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Ecology and behavior of juvenile *Mugil cephalus* in Hawaiian streams

By

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A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Environmental Science, Policy, and Management

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Graduate Division

of the University of California, Berkeley

Committee in charge:

Professor Stephanie M. Carlson, Chair

Professor Mary E. Power

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## Abstract

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By

Kauaooa Matthew Sam Fraiolo

Doctor of Philosophy in Environmental Science, Policy and Management

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Professor Stephanie M. Carlson, Chair

Streams and rivers are ecosystems that are tightly coupled with adjacent ecosystems, such as forests, estuaries, and oceans. The freshwater fauna found in streams of the high Pacific Islands are all marine-derived, and many animals found in these streams have complex life cycles that involve movements between marine and freshwater ecosystems. Movements between adjacent ecosystems can provide organisms with many benefits, such as access to abundant food resources or escape from predators and competitors. The striped mullet (*Mugil cephalus*) is one such organism. *M. cephalus* is a euryhaline fish that spawns in the marine environment, but which utilizes freshwater ecosystems as feeding habitat to varying degrees across its range. Striped mullet is a species of great concern given its important nutritional, economic, and cultural roles. In this dissertation, I delve into the ecology and behavior of *M. cephalus* in Hawaiian streams across large and small spatial scales.

Broad-scale factors can often be useful for understanding the distribution of stream fishes across the landscape. I start my dissertation at these broad spatial scales, where I investigated the watershed-scale factors that influence the presence-absence of *M. cephalus* among 33 Hawaiian watersheds. I complemented this large-scale study with a field study examining how variables at the reach-scale compared between a "mullet stream" and neighboring "non-mullet streams." For my investigation of large-scale factors, I used a model selection framework to compare 11 generalized linear models, including slope, discharge, watershed area, and watershed health. My results showed that slope was highly influential and inversely related to the occurrence of *M. cephalus* in lower stream reaches. In addition, discharge was also influential, but to a lesser degree, and had a positive relationship with *M. cephalus* occurrence. For the reach-scale comparison, I sampled depth, velocity, wetted width, and canopy cover systematically within the lower reaches of the three non-mullet streams and one neighboring mullet stream. Results from this reach-scale analysis revealed that non-mullet streams were significantly narrower and shallower, but similar to mullet streams in terms of velocity and canopy cover. This study demonstrates that slope and discharge are associated with the distribution of *M. cephalus* among watersheds and that, at smaller scales, mullet are associated with streams that are relatively wider and deeper.

At finer spatial scales (i.e., microhabitat), local environmental variables influence the daily decisions made by organisms, such as where to feed. Through a field study, I examined *M. cephalus* feeding microhabitat use within a lowland Hawaiian stream. In this section, I assessed *M. cephalus* feeding behavior by comparing the microhabitat variables (i.e., depth, velocity, substrate, and canopy cover) from locations where *M. cephalus* were observed feeding to the full suite of microhabitats available in Waiāhole Stream, O‘ahu. Data from feeding sites were collected from repeated stream bank surveys, and habitat availability data for the same study reach were collected over a two week period prior to stream bank surveys. I then used a chi-squared test to statistically compare habitat used by mullet to the habitat available. Additionally, because many of the variables are correlated with one another, I used Principle Components Analysis (PCA) to visually assess evidence of habitat selection using the suite of habitat variables. I found clear evidence that mullet are selecting specific microhabitats for feeding. The selected microhabitats were characterized by moderate depths and velocities, moderate to large substrate, and open canopies. Visual examination of PCA plots confirmed these findings. These findings suggest that *M. cephalus* are actively selecting their feeding locations, with individuals selecting erosional type habitats and avoiding more depositional habitats.

Finally, because I am interested in how euryhaline and/or catadromous fishes connect ocean and stream ecosystems, I studied the movement of juvenile mullet between Waiāhole Stream and the downstream estuary. Specifically, I quantified the feasibility of using Passive Integrated Transponder (PIT) tag technology to tag for studying the movement behaviors of *M. cephalus*. As a first step, I quantified retention for PIT tag and VIE (Visible Implant Elastomer) tags through a laboratory study. For the component, I tagged juvenile *M. cephalus* (7-10 cm) with PIT tags implanted in the peritoneal cavity, and VIE tags placed in post-orbital adipose tissue. Fish were kept for 48 days at the Anuenue Fisheries Research Center. I found that retention rates for PIT tags were perfect (100%) but failed completely (0%) for VIE tags. Within this information in hand, I next conducted a field study where I inferred the movement of individual *M. cephalus* between Waiāhole Stream and its estuary using PIT tags and a stationary RFID antenna array. For the field portion of the study, I tagged fish from Waiāhole Stream and monitored their movements between the stream and estuary using a stationary Radio-Frequency Identification (RFID) antenna array. I found that this system worked well with *M. cephalus*. Analysis of detections at the antenna indicated that individuals were making forays into the estuary at varying frequencies and of varying length. For example, longer forays were occurring primarily at night. Results from this work suggest that PIT tag technology is well suited for mark-recapture studies with juvenile *M. cephalus*, and that individuals in Waiāhole Stream made frequent forays into the estuary.

In conclusion, my dissertation work has contributed to our knowledge of the freshwater ecology of striped mullet, a globally distributed fish species that supports commercial fisheries throughout its range. Specifically, my research suggests that at large spatial scales, mullet are associated with streams that are low gradient in the vicinity of the mouth and with relatively high discharge. At smaller scales, my research suggests that mullet are feeding in erosional habitats and may be moving back and forth to the downstream estuary more regularly than has been recognized previously. Whether these patterns extend beyond the Hawaiian streams where I studied mullet is currently unknown. However, recent work suggests a high degree of genetic population structure across the global range of *M. cephalus*, and other work has suggested

considerable life history variability across their range, including the degree of dependence on freshwater rearing habitat. In the Hawaiian streams that I studied, mullet are actively feeding and growing in freshwaters, suggesting the need for continued management efforts to conserve (and restore, where necessary) connectivity between streams and their downstream estuaries and the need for further studies to understand how these organisms use and benefit from freshwater streams and rivers across their range.

**This dissertation is dedicated to the streams and rivers of Hawai'i.**

## TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	iii
CHAPTER 1	
General Introduction.....	1
CHAPTER 2	
The influence of large scale environmental factors on <i>Mugil cephalus</i> occurrence in Hawaiian watersheds.....	7
CHAPTER 3	
Feeding microhabitat use and selectivity of juvenile <i>Mugil cephalus</i> in a Hawaiian stream.....	20
CHAPTER 4	
Feasibility of using Passive Integrated Transponder technology for studying the ecology of juvenile striped mullet ( <i>Mugil cephalus</i> ) in streams.....	33
CHAPTER 5	
Conclusions, Conservation Implications, & Directions for Future Research.....	49
LITERATURE CITED.....	54



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# Chapter 1

# Chapter 1

## Introduction

People have been interested in the distribution and movement of animals through space and time since the beginning of our species (Berkes et al. 2000), and those interests have become cornerstones of modern ecological science in the past century. The question of what influences the distribution of a species is of great interest to both scientists and resource managers. The answer to this question is dependent upon a suite of biotic and/or abiotic variables that act at one or more spatial and temporal scales. Stream ecologists, for example, have long embraced a hierarchical perspective on what influences the distribution of fish species within stream networks and across the landscape (Frissell et al. 1986; Poff and Ward 1990). In particular, there has been growing appreciation for how the habitat available to stream fishes at fine-scales (e.g., pool, sand bar, etc.) is influenced by factors operating at larger scales (e.g., watershed-scale land use). Early scientific work on the environmental factors limiting fish species distributions in streams ecosystems was dominated by work at these finer scales (Fausch et al. 1988), and provided much insight into how biotic and abiotic interactions influenced the distribution of fishes in the stream. However, characterizing habitat at this fine scale is time intensive and applying this approach to larger areas can be prohibitively expensive. Recent advances in remote sensing technology and the advent of geographic information systems (GIS) have given researchers access to a wealth of landscape-scale data, and opened the doors to working at larger spatial scales. Today, there is a greater appreciation for the influence of both landscape scale factors and local scale factors in determining the distribution on fishes in streams. My dissertation research considers how environmental factors across a gradient of spatial scales – from the entire watershed to the microhabitat available to the organism – influence how one particular fish species (*Mugil cephalus*) is distributed in Hawaiian streams.

