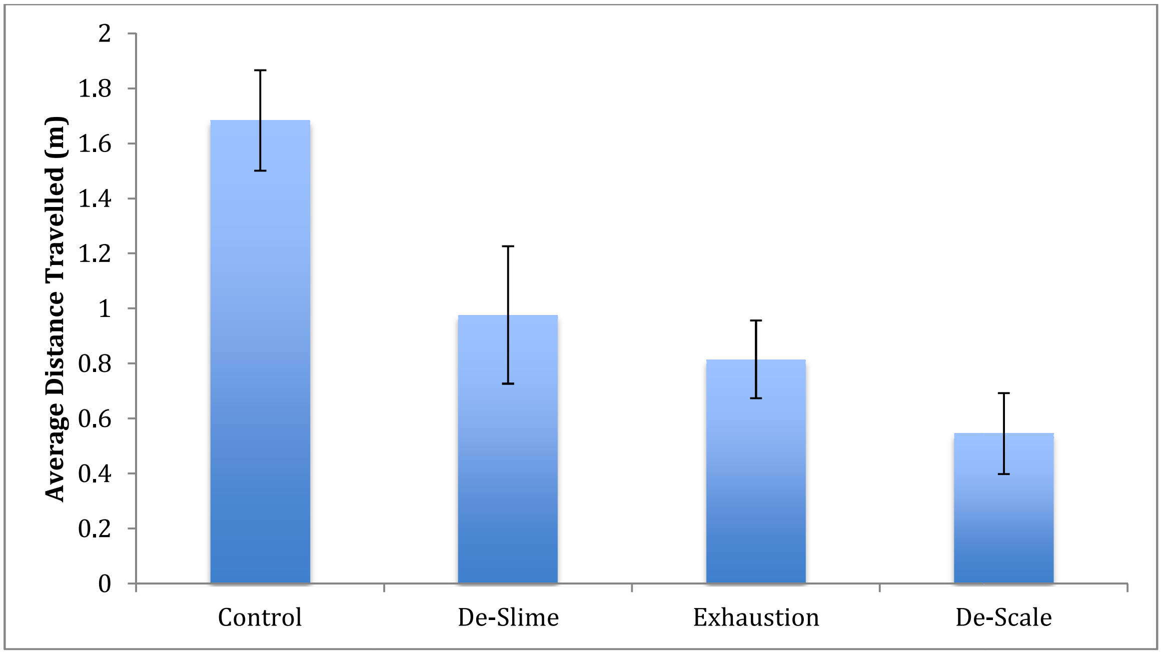

Figure 8: The average distance travelled by zebrafish (n = 6) in a school when the target fish was stressed by de-sliming, exhaustion, and de-scaling. Error bars represent standard error.