Another element of my research considers how organisms with complex life cycles can connect adjacent ecosystems. As I will describe in more detail below, *M. cephalus* move from ocean breeding habitat into adjacent freshwater streams to feed and grow. Whether these movements should be considered migrations or facultative movements to take advantage of feeding opportunities in streams is still under debate (see below). Regardless, it is important to keep in mind that movements can be categorized by the scale at which they take place. For example, many animals make seasonal migrations (large scale) over the course of the year between feeding and breeding areas, or the movements can be at a much smaller spatial and temporal scales (i.e., over the course of a day). These movements over the landscape can be driven by changes in food resource availability, ontogeny, or even avoidance of predators. At the ecosystem level, these movements can link the dynamics and processes of otherwise separate ecosystems (e.g., streams-estuaries-oceans or streams-forests). Therefore knowing the movement patterns of individuals at various time-scales can be critical to the conservation and management of populations and ecosystem function.

### ***The system: Mugil cephalus***

The overall goal of this study is to advance our understanding of the freshwater ecology of *Mugil cephalus*. *M. cephalus* has a circumtropical distribution and is widely distributed between the latitudes 42N to 42S. Genetic work on based on lineages created from mitochondrial markers, has found considerable genetic structure among populations from different parts of the world, and suggest that this species could actually be a species complex. Its ability to tolerate a wide range of salinity and water quality conditions and because it feeds low on the food chain (e.g., on detritus and algae) are both thought to be reasons for its high productivity. Because of its affinity for estuarine environments and its schooling behavior, *M. cephalus* are also relatively easy to collect, and it is thus an organism that can be commercially harvested by people with a wide range of resources from small-scale family operations to larger commercial enterprises. Not surprisingly, therefore, *M. cephalus* supports commercial fisheries around the globe, with the FAO reporting global capture of 130,139 tonnes in 2012. In the past 18 years, the global aquaculture production of this species has increased dramatically to ~141,730 tonnes in 2012.

Spawning for this species typically occurs in nearshore marine environments, and after larvae feed and grow in the plankton they recruit to the shore line as early-juveniles where they begin to form schools. Schools then begin to move into the estuary as fry, at which point they have the option to move upstream into adjacent freshwater ecosystems. After a period of time feeding and growing, individuals that have attained appropriate reproductive size will then school together and move to nearshore spawning grounds. After spawning, individuals resume feeding activities in the marine, estuarine, or freshwater environments, and will then spawn again in future years (i.e., they are iteroparous). In larger river systems, *M. cephalus* can be found hundreds of kilometers up river, and can spend most of their life in freshwater, only returning to the sea to spawn (Thomson 1966; Wang et al. 2010).

How and when *M. cephalus* use freshwater ecosystems is highly variable, which leads to some confusion about whether these organisms should be considered euryhaline or whether a catadromous or marine-amphidromous designation is more appropriate. Catadromous and marine-amphidromous fishes are two groups of migratory fishes that move between marine and freshwater environments (McDowall 1988). These fishes breed in the marine environments and then migrate to freshwater ecosystems to feed and grow, and then return to the sea to spawn. The difference between these two groups is that catadromous taxa spend most of their life in freshwater and migrate back to the sea to spawn (e.g., the American eel, *Anguila rostrata*), while marine-amphidromous fishes migrate into freshwater for only a short period of time and do not necessarily return to marine environments to spawn. For example, *Kuhliidae* fish in many pacific island streams which have a marine-amphidromous/catadromous life histories (McDowall 1988). *Mugil cephalus* is a species that breeds in the marine environment and can move into freshwater streams and rivers to feed and grow. However, *M. cephalus* does not fall neatly into one of the above categories. Many have classified *M. cephalus* as euryhaline and facultative because their movements into freshwater environments are not necessary to complete its life cycle (e.g., Moyle 2002). McDowall (1988) suggests that *M. cephalus* seem to be catadromous, but to what extent is not clear. He goes on to say that “*Behavior of M. cephalus is very variable; sometimes it is present in freshwater only in the juvenile stages, ... but in other populations the fish grow to adult size in freshwater before going to sea to mature and spawn.*”

The former behavior might suggest they should be considered marine-amphidromous, while the latter behavior suggests a catadromous designation might be appropriate.

Whether or not movement into freshwater ecosystems is mandatory to complete their life cycle is therefore still under some debate, but it is likely that their dependence on freshwater ecosystems varies among regions. In some regions, for example, it has been observed that *M. cephalus* can complete its life cycle in strictly salt water (Whitfield et al. 2012), whereas in other places their densities have been shown to be highly dependent on freshwater rearing habitat (Marais 1983). Despite these uncertainties, there is strong consensus among researchers that *M. cephalus* does not breed in freshwater, and that it has a high affinity for freshwater, especially in their juvenile life stages.

To date, most of the research on *M. cephalus* has taken place in marine or estuarine environments, with far less research focusing on the ecology of mullet in freshwater ecosystems. My dissertation research aims to fill this knowledge gap. This question has its roots in the many encounters I had with *M. cephalus*, *Kuhlia sandwichensis* and *Kuhlia xenura* in the estuaries and streams during my childhood growing up in Hawai‘i. While I was an undergraduate I became interested in natural science, and it was until later while I was a graduate student at the Institute of Ecology (now the Odum School of Ecology), University of Georgia that I realized the ecology of *M. cephalus* in streams was an interesting and important question. That feeling was confirmed upon my completing my migration home to my natal streams of Hawai‘i and seeing schools of mullet grazing throughout Waiāhole Stream.

### ***The setting: streams of the Hawaiian Islands***

This research was conducted in the main Hawaiian Islands. Hawai‘i consists of a chain of oceanic islands in the “middle” of the Pacific Ocean. It has eight main islands that range in area from 10,432 to 115 km<sup>2</sup>, and in height from 380 to 4,200 m. Five of the main Hawaiian Islands have perennial streams (~300) flowing down their slopes. There is a large amount of heterogeneity between streams in Hawaiian Islands due to a combination of differences in precipitation among watersheds on the same island and differences in island age. For example, windward facing slopes tend to be wetter than leeward facing slopes, resulting in higher discharge and more eroded watersheds compared to leeward facing slopes. Younger islands tend to be larger, have taller mountains and shallower soil layers, which results in larger watersheds with higher slopes, larger substrate, and more waterfalls.

The community of native stream fish in Hawai‘i is all migratory and marine-derived, and consists of both amphidromous and catadromous taxa (Kinzie 1988). The freshwater-amphidromous species include a species of sleeper (*Eleotris sandwichensis*) and 4 gobies (*Stenogobius Hawaiiensis*, *Awaous staminous*, *Sicyopterus stimpsoni*, and *Lentipes concolor*). The catadromous fishes include *Mugil cephalus* (*Mugilidae*), as well as *Kuhlia sandwichensis* and *K. xenura* (*Kuhliidae*). *Mugil cephalus* and the two native *Kuhlid* species, along with the native sleeper and two native gobies (*Stenogobius Hawaiiensis* and *Awaous staminous*), are commonly found in the lower to middle reaches of streams in Hawai‘i, but *M. cephalus* is the only grazer among this group. *M. cephalus* can reach high densities in the lower reaches of streams and spends its days scrubbing the surface of rocks within the stream. They are found in

the stream year round, however the population size structure changes through time suggesting growth in the freshwater environment. For example, the population is dominated by small-sized individuals (~5 cm) in the late summer, by medium-sized individuals (~11 cm) in spring, and by large individuals (~18 cm) in summer. These large fish can weather even the largest floods with ease.

*Mugil cephalus* has long been an important species in Hawai‘i. It has many names in Hawaiian (e.g. ‘Aanae, ‘Ama‘ama, Kahana, Po‘olā) signifying the high status of this fish in indigenous Hawaiian culture, a feeling that is echoed by many of the settler decedents that now call Hawai‘i home. In pre-European times, Hawaiians invented an ingenious method of raising mullet that involved enclosing areas of shallow coastal waters using stonewalls with sluice gates where mullet could be easily collected and retained. Some of these fish ponds still remain and can be seen easily from satellite images. The strong relationship between people and mullet in Hawai‘i continues today, with some of the first work on mullet husbandry (Shehadeh and Ellis 1970) and the only work looking into the possibility of stock enhancement through hatchery production conducted in Hawai‘i (Leber et al. 1996). It thus seems fitting that my research, which represents some of the first work on the ecology and behavior of *M. cephalus* in freshwaters, was conducted in Hawai‘i as well. With Hawai‘i’s freshwater ecosystems under the pressure from modern society and changing weather patterns, understanding how our ‘Aanae use freshwater ecosystems is urgently needed.

### ***Research questions***

Here, I seek to shed light on the ecology and behavior of juvenile *M. cephalus* in streams by investigating the following research questions:

1. How do large scale environmental factors influence the occurrence of *M. cephalus* among watersheds in Hawai‘i?
2. Are juvenile *M. cephalus* selecting particular the feeding microhabitats in a lowland Hawaiian stream?
3. Is Passive Integrated Transponder (PIT) tag technology a feasible way to study the ecology of juvenile *M. cephalus* in streams, and how can this technology inform our understanding of mullet movement between streams and adjacent estuaries?

### ***Dissertation outline***

This research was conducted in Hawai‘i and explores the ecology and behavior *M. cephalus* in streams at large and small spatial scales. To do this, I utilized a mixture of data gleaned from the other sources and from my own field studies. This work represents an important contribution to our understanding of mullet ecology by focusing on the understudied freshwater phase of their life history. The research is also locally important in Hawai‘i, by providing useful information to resource managers making decision regarding Hawai‘i’s streams and provides a foundation for future work in this region.

In chapter two, I constructed generalized linear models (GLMs) to accommodate the binary nature of the response variable (i.e., mullet presence or absence). I then developed 11 candidate models, including combinations of 4 variables (channel slope, watershed area, watershed health, and discharge) to explain *M. cephalus* distributions among 33 Hawaiian watersheds. The overall aim of this chapter was to provide an empirical description of the large scale factors that influence the distribution of *M. cephalus* in Hawaiian streams. This information is useful in a management context for identifying streams with characteristics suitable for supporting mullet, which might help focus restoration efforts in watersheds that are seemingly suitable but currently lack mullet.

In chapter three, I report the results of a field study in which I made observations on the characteristics of the microhabitat where juvenile *M. cephalus* were observed feeding within a stretch of a lowland stream on the windward side of Oahu. I then quantified the habitat that was available in this same stretch of stream. By comparing the microhabitat that mullet selected to those that were available to them, I was able to document microhabitat selection by feeding mullet.

In my fourth chapter, I investigate the feasibility of using Passive integrated Transponder (PIT) tag technology – a uniquely coded tag – with juvenile *M. cephalus*. This technology has been used in several other systems to study the dynamics and behavior of stream fishes, but had yet to be applied to the study of mullet ecology. To do this, I first conducted a laboratory study that was carried out at Anuenue Fisheries Research Center to assess the retention rates and effects of tagging on the health of juvenile *M. cephalus*. After confirming via the lab study that mullet retain these tags at a high rate and grow well compared to untagged control fish, I shifted my focus to a study of their movement behavior in streams. Specifically, I tagged individuals in the field and installed a stationary PIT-tag antenna to monitor the movement of tagged individuals between the stream and the downstream estuary.

Finally, in my fifth chapter, I conclude my dissertation with a discussion of the main conclusions from this body of work and my thoughts on future directions that would continue to expand our knowledge of the biology, behavior, and ecology of *M. cephalus*.

## **Chapter 2**



## Chapter 2

### **The influence of large scale environmental factors on *Mugil cephalus* occurrence in Hawaiian watersheds.**

#### **ABSTRACT**

Broad-scale environmental factors are often useful in explaining the distribution and abundance of stream fishes within and among watersheds. In this study, we investigate how watershed-scale factors influence the presence of *Mugil cephalus* among Hawaiian watersheds, and we explore how reach-scale variables compare between neighboring streams that do and do not contain mullet. For the watershed-scale analysis, we used a model selection framework to test the relative support of 11 models including combinations of the variables channel slope, discharge, watershed area, and watershed health to describe mullet occurrence, using mullet presence-absence and watershed-scale data from 33 streams distributed around Hawai'i. Our results suggest that slope is the single most influential variable and that mullet tend to occur in watersheds with a lower stream slope in the vicinity of the mouth. Discharge was also important in determining mullet occurrence, but to a lesser degree than slope, with mullet occurring in watersheds with relatively higher discharge. To complement the large-scale analysis, we conducted a reach-scale analysis by comparing the depth, velocity, canopy cover, and wetted width measurements from four neighboring streams that all drain into Kāneʻohe Bay, Oʻahu, only one of which is used by mullet. Our results suggest that the non-mullet streams tend to be narrower and shallower than the one stream containing mullet, likely related to the higher discharge in this watershed. In contrast, we found no consistent differences in stream velocity or canopy cover among these neighboring streams. Overall, our results suggest that slope and discharge are important drivers of *M. cephalus* presence in Hawaiian streams and that stream width and depth at the reach-scale may play a role as well. These results suggest that declining baseflows due to diversions or climate change may negatively impact mullet populations by decreasing access to freshwater streams and also points to the potential benefits of restoration efforts, such as barrier removals or flow restoration, for conserving *M. cephalus* in Hawaiian streams.

## INTRODUCTION

Understanding the factors that influence the distribution of species across the landscape is a basic goal in ecology with implications for conservation and management. Correlating species distributions to environmental factors has the added benefit of allowing predictive mapping based on statistical models (Porter, Rosenfeld, and Parkinson 2000). Such predictive mapping has been used to forecast patterns of invasive fish, such as round goby in the Great Lakes area (Kornis and Vander Zanden 2010). These models can also be used to identify important sites for conservation or restoration efforts targeting stream fishes (Filipe et al. 2004; Dauwalter and Rahel 2008). For example, in the Pacific Northwest, Steel et al. (2004) used predictive models based on landscape variables to identify potential breeding habitat of winter steelhead that helped prioritize restoration activities to increase access to these sites.

Several studies have focused on the large-scale variables influencing the freshwater distribution of anadromous fishes such as Pacific salmon and trout (e.g., Porter, Rosenfeld, and Parkinson 2000; Pess et al. 2002; Fransen et al. 2006; Kruse, Hubert, and Rahel 1997). For example, in the Snohomish River basin in Washington, coho salmon abundance is negatively related to the proportion of the watershed that has been developed (e.g., urbanized or in agricultural production) (Pess et al. 2002). As another example, Bozek and Hubert (1992) found stream size (i.e., wetted width) was significantly related to the distributions of cutthroat, brook, and rainbow trout in Rocky Mountain streams.

Here we explore the large-scale factors influencing the distribution of a catadromous fish, *Mugil cephalus*, in streams of the Hawaiian Islands. Like other catadromous fishes, mullet spawn and spend most of their life in saltwater, but make migrations into freshwater at some point in their life (McDowall 1988; McCormick, Farrell, and Brauner 2013). Despite a substantial amount of literature surrounding its ecology, physiology, and culture, little research has focused on the ecology of mullet during their freshwater rearing phase (Whitfield, Panfili, and Durand 2012).

The overarching goal of this study is to identify the large-scale factors influencing the distribution of *Mugil cephalus* in streams of the Hawaiian Islands, U.S.A. We addressed this goal through a two-pronged approach. First, we explored the influence of watershed-scale variables (channel slope, discharge, watershed area, and watershed health) on *M. cephalus* distributions among watersheds distributed across the State of Hawai‘i. We hypothesized that mullet presence in Hawaiian streams is positively associated with discharge, drainage area, and watershed health, and negatively associated with stream slope. In general, we assumed that watersheds needed to be of a minimum size (in terms of area and discharge) to support mullet. Moreover, we hypothesized that watersheds with higher overall watershed health would be more likely to support mullet because activities in the watershed can influence the quality of stream habitat (Allan 2004). Finally, we hypothesized that streams with a high gradient near the mouth would be less likely to support mullet as steep slopes could be a barrier limiting the access of these water-column swimmers. The second objective of this study was to compare how reach-scale variables differed between neighboring streams where mullet do and do not occur, all of which drain to the same bay and have open estuaries suggesting that mullet could in theory access the

full suite of streams. This allowed us to identify the local-scale factors that may be contributing to the larger-scale patterns of mullet distributions throughout the State of Hawai‘i.

## **MATERIALS AND METHODS**

### ***Study site***

The Hawaiian archipelago is located in the central Pacific Ocean (latitude: 18°55' N to 28°27'N, longitude: 154°48'W to 178°22'W) and consists of a chain of numerous atolls (northwestern half) and eight high islands (southeastern half). Across the five high islands, there are ~300 watersheds with perennial streams draining the slopes, ranging in discharge from ephemeral to 5.9 m<sup>3</sup>/s (mean annual discharge, USGS gauge 16713000). The precipitation is highly heterogeneous among Hawaiian watersheds due to orographic effects that cause rain to fall on windward facing slopes and leave leeward facing slopes dry. Differences in island age also contribute to watershed heterogeneity across the Hawaiian islands, with younger islands being taller, larger in area, and consisting of more subsurface flow (Lau and Mink 2006), while older islands are shorter, smaller in area, and have less porous soils.