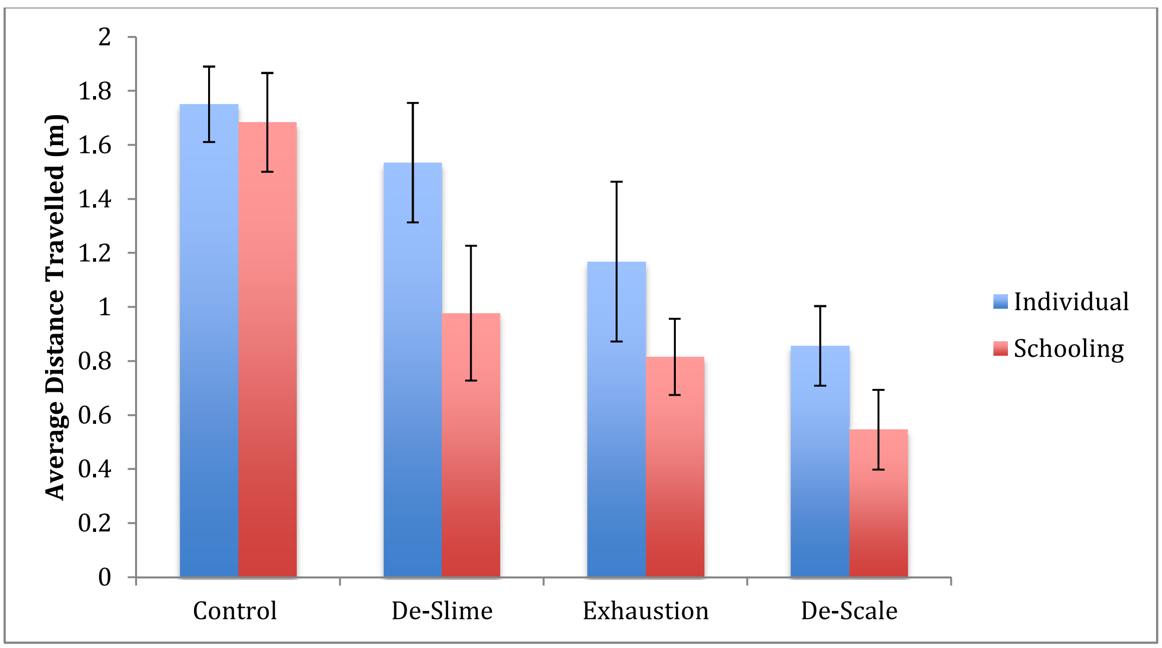

Figure 9. The average distance travelled by individual zebrafish and zebrafish in schools when they were stressed by de-sliming, exhaustion, and de-scaling. Error bars represent standard error.

Distance to nearest neighbor

Analysis of variance with the Tamhane post hoc test was used to compare the distance to closest neighbor for the four different treatments (n = 6). The ANOVA was marginally significant (F = 3.099, P = .050), but there were no significant differences among the four treatments. There is an interesting trend in the data, with the control treatment yielding the closest distance to the nearest neighbor, followed by the de-sliming treatment, the exhaustion treatment, and finally the descaling treatment, which yielded the highest distance to the nearest neighbor (Figure 10). There were high levels of variation in the results for all of the treatments.

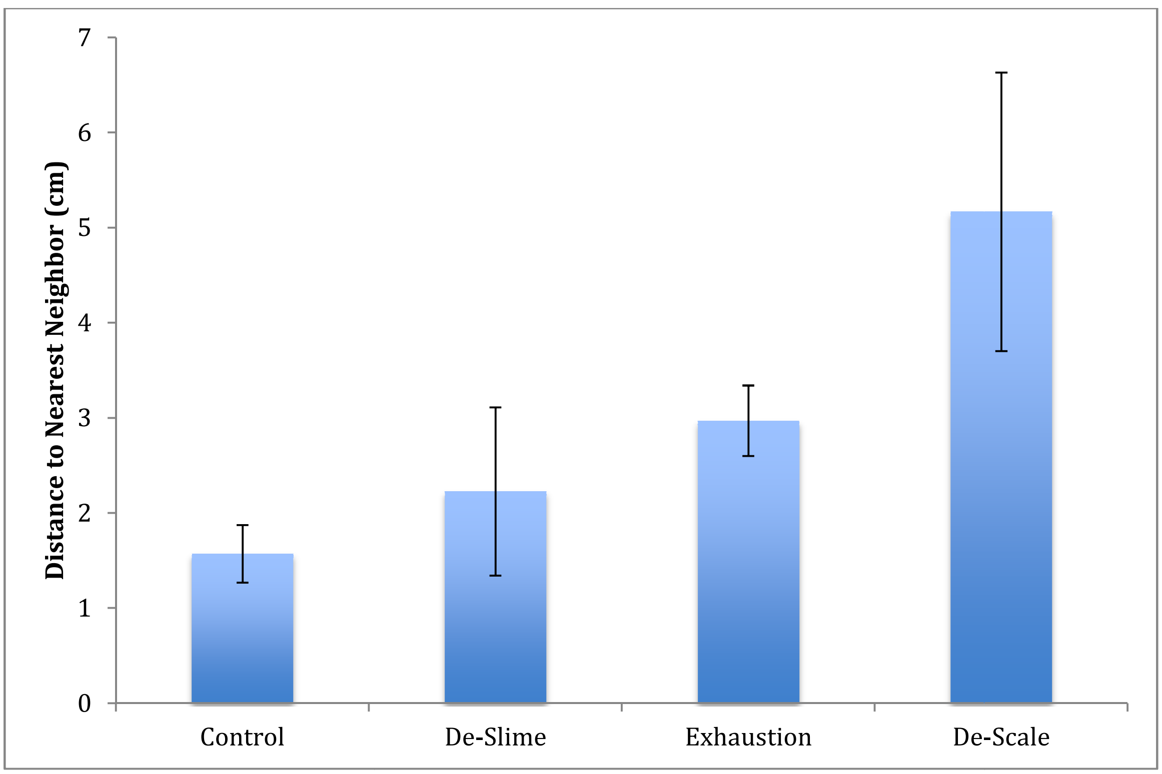

Figure 10. The average distance to the nearest neighbor of zebrafish (in a school, n = 6) stressed by de-sliming, exhaustion, and de-scaling. Error bars represent standard error.