### ***Study organism***

*Mugil cephalus* is a coastal fish species distributed around the world from 42N to 42S. Its ability to tolerate a wide range of salinities (i.e., fresh to hypersaline waters) allows it to utilize many different habitats, such as coral reefs, estuaries, and freshwater streams and rivers (Thomson 1966). An abundant species, *M. cephalus* is important both as a protein source and for supporting commercial fisheries (Thomson 1966). *M. cephalus* spawns in the marine environments, and after some time feeding and growing in the ocean, fry move into the estuary and eventually upstream into freshwater habitats (i.e., streams) as juveniles and sub-adults (Whitfield et al. 2012). In Hawai‘i, *M. cephalus* is ubiquitous in estuaries and often enters the lower reaches of Hawaiian streams (Yamamoto and Tagawa 2000). For example, in Waiāhole stream, Oahu, juvenile *M. cephalus* (5 – 7cm standard fork length) begin to move into the lower 1.5 km of the stream between the August and early October (K. Fraiola, personal observation), where they graze algae from the stream substrate (Fraiola and Carlson in prep). After spending approximately 10 months in the stream, they begin to leave the stream in early summer as sub-adults (14 – 18 cm) at the same time a new cohort of juvenile fish begin to arrive in the stream. Thus, mullet are present year round - at different size/life stages - in the streams where they occur.

### ***Watershed scale***

Our goal was to relate mullet presence or absence (response variables in our analyses) to four watershed and reach-scale variables: channel slope, annual discharge, watershed area, and a watershed health index. We were able to find 33 streams for which data on all four variables were available (described below) and which had no barriers between the estuary and stream that limited mullet access. State records indicated that mullet were detected in 14 of the 33 streams based on data reported in the “Atlas of Hawaiian watersheds and their aquatic resources”

(Parham et al. 2008), including streams on the islands of Kaua‘i, O‘ahu, Moloka‘i, and Hawai‘i. We focus on these 33 streams for our watershed-scale analysis (see Appendix 1).

A generalized linear model (GLM) framework was used to explore how the presence and absence of *M. cephalus* in Hawaiian streams is influenced by channel slope, annual discharge, watershed area, and watershed health. We determined channel slope for each of our focal watersheds from 1:24,000 USGS topographic maps. Specifically, we used the lower channel slope in analyses, which was calculated as the increase in elevation between zero and 200 feet or to the lower isocline nearest a barrier (e.g., a waterfall) divided by the length of stream between those two points. We determined the annual discharge for each of our focal watersheds using USGS gauge data. When more than one year of data were available (32 of 33 cases), we calculated the average discharge across years for analyses. Some streams (n=2) did not have a mainstem gauge but did have gauges on all tributaries; in these cases, we estimated discharge by summing the discharge from all of the gauged tributaries. We used watershed area (km<sup>2</sup>) data presented in (Parham et al. 2008) in our analyses. Finally, we included an index of watershed health based on earlier work by (Rodgers et al. 2012) that consisted of 9 weighted metrics developed from land cover classes (e.g., native forest, alien forest, grasslands, open sparse vegetation, very sparse un-vegetated, agriculture, high intensity developed, low intensity developed, roads, water bodies.).

We constructed 11 generalized linear models to test our hypotheses regarding the influence of slope, discharge, watershed area, and watershed health. These included models of the main effects, their pairwise combinations, and a full model. The pairwise combinations were included to explore the relative importance of the variables and to account for possible correlations between variables in a multi model inference framework. We ranked competing models using AIC<sub>c</sub> values because our ratio of sample size to parameters (i.e., n/4) was < 40 (Burnham and Anderson 2002). We calculated “weighted AIC<sub>c</sub>” ( $w_i$ ) as:

$$w_i = \frac{e^{-0.5\Delta_i}}{\sum_{r=1}^R e^{-0.5\Delta_r}}$$

(Burnham and Anderson 2002). We performed all analyses using the statistical packages “glm” and “AICcmodavg” in R (R Core Team 2014; Mazerolle 2015).

### ***Reach-scale comparison***

As a complement to our watershed-scale analyses, we explored the influence of reach-scale factors in determining mullet presence in streams by focusing on several streams from neighboring watersheds (i.e., stream mouths within 2.4 km of one another) on the windward side of O‘ahu Island, Hawai‘i (latitude: 21.48°N, longitude: -157.85°W) and that have similar aspect (east), environmental regimes, and geologic histories. They also flow into the same embayment (Kāne‘ohe Bay), and are similar in that they have estuaries that are open to the bay. Additionally, they have similar channel slopes over the lowest portion of the stream (1.5% – 2%). However, they differed in terms of discharge and whether or not mullet were present, with the one “mullet stream” having a much higher discharge (average annual, 1.1 m<sup>3</sup>/sec ± 11 (SD)) compared to the three surrounding “non-mullet streams” (Table 3).

We compared the mean values of microhabitat variables taken from the lower reaches of the one stream containing mullet (Waiāhole) to neighboring streams that lacked mullet (Waikāne, Ka‘alaea, and Waihe‘e). Reach-scale variables (depth, velocity, canopy cover, and wetted width) were measured along systematic transects of the lower portions of each of these four streams. Velocity was measured at the 60% depth, and canopy cover was estimated using a convex spherical densitometer. Transects were placed perpendicular to the direction of stream flow, and depth and velocity were taken at regular intervals along each transect (i.e. wetted width), and one canopy cover per transect. The spacing of transects and sample locations along transects were roughly proportional to the relative length and width of each habitat, such that we had approximately 3 transects with approximately 4 sample locations (e.g. for depth and velocity) for moderately sized mesohabitats (e.g., pools, riffles, etc.). We sampled 38 transects on Waiāhole Stream, 27 on Waihe‘e Stream, 19 on Waikāne Stream, and 20 on Ka‘alaea. We tested for differences between the mean values for each variable between each pairwise combinations of streams using a generalized linear modeling approach implemented within R using the package “glm”.

## RESULTS

### *Watershed-scale analysis*

Streams where mullet occur had an average watershed health index of  $73 \pm 17$  (SD) (range: 42 – 95), channel slope of  $1.16\% \pm 0.4$  (SD) (range: 0.5 – 2), average annual discharge of  $2.0 \text{ m}^3/\text{s} \pm 1.63$  (SD) (range: 0.13 – 5.9), and average watershed area of  $58.6 \text{ km}^2 \pm 58.7$  (SD) (range: 6.1 – 222.6). Streams without mullet had a slightly lower average watershed health index score of  $64 \pm 20$  (SD) (range: 30 – 92), and higher channel slope of  $2.6\% \pm 2$  (SD) (range: 0.9 – 8.9), a lower average annual discharge of  $0.69 \text{ m}^3/\text{s} \pm 0.86$  (SD) (range: 0.04 – 3.46), and a lower watershed area of  $26.5 \text{ km}^2 \pm 33.9$  (SD) (range: 3.1 – 151.7).

The top three models explaining mullet presence in Hawaiian streams were the slope + discharge, slope, and slope + watershed health models. All of these models were within 2  $\text{AIC}_c$  units of one another, suggesting some support for each of these (Table 1). Thus, the top models all included slope and the second most supported model included the variable slope alone. These results suggest that overriding importance of slope in determining mullet presence in Hawaiian streams. This is supported by the fact that the next closest models were slope + watershed area and the full model ( $3.7 \Delta\text{AIC}_c$ ), both of which also included the variable slope (Table 1). In looking at the associated parameter estimate for slope in each of these models, slope is consistently negatively associated with mullet presence (Table 2).

### *Reach-scale comparison*

As a reminder, for our reach-scale comparison, we compared reach-scale variables between neighboring streams with mullet (Waiāhole) to those without mullet (Waikāne, Ka‘alaea, and Waihe‘e). The mullet stream (Waiāhole) had a mean ( $n = 38$ ) wetted width of  $7.9 \text{ m} \pm 2.2$  (SD), velocity ( $n = 158$ ) of  $0.35 \text{ m}/\text{sec} \pm 0.32$  (SD), depth ( $n=158$ ) of  $0.64 \text{ m} \pm 0.60$  (SD), and canopy cover ( $n=38$ ) of  $67\% \pm 26$  (SD). The average wetted widths (sample sizes

equivalent to canopy cover) and depths (sample sizes equal to that of forth coming velocity measurements) of each of the three non-mullet streams were significantly less than that of the mullet stream (Figure 1). The average velocities of the non-mullet streams were equivalent ( $0.35 \pm 0.25$  m/sec, and  $0.33 \pm 0.27$  m/sec, Waikāne (n=78) and Waihe‘e (n=101) respectively; mean  $\pm$  SD) or slightly less ( $0.14 \pm 0.19$  m/sec, Ka‘alaea (n=51); mean  $\pm$  SD) than that of the mullet stream. Average canopy cover was variable among the streams, and the average canopy cover of the mullet stream ( $67 \pm 26\%$ , mean  $\pm$  SD, n = 38) was significantly lower than Waikāne (n=19), similar to Waihe‘e (n=27), but higher than Ka‘alaea (n=20) ( $89 \pm 12\%$ ,  $58 \pm 23\%$ ,  $51 \pm 24\%$ , respectively; mean  $\pm$  SD).

## DISCUSSION

While the occurrence of *M. cephalus* in streams and rivers of the Hawaiian Islands is well known (Leber et al. 1995; Yamamoto and Tagawa 2000), they do not occur in all watersheds. Our goal was to try and elucidate some of the factors associated with mullet presence in Hawaiian streams. Our findings suggest that stream slope was strongly associated with mullet presence, supporting our hypotheses that mullet will be excluded from streams with high gradients near the mouth. Discharge was also important, but to a lesser degree, and was positively associated with mullet presence. The findings from our reach-scale comparisons suggest that mullet occur in streams that are wider and deeper, on average, than nearby streams where mullet do not occur.

Our results indicate that slope in the lowermost sections of watersheds near the estuary has a negative influence on *M. cephalus* presence in a given watershed. Stream slope is widely appreciated as a factor limiting the upstream distributions of stream fishes – e.g., salmonids (Chisholm and Hubert 1986; Kruse et al. 1997) and gobies (Schoenfuss and Blob 2003; Kido 2007). In Hawaiian streams, there is often strong longitudinal zonation of fish communities, with distinct fish communities occupying lower, middle, and upper reaches of watersheds (Kido 2007). These longitudinal patterns are believed to be related to differences in the ability to surmount barriers (Yamamoto and Tagawa 2000). Lower reaches are often a mixture of specific subset of amphidromous taxa (i.e., gobiidae or eleotris sp.) and juvenile euryhaline fishes from one of three taxa that enter the stream from the estuary (i.e., *Mugil cephalus*, *Kuhlia sandwichensis*, and *Kuhlia xenura*). *M. cephalus* has a torpedo shaped body (Moyle 2002) typical of a mobile water column fish and although they are known to jump often, they are not able to ascend barriers (Bok 1979). In contrast, the dorsally compressed body shape and sucking disk of gobies allows these fish to access high elevation, upper portions of these watersheds. We posit that the lower energy habitats in low slope reaches that are dominated by the riffle-pool channel morphology may be more conducive to mullet morphology and behavior than higher energy reaches that are characterized higher slopes (e.g., step-pool and cascade channel types) (Montgomery and Buffington 1997; Thomson et al. 2001). Another possibility is that mullet distributions in streams is partly determined by feeding opportunities. Recent work on the feeding microhabitat use of *M. cephalus* has shown that *M. cephalus* select grazing sites characterized by moderate depth and velocities, while under-selecting microhabitats that are at the extremes of the depth and velocities spectrum available (Fraiola and Carlson in prep). Future

work investigating how these preferred microhabitats change with increasing slope would help us better understand how slope influences *M. cephalus* presence in a stream.

We found that discharge was positively associated with *M. cephalus* presence and may be related to changes in habitat composition at both reach and microhabitat scales. As discharge decreases in a stream, high and moderate velocity habitats are significantly reduced relative to other mesohabitats (Hauer et al. 2009), shifting the microhabitat composition away from the moderate velocities which *M. cephalus* select for feeding (Fraiola and Carlson in prep).

Despite the influence of discharge on mullet occurrence in watersheds and the well-established relationship between discharge and watershed area (Dunne and Leopold 1978), we found that watershed area had almost no support as a factor influencing mullet presence. This may be due to a weak relationship between watershed area and discharge in Hawaii, as a result of the highly heterogeneous precipitation patterns among watersheds (Lau and Mink 2006). Additionally, the lack of correlation may be a result of the strong orographic nature of rainfall in the Hawaiian islands, often resulting in a “wet side” on the northeastern mountain slopes and a “dry side” that is found in the rain shadow of the mountain (Lau and Mink 2006). The younger islands, like Maui and Hawai‘i Island are also larger and taller, and therefore can have fairly large watersheds, with high and low flows depending on what side of the island they are on. For these reasons, it is possible to find mullet in streams with high discharge, but which vary greatly in watershed area.

The influence of watershed health on stream fish, mediated through effects on water quality and habitat, has been long recognized (Wang et al. 2001; Meador and Goldstein 2003; Allan 2004). In our study, we found that the watershed health did not significantly influence mullet occurrence in Hawaiian streams. The weakness of this relationship may, in part, be due the difficulty of translating broad-scale land cover estimates to an index of stream health that are relevant to specific components of the stream community (King et al. 2005). By learning more about the water quality conditions that influence *M. cephalus* distributions in streams, we may be able to better utilize broad-scale land cover data to model those factors. For example, recent work has on *M. cephalus* in a Hawaiian stream has shown that *M. cephalus* select moderate size substrate (e.g., gravel, pebble, cobble, etc.) as feeding sites, which suggests that *M. cephalus* may be sensitive to changes in watershed land use that influences sediment composition, like soil erosion. Future work expanding on how *M. cephalus* interact with the physical habitats in streams along with work on how different water quality parameters influence *M. cephalus* in streams will greatly improve our ability to conserve and manage watersheds to support these iconic fish.

The smaller-scale comparisons of neighboring streams draining into the same bay offered additional insights into the importance of discharge. Since the non-mullet streams varied little in terms of slope or land use and all were open to the same bay but had lower discharges than the one “mullet stream”, these smaller-scale results reaffirm the importance of discharge in determining mullet use of a particular stream. When we then compared the reach-scale habitat across these systems that differed in discharge and mullet use, we found that the higher discharge/mullet site tended to be deeper and wider. Perhaps these conditions are associated with better feeding opportunities or less risk from predators. For example, smaller stream habitats

force fish into shallow water where they are more vulnerable to terrestrial predators, such as wading birds (Power 1984). An alternative could be that as overall depth decreases that the shallower habitats, like riffles, become difficult to navigate, no new mullet recruited the stream. Future work examining how different environmental factors change in relation to discharge would help illuminate how abiotic and biotic variables interact to influence *M. cephalus* distributions in Hawaiian streams.

### ***Conclusion***

Stream biota in Hawaiian streams, including mullet, face a number of challenges from decreasing base flows in streams (Oki 2004), increased sediment supply (Laws and Ferentinos 2003), and channelization (Timbol and Maciolek 1978). Our results suggest that decreasing base flows in Hawaiian streams could shift streams away from ones that support mullet. The understanding gained in our study could help resource managers identify streams that are suitable for mullet, and better understand how certain restoration activities – e.g., the removal of barriers or the restoration of flow to a stream - might alter which streams are utilized by *M. cephalus*.

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## TABLES

Table 1. Models selection results for analysis of *Mugil cephalus* presence-absence data from 33 Hawaiian based on combinations of watershed area, discharge, stream slope, and watershed health (see Methods) streams. Models are presented in rank order based on their AIC<sub>c</sub> values.

Model	$p$	AIC <sub>c</sub>	$\Delta$ AIC <sub>c</sub>	Rank	$w_i$
Slope + Disharge	2	33.237	0.000	1	0.297
Slope	1	33.281	0.043	2	0.291
Slope + Watershed health	2	33.490	0.253	3	0.262
Slope + Watershed area	2	35.622	2.384	4	0.090
Full	4	36.906	3.669	5	0.048
Disharge	1	40.937	7.700	6	0.006
Discharge + Watershed area	2	43.331	10.094	7	0.002
Watershed health + Waterhed area	2	43.364	10.127	8	0.002
Watershed area	1	45.329	12.092	9	0.001
Watershed health + Waterhed area	2	46.413	13.175	10	0.000
Watershed health	1	47.263	14.026	11	0.000

Table 2. Parameter estimates associated with each of the 11 models describing *Mugil cephalus* presence-absence from 33 Hawaiian streams.

‘.’ = 0.1 > P > 0.05, ‘†’ = P < 0.05.