Discussion

Scientists are just beginning to understand the impacts that bycatch has on the marine environment. Few studies have focused on escapee mortality and the behavioral changes that occur when fish come in contact with fishing gear (Gilman et al., 2013). This lack of research has made for uninformed and ineffective fishery policies. In this study I isolated the variables of scale loss, slime loss, and exhaustion, which have all been found to be part of the experience of a fish escaping through fishing gear. The goal was to explore whether escapees experience changes which alter their startle response, and therefore their ability to escape from nearby predators. The results indicate that stressed zebrafish travelled shorter distances than the control zebrafish, supporting my hypothesis.

The pilot study showed that a model predator could evoke a startle response from zebrafish. Previous studies by Chanin et al (2012) revealed that the distance travelled is an appropriate measure of the startle response of zebrafish, so this metric was used in both the pilot and full-scale experiments. Individual fish in each of the four trials were exposed to a 6-inch replica of a largemouth bass, a shadow, and a control. Previous studies have used other stimuli, such as sound and vibration (Chanin et al., 2012), but these stressors do not model the visual impact of an approaching predator. It was hypothesized that the fish replica would produce the largest startle response, as it is the most intrusive of the predator models. Individual zebrafish confronted with the replica travelled at a significantly higher velocity than fish exposed to a shadow or the control, supporting this hypothesis.

Experiments with Single Fish

In the individual experiment, the effects of scale loss, slime layer loss, and exhaustion on the startle response of individual zebrafish was examined. It was hypothesized that the most impactful stressor would be scale loss, and that this treatment would result in the shortest distance travelled. This hypothesis was supported, as descaled zebrafish travelled significantly shorter distances when compared to the control group. Scale loss has previously been shown to be physiologically devastating to fish. Many studies have shown that scale loss due to interactions with fishing gear caused increased mortality, predation, and disease (Suuronen et al., 1996, Broadhurst et al., 1997, Ryer, 2004, Butcher et al., 2009). Olsen et al (2012) concluded that descaling causes loss of osmoregulatory ability, leading to injury and often mortality. Scale loss also led to large cortisol responses, indicating a significant stress response, which was not seen in the control tests. In the same study many descaled fish swam significantly faster than the control. I did not observe this, perhaps because zebrafish are much smaller, and presumably more delicate, than the herring used in the previous study. The tanks used in the previous study were also much larger, which could have affected the behavior of the fish.

There were no significant differences in the distance travelled between the slime loss group and the control group, indicating that slime layer loss was not impacting the zebrafish in a harmful way. There was no significant difference between the de-slimed group and the control group, as the de-slimed fish travelled on average only about 0.2 m less than fish not exposed to a predator. This similarity could have been due to the methods used for de-sliming. Previous studies have used 70% ethanol to wipe away the external slime layer (Bradford et al., 1994), so the use of a wet Kimwipe to remove the

slime layer may not have been sufficient. There is a lack of information on the role of the slime layer in zebrafish, but its importance has been more extensively explored in other species such as brook trout (*Salvelinus fontinalis*), haddock (*Melanogrammus aeglefinus*), and hagfish (*Myxinidae*). This mucus layer contains many antimicrobial and antifungal agents to offer effective defense against pathogens, and has even been suggested as an antimicrobial agent for humans (Subramanian et al., 2008). Mucus also lubricates fish, helping them to be more streamlined in the water. Because the mucus aids in movement, it seems that the loss of the slime layer could potentially make individuals more susceptible to predators. This was not found in the present study, most likely because the slime loss was not excessively severe. Bruce Barton (2002) writes, “Physical, chemical and perceived stressors can all evoke non-specific responses in fish, which are considered adaptive to enable the fish to cope with the disturbance and maintain its homeostatic state.” Perhaps the slime layer loss was able to heighten the individual’s senses and make them more alert to the potential predator, causing them to move more quickly despite the loss of streamlining. Scale loss, on the other hand may have been damaging enough to the fish that they were unable to reestablish homeostasis. While animals in the control and de-slime treatments seemed relatively unharmed, fish in the exhaustion and descaling groups seemed to display more intense behavioral changes.

Though the exhaustion treatment did not yield a statistically significant difference from the control, the results suggest that exhaustion may be more damaging than either slime loss or the absence of a stressor. Fish exhaustion has been thoroughly researched in the past 50 years. This research has suggested that fish in general are very successful swimmers with a large aerobic capacity (Kieffer, 2000). During sustained (and

comfortable) swimming, highly vascularized red muscle is used. However, in this study, individuals were forced to move at high speed in a “burst-type” of swimming for 5 minutes. This type of swimming uses white muscle, and ends in fatigue or exhaustion. Zebrafish, a small minnow-type fish, is not adapted to this type of swimming. Factors such as small body size and lack of training exacerbate this exhaustion (Kieffer, 2000). This suggests that larger fish often found caught in commercial fishing nets may be able to avoid exhaustion for longer. However, these larger fish are much less likely to escape through the nets they are caught in. From general observational made during the experiment, there was a clear separation between behaviors in the control and de-slime groups and the exhaustion and descale groups.