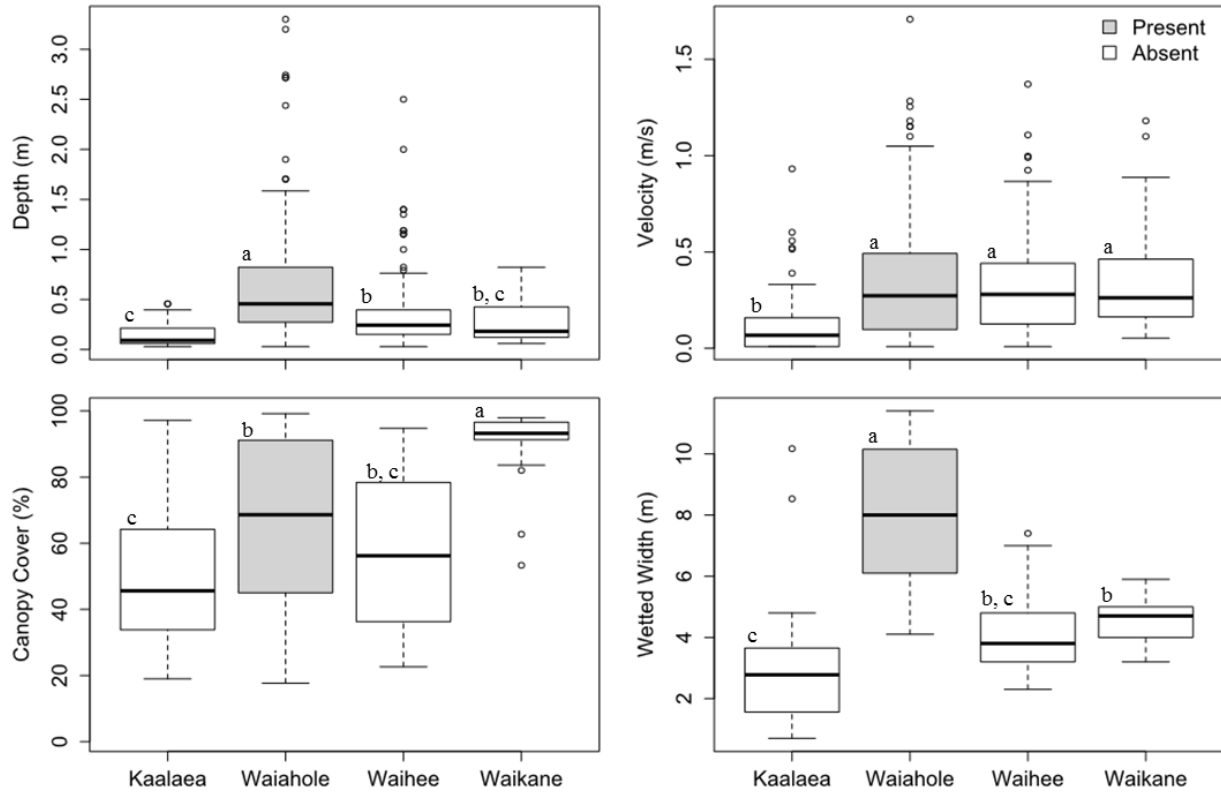
Model	Parameter estimates			
	HI	SI	Di	DA
Slope + Discharge	--	-2.39 <sup>†</sup>	0.86.	--
Slope	--	-3.31 <sup>†</sup>	--	--
Slope + Watershed health	0.04	-2.59 <sup>†</sup>	--	--
Slope + Watershed area	--	-3.57.	--	0
Full	0.01	-3.03	1.18	-0.02
Discharge	--	--	0.96 <sup>†</sup>	--
Discharge + Watershed area	--	--	0.90	0
Watershed health + Watershed area	0	--	0.97 <sup>†</sup>	--
Watershed area	--	--	--	0.02
Watershed health + Watershed area	0.02	--	--	0.02
Watershed health	0.03	--	--	--

Table 3. Large-scale variables of the one “mullet” stream (Waiāhole) compared to three “non-mullet” streams. \* indicates a spot measurement at the time that sampling occurred.

Stream	Aspect	Channel Slope (%)	Discharge (m <sup>3</sup> /s)
Waiāhole	East	1.5	1.1 ± 0.31
Waihe‘e	East	2	0.45 ± 0.07
Waikāne	East	1.5	0.31 ± 0.10
Ka‘alaea	East	2	0.02*

## FIGURES

Figure 1. Microhabitat variables (depth, velocity, canopy cover, and wetted width) measurements for the mullet stream (Waiāhole, light grey fill) compared to the three adjacent non-mullet streams (Ka‘alaea, Waihe‘e, and Waikāne, no fill). Similar (different) letters denote streams with statistically insignificant (significant) differences in their means at  $\alpha=0.05$ .



## **Chapter 3**

## Chapter 3

### Feeding microhabitat use and selectivity of juvenile *Mugil cephalus* in a Hawaiian stream

#### ABSTRACT

Understanding the microhabitat use of an organism is an important first step to understanding its ecology and also provides critical information for guiding management and conservation of riverine fishes. Most microhabitat studies focused on freshwater fishes have focused on temperate systems, with far less research on fish microhabitat use in tropical streams. Here, we studied the microhabitat use and selectivity patterns of juvenile *Mugil cephalus* within a Hawaiian stream, USA (Waiāhole stream, latitude: 21.48°, longitude: -157.85°). We measured the depth, velocity, substrate composition, and canopy cover at locations where *M. cephalus* were observed feeding, and compared the attributes of these locations to the microhabitat available to the fish. We found significant differences in the kinds of microhabitats where mullet fed when compared to the habitat available, with feeding over-represented in microhabitats with moderate velocities (0.21 – 0.60 m/s), shallow to moderate depths (0.31 – 0.80 m), gravel and pebble as dominant substrates, and low canopy cover. The results of our study suggest that juvenile *M. cephalus* are selecting for erosional type habitats and avoiding depositional habitats, a result that was also supported by a multivariate analysis of habitat selectivity. Our data corroborates the results of other studies that have found fish grazers select habitats that are clean (erosional) rather than silted (depositional). Knowing how *M. cephalus* in streams and rivers use the habitats available is important for understanding how to manage nursery freshwater habitat for juvenile *M. cephalus* in the face of increasing anthropogenic impacts and a changing climate.

## INTRODUCTION

Understanding how organisms use the available habitat is an important first step in learning about their ecology. In streams and rivers, microhabitat (e.g. velocity, depth, and substrate) use studies have helped reveal the habitat requirements of fish and the degree to which sympatric fish species partition stream habitat (Rosenfeld 2003). For example, studies of microhabitat use have been used to assess the potential success of planned fish reintroductions and to better understand why past reintroductions may have failed (Harig and Fausch 2002; Mundahl et al. 2012). Researchers have studied microhabitat use to investigate the role competitive interactions between native and invasive fish play in decreasing the population densities of native stream fish (Nakano et al. 1998). Microhabitat use information can also serve as input to models that predict how changes in stream discharge due to diversions or climate change will affect fish populations. For example, the “instream flow incremental methodology” model is widely used to predict how populations of aquatic biota will respond to changes in stream discharge and relies upon habitat suitability curves generated from microhabitat use and availability data (Bovee 1982).

Of the many fish functional feeding groups in stream and river ecosystems, grazers and detritivores have received much less research attention than other fish functional feeding groups (Matthews et al. 1987), such as drift feeders (Young et al. 1998; Anglin and Grossman 2013), especially in regards to their microhabitat use (e.g., selection, preference, association). This is despite the potentially important impacts that fish grazing can have on the structure and function of stream ecosystems (Power et al. 1985; Flecker 1992). Furthermore, most of the published research on microhabitat use has focused on taxa from temperate streams and rivers, with very few examples from tropical systems, where these functional feeding groups comprise a significant portion of the fish community (Winemiller et al. 2008).

*Mugil cephalus* (Linnaeus, 1758) is a coastal fish species distributed around the globe from 42 North to 42 South latitude (Thomson 1966). This species is important ecologically, nutritionally, and economically (Blaber 1997). *M. cephalus* tolerates a wide range of salinities, so uses a wide range of habitats, such as coral reefs, estuaries, and streams/rivers (McDowall 1988). Because of its economic importance, *M. cephalus* has been the subject of numerous scientific investigations (Whitfield et al. 2012). Most of this research, however, has focused on *M. cephalus* in marine or estuarine environments (McDonough and Wenner 2003; da Silva Rocha et al. 2005; Cardona 2006; Shen et al. 2011).

While some aspects of *M. cephalus* ecology in freshwater have been explored, such as movements between rivers and coastal environment (Thomson 1955; Wang et al. 2010), there has been a dearth of research exploring their microhabitat preferences (e.g. depth, velocity, substrate, etc.). Insights into microhabitat use of juvenile *M. cephalus* in streams will help us better understand how changes in stream and river ecosystems, such as reduced flow and invasive species, might impact native *M. cephalus* populations.

The objectives of this study are 1) to characterize the microhabitat used by *M. cephalus* in a small lowland tropical stream in Hawai‘i, and 2) to examine evidence of microhabitat selection.

## MATERIALS AND METHODS

### *Study site*

Our habitat measurements and fish observations were collected in Waiāhole Stream, Hawai‘i, U.S.A. Waiāhole Stream is a 2<sup>nd</sup> order perennial stream on the Eastern side of O‘ahu Island. It has an average annual discharge of 1.2 m<sup>3</sup>/s (42 ft<sup>3</sup>/s, 2002 – 2012 water years, USGS 16294100), a drainage area of approximately 10.4 km<sup>2</sup>, and its predominant surrounding land use is scrub/shrub and evergreen forest (Parham et al. 2008). Waiāhole Stream also ranks high among the streams of O‘ahu in terms of native biodiversity (Parham et al. 2008). We studied a 54-m reach of stream with its downstream boundary approximately 200 meters upstream from the estuary. Here, the tide had negligible effects on the stream’s depth and velocity. The study reach was within the mullet’s longitudinal range and encompasses the range of depths, substrates, velocities, and canopy cover available to the fish through the entire stream. Baseflow at the time of observations ranged between approximately 0.7 - 0.6 m<sup>3</sup>/s (USGS 16294100), and there were no significant fluctuations in baseflow due to rain events during the study period.