Schooling Experiment

In the schooling experiment, the effects of scale loss, slime layer loss, and exhaustion on the startle response of an individual zebrafish within a school was examined. This experiment was necessary because zebrafish are a naturally shoaling species, and their behavior in response to a predator is likely defined by the schools they are commonly found in. As in the study of individual zebrafish, it was hypothesized that the most impactful stressor would be the scale loss, and that this treatment would result in the shortest distance travelled. This hypothesis was supported, as descaled zebrafish travelled a significantly shorter distance than those in the control group. Significantly lower distances travelled were also found in the exhaustion and de-slime treatments. Though there were no significant differences found between the distances travelled by schooling fish and individual fish, the trend in the data suggests that being in a school

allows stressed zebrafish to travel smaller distances in response to a model predator. This may also mean that the fish confronted with these stressors in the individual study were more distressed than they appeared, and that they were in a way forced to over compensate because they were not in a school.

These results support Stankowich and Blumstein (2005), who concluded that fish tolerated closer approaches by a predator when in schools than when alone. The individual fish in the group “gained an increased perception of safety when aggregated” (Stankowich and Blumstein, 2005). The distance to nearest neighbor was also explored as a way to analyze the schooling behaviors of the zebrafish. While the findings from this experiment were only marginally significant, the general pattern in the data suggests that increasing intensity of the stressor creates a less intact school or an individual fish that is unable to keep up with the rest of the school. The lack of significance may be due to the high levels of variation in the data for all of the treatments. Further studies, with larger sample sizes (perhaps 15 trials, which yielded significant results in the study of single fish) are needed to further explore the effects of these stressors on the behavior of schooling fishes confronted with a model predator.

Limitations

There were limitations to this study that should be addressed. Though zebrafish model the correct size of some bait fishes, it would be more appropriate to use a larger marine species to estimate the effects of scale loss due to trawling. We did not have the appropriate facilities to house such fishes, but further study should be done to determine whether the results would be similar with larger species of fishes. Even in the present

study, it appeared that larger zebrafish were less impacted by stressors than their smaller counterparts. In future studies it would be interesting to use a living predator, either behind a partition, or ideally interacting with the stressed fish themselves. This could be done in the form of actual predation and consumption (Herting and Witt, 1967, Mesa, 1994, Ryer, 2002) or in the form of disabled predators that are cold stunted (Ryer, 2002). In an effort to use as few individuals as possible, sample sizes were kept small, especially in the schooling experiments. These small sample sizes could lead to inconclusive results when there is high variability in responses, and in the future larger sample sizes should be used. In future studies, it would also be interesting to look at different biological measures of stress such as blood chemistry levels or other behavioral measures like freezing and highly mobile duration, or the time zebrafish spent travelling faster than their average speed.

Conclusion and Conservation Implications

The results of this study suggest that scale loss and exhaustion have significant effects on the behavioral responses of zebrafish to a model predator. These results may in turn suggest that fish escaping from commercial fishing nets are less able to defend themselves against a nearby predator. Scales are lost during fishing operations. Researchers have even noticed, "the sea sprinkled with scales" after a trawl (Olsen et al., 2012). The purpose of this study was not to model an entire trawling experience; rather it was to isolate three of the variables fishes endure during an interaction with fishing gear: scale loss, slime layer loss, and exhaustion. By showing the impact of these three variables, it is possible for researchers and fisheries managers to better informed about

hidden, and wasteful escapee mortality. Also, the general conclusion among fishers that bycatch problems can be solved via gear modifications, such as enlarging mesh sizes, is challenged by the results of this study. If gear modifications allow for the release of non-target species but cause scale loss, then managers should consider focusing on other management methods, such as area closures and stricter quotas. Scale loss can also occur when fish are handled with dry hands (Butcher et al., 2009). The fact that scale loss had such a negative impact in this study could lead to better handling practices in recreational and commercial fishing (i.e., using gloves while handling). By understanding how interactions with fishing gear influence fish behavior and physiology, fisheries managers could learn the true impact that bycatch is having on stocks, and they would be able to make better management decisions, potentially saving the lives of millions of fish.

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