### *Study species*

*Mugil cephalus* spend the majority of their adult life in brackish to salt water habitats near shore, but a range of size/age classes of juveniles can inhabit freshwater streams and rivers for extended periods (Thomson 1955; Thomson 1963; Cardona 2000; Wang et al. 2010). It is also known that some large river systems can support significant resident adult populations of *M. cephalus* and that adults can undergo annual migrations between rivers and offshore marine spawning grounds (Thomson 1955; Wang et al. 2010).

Both adult and juvenile *M. cephalus* are considered bottom feeders, consuming a variety of benthic food items including detritus, attached algae, and meiofauna (Thomson 1963; Blaber 1976), although they have also been observed feeding in the water column (Odum 1968) and will feed readily on floating pelletized fish food in aquaculture settings (Lee et al. 1992). At our study stream, we have observed juvenile *M. cephalus* feeding by scraping the surfaces of rocks. To do so, they angle their heads downward and push their lips against a rock below them, they then swing their head in a side-to-side motion in order to vigorously scrub the rock’s surface (K. Fraiola, personal observation). This feeding behavior is similar to their feeding behavior on soft sediments in estuarine environments (Thomson 1955).

In the main Hawaiian Islands, mullet spawning typically occurs in the marine environment from December – February. Fry then move into near shore estuarine areas to feed and grow. In Kāne‘ohe Bay, the bay into which Waiāhole Stream drains, estuarine conditions including steep gradients in salinity from surface to bottom are found primarily at stream mouths (Bathen 1968). These stream-mouth estuaries and the area immediately surrounding them are the primary habitat for juvenile *M. cephalus* in the bay (Leber et al 1996; Nishimoto et al 2007; Parham et al 2008). Upstream of the estuary in Waiāhole Stream, juvenile mullet (5 – 7cm standard fork length) enter the stream between August and late September, are generally found in schools ranging from the estuary to approximate 1.5 km upstream (personal observation, K.



Fraiola). Schools of fish can move quickly within and between stream channel units (e.g., riffles, runs, pools) and the number of individuals as well as the size structure of schools are continually changing, ranging from a single individual to 25 individuals. Schools can merge or split into smaller schools in as little as 15 – 20 minutes.

### ***Microhabitat use***

The microhabitat use of juvenile mullet was assessed using visual surveys from above the water (i.e., from the adjacent bank or bridge (see below)). Microhabitat use observations were made twice a day (morning and afternoon) on three different days spread out over three days in the summer of 2013 (6/28, 7/2, 7/5). We recognize that by repeating our sampling at the same location over multiple times we may have sampled individuals or schools more than once, but we believe that by staggering the observations by 7-8 hours during a day and spacing our sample days over the course of 8 days that we minimized the possibility of repeat counting.

Visual surveys from above water were necessary because *M. cephalus* avoid feeding in areas with divers. Good bankside overviews, combined with the stream's clear water, shallow depths (mean of 1m, SD = 0.84 m), and low gradient made locating and identifying feeding individuals feasible. To make observations, the lead author would first slowly approach the viewing area, then the location, number of fish, and approximate size of individual fish feeding in an area were recorded on a map of the site. Following each observation, a labeled weight identifying school or individual fish was placed to mark the location where the school or individual fish were seen feeding. Fish were assigned to one of three size categories: small (< 11 cm), medium (11 - 16 cm), and large (>16 cm). These size bins were determined by dividing the size range of mullet found in Waiāhole Stream over the course of year into three bins of equal size. The primary activity of juvenile *M. cephalus* in Waiāhole Stream was feeding, which was easily identifiable based on the characteristic feeding behavior (described above). Other easily identifiable behaviors include both cleaning and resting, but these observations were excluded from analyses due to very low numbers of observations for both of these behaviors.

The day following the fish observations, microhabitat use measurements were taken at each of the deployed markers. At each marker, water velocity, depth, substrate composition, and canopy cover were measured. Water velocity was measured at the 0.6 depth using a Pygmy Flow meter. The percent composition of the substrate size classes was estimated visually within a 0.25 m x 0.25 m quadrat (centered on each marker). Sediment were placed into one of seven size classes based on a modified Wentworth scale (Cummins 1962), including silt (<0.06 mm), sand (0.06 – 2 mm), gravel (2 – 16 mm), pebble (16 – 64 mm), cobble (64 – 256 mm), boulder (>256 mm), and bedrock or cement. Canopy cover (%) at each marker was estimated using a spherical densiometer.

### ***Microhabitat available***

The microhabitat available to the fish was quantified across the study reach using the following systematic approach. Transects perpendicular to flow were placed every 2 meters along the stream reach. Microhabitat measurements were then taken every meter along transects. At each sampling point, the water velocity, depth, dominant substrate size class, and canopy

cover were recorded. The number of habitat measurements recorded for the entire study reach was 286 (27 transects, averaging 10 m wide), which represented approximately 25% of the total stream bottom area within the focal reach (based on quadrats of 0.5 m x 0.5 m).

### ***Statistical analyses- Microhabitat selection***

To test for evidence of microhabitat selection we used a chi-squared goodness of fit test, which allowed a test of the differences between the frequencies of microhabitat available (expected) and the frequencies of microhabitats used (observed) for feeding by juvenile *M. cephalus* (Zar 2010). Each habitat parameter (depth, velocity, substrate, and canopy cover) was tested separately and then visualized using a suite of correlated variables (see next paragraph). Habitat parameter values were divided into categories for the chi-squared test, which were determined such that they captured meaningful ranges and so that the average expected frequencies of categories was greater than 6 (Zar 2010). The substrate data was simplified into a categorical variable, dominant substrate size (i.e. the substrate size which accounted for highest proportion the sample).

Evidence of selectivity was also explored visually using Principle Components Analysis (PCA). This approach is often used in studies of microhabitat use because the inherent correlation of the microhabitat factors to one another is preserved (Grossman and Freeman 1987; Anglin and Grossman 2013). The microhabitat data did not meet the assumptions of normality required for PCA, and transformations were ineffective in alleviating this. Therefore, we chose to use the untransformed data in our PCA and to forego any statistical hypothesis testing using our PCA results. Instead, we extracted principle components from the “available microhabitat” data and then superimposed the “observed microhabitat” data on top of the two major components retained. Components with eigenvalues >1 and that were ecologically meaningful were retained (Grossman and Freeman 1987). To perform the PCA, we used the statistical package “FactoMineR” version 1.04 (Husson et al. 2014) in statistical package R (R Core Team 2014).

## **RESULTS**

A total of 54 observations of feeding individual juvenile *M. cephalus* or schools (ranging from 2-25 individuals) were made over the course of this study (Table 1). Juvenile *M. cephalus* observed in the focal schools ranged from 10 – 21 cm (standard fork length). The median size of a school was approximately 3 individuals, and the modal school size was 3 and 4 individuals. 45% of the schools were mixed size schools (medium and large individuals) and 55% were homogeneous schools with either only medium- (11 - 16 cm) or large- (>16) sized individuals. Observations of individuals feeding alone made up approximately 25% of the total feeding observations (14 individuals, Figure 1), 10 of which were large individuals and four of which were medium-sized.

### ***Microhabitat availability***

When considering the microhabitats available to the fish, the study reach was characterized by broad range of depths (0.03 – 2.9 m, Figure 1), with a mean depth of 0.99 m

(SD = 0.84). Velocity ranged from 0 – 0.9 m/s (mean = 0.16 m/s, SD = 0.22, Figure 1). All substrate types were documented in our microhabitat availability study (Figure 1). Across sample points, the average percentage of each substrate type was as follows: gravel (avg. = 26%, SD = 32%); silt (avg. = 25%, SD = 39%); pebble (avg. = 10%, SD = 16%); cobble (avg. = 8%, SD = 16%); sand (avg. = 8%, SD = 24%); detritus (avg. = 18%, SD = 34%); bedrock 4% (SD = 17%); and boulder 1% (SD = 5%). The proportion of samples that were dominated by gravel was 31.1% (Figure 1), followed by silt (25.9%), detritus (16.8%), sand (8.0%), cobble (7.7%), pebble (5.6%), bedrock (2.8%), and boulder (2.1%). Percent canopy cover of available habitat ranged between 21 – 100% cover (mean = 71%, SD = 20, Figure 1).

### ***Microhabitat use***

Habitats used by feeding *M. cephalus* ranged in depth from 0.18 – 1.13 m and had a mean depth of 0.53m (SD = 0.23). Feeding habitats ranged in velocity from 0.08 – 0.81 m/s with a mean velocity of 0.39 m/s (SD = 0.16). Samples at mullet feeding sites were composed of mixtures of substrate types. Specifically, at sites where mullet were actively feeding, gravel averaged 61% (SD = 22%) of the sample, pebble averaged 18% (SD = 14%), cobble averaged 16% (SD = 15%), bedrock and sand both averaged 3% (SD = 15% and 5%, respectively), and silt was nearly absent (avg. = 0%, SD = 0%). The majority of sites where mullet were observed feeding were dominated by gravel (83% of the total feeding sites, Figure 1) and were associated with canopy covers that ranged between 31 – 60% (mean = 51%, SD = 14).

### ***Habitat selection- Individual habitat variables***

We detected evidence of habitat selection by comparing the habitat available to juvenile *M. cephalus* with the habitat that they used, for depth, velocity, substrate, and percent canopy cover. In particular, juvenile *M. cephalus* feeding was over-represented in depths ranging from 0.31 – 0.80 m, and under-represented in depths < 0.3 m or > 0.91 m ( $\chi^2_{0.025,13}$  p <0.0001, Figure 1). In terms of velocity, feeding tended to be over-represented in velocities ranging from 0.21 – 0.60 m/s and under-represented in velocities < 0.20 m/s and > 1.21 m/s ( $\chi^2_{0.025,8}$  p <0.0001, Figure 1). For the dominant substrate, feeding was most over-represented on gravel substrates, while feeding was under-represented on substrates sand, silt and detritus ( $\chi^2_{0.025,7}$  p <0.0001, Figure 1). In relation to percent canopy cover, feeding individuals were over-represented in habitats with < 50% canopy cover and under-represented in habitats > 80% canopy cover ( $\chi^2_{0.025,7}$  p <0.0001, Figure 1).

### ***Habitat selection - Suites of correlated variables***

The first two principle components were the only ecologically interpretable components, and represented 45% of the total variation in the habitat available. Component 1 (PC1) captured the bulk of the variation (31%) and represented a gradient from shallow and fast habitats with moderately sized substrate to deeper slow moving habitats with small sediment sizes and detritus (Figure 2). Component 2 (PC2) captured 14% of the variation and represented a gradient from high canopy cover to low canopy cover (Figure 2). When super imposed upon the available microhabitat ordination space, feeding observations were clustered over a small portion of the microhabitat ordination space characterized by shallower depths; higher velocities, % pebble, %

gravel, and % cobble; and moderate % canopy cover. The mean component score for the habitat used by juvenile *M. cephalus* on PC1 was 1.9 (SD = 0.60) and 0.05 (SD = 0.06) for PC2.

## DISCUSSION

Our results suggest that 10-21 cm long juvenile *M. cephalus* in a Hawaiian stream are selecting moderate velocities, shallow to moderate depths, gravel, pebble, and, cobble substrate, and low canopy cover. While the results are from a single stream system, this work provides important information on the relationship between juvenile *M. cephalus* feeding behavior in a nursery streams and has implications for their conservation given pending threats in the form of invasive species and climate change.

The selection of erosional habitats is a behavior that has also been observed in other benthic grazer/detritivorous fish species in lotic freshwater ecosystems, in both temperate and tropical systems. In temperate systems, for example, juvenile *Catostomus warnerensis* (Snyder, 1908) from high-desert streams where Oregon, Nevada, and California meet. Kennedy and Vineyard (2006) found that young *C. warnerensis* were present primarily in riffle/run habitats as opposed to pool habitats and that they selected for moderate flows and relatively shallow depths. Selection of erosional type habitats was also observed in Grossman and Freeman's (1987) microhabitat use study in a southern Appalachian stream where they found that the Central Stoneroller (*Campostoma anomalum*; Rafinesque, 1820), a benthic grazer (Kraatz 1923; Matthews et al. 1986), also showed signs of selection of erosional type habitats. While these studies found similar results to our study, it is unclear how the selection of these habitats relates to specific behaviors, such as feeding, nest site selection, or sheltering.

Similarly, several studies from tropical systems have found that grazing fish select erosional habitats. On Reunion Island in the Indian Ocean, for example, a tropical grazing gobiid fish, *Sicyopterus lagocephalus* (Pallas, 1770) was found to select highly erosional (Donaldson et al. 2013) microhabitats, possibly as a result of its ability to cling tightly to rocks using the characteristic fused pelvic fins found in the goby family. In the same study, the authors propose that *S. lagocephalus*'s ability to utilize high velocity microhabitats has given it access to a "rich food resource" not available to other fishes. Grazing species of another family of tropical lotic freshwater fish, the loach (Balitoridae), have also been found to inhabit high velocity erosional habitats like riffles in eastern Sabah, Malaysia (Martin-Smith 1998). A study by (Sheldon 2011) that compared the microhabitat use of a native grazing cyprinid (*Paracrossochilus acerus*; Inger & Chin, 1962) with several sympatric native grazing loach in Sungai (River) Belalong on the island of Borneo provides microhabitat data supporting Martin-Smith's observation that loaches occupied erosional microhabitat with high velocities and that despite the loaches being able to occupy microhabitats with slightly higher water velocities that there was significant overlap in microhabitat use with the benthopelagic *P. acerus*.

Factors that might explain the selection of erosional habitats for feeding grounds by *M. cephalus* and other grazing fish could include higher benthic primary productivity and the distribution of fine sediment. For example, in Panamanian streams, Power (1983, 1984) found that the densities of armored catfish in pools were proportional to the productivity of benthic

algae in a given pool, which in turn was positively correlated with the openness of the canopy. The over-representation of mullet in microhabitats with lower canopy cover provides supporting evidence that primary productivity may be one of the primary factors driving microhabitat selection in juvenile mullet. Sediment could also be a major factor influencing the microhabitats selected for feeding by *M. cephalus*. Power (Power 1984c) found that sediment deposited upon benthic surfaces could impose a metabolic cost upon individual armored catfish and could be a significant factor during summer low flows when sediment deposition rates increased in pool habitats due to decreases in water column velocities in pool habitats. The under selecting of low velocity habitats by *M. cephalus* where fine sediment was higher and where algal standing stocks were lower (personal observation) could be a sign that fine sediment in Waiāhole Stream is not metabolically worthwhile to feed on despite the lower energy cost of gathering food in habitats with slower water velocities.

Biotic factors could also influence microhabitat use. For example, in the same Panamanian stream ecosystem, Power observed catfish avoiding the shallowest habitats along the margins of the stream where they were most vulnerable to bird predators (Power 1984a), a behavior that is also exhibited by *C. anomalum* in Ozark Streams in North America (Matthews et al. 1986). At our study site there is one potential predator of juvenile mullet, the ‘Auku‘u (*Nycticorax nycticorax*; Linnaeus, 1758), also known as a “Night Heron.” While we have observed night herons fishing in the area during the day, such observations were extremely infrequent. The impacts of these and other wading birds on *M. cephalus* in Hawaiian streams have not been explored and deserve further attention as an additional factor that could be influencing mullet microhabitat use.

Biotic interactions other than predation, such as territoriality and dominance hierarchies can also significantly affect a stream fish’s selection of specific microhabitats. For example, in stream dwelling salmonid populations it has long been observed that habitat use for feeding can be segregated by size of individual, with larger individuals occupying more productive feeding grounds (Chapman and Bjornn 1969; Fausch 1993; Nakano et al. 1995). Interspecific interactions between fish species, such as competition for feeding positions has also been shown to be able to potentially significantly influence a stream fish’s habitat selection and is well illustrated by salmonid examples from different parts of the world (Fausch and White 1981; Fausch et al. 1997; Nakano et al. 1998). Evidence of such inter- and intra-specific competition was not observed during our study. Intraspecific interactions for feeding sites may never be a problem for *M. cephalus* in Waiāhole Stream since they are a schooling fish and are one of the only native fish species in low gradient Hawaiian Streams that specialize on grazing benthic surfaces, where as in higher gradient stream reaches in Hawai‘i the grazing gobiid *Sycipoterus stimpsoni* is found in high abundances (Yamamoto and Tagawa 2000). However, this may change because many Hawaiian streams are currently being invaded by invasive armored catfish (*Ancistrus* c.f. *temmincki*, *Hypotomus* c.f. *watwata*, and *Liposarcus multiradiatus*) from the aquarium trade, which feed by scraping the surface of rocks and other hard substrates in streams (see previous paragraph) and, thus, is highly likely to compete with native mullet for food resources. Research on how benthic primary productivity, sedimentation rates, and predation risk relate to microhabitat selection of juvenile *M. cephalus* in streams will help us better understand the factors that might be driving the selection of erosional habitats by *M. cephalus* in Waiāhole

Stream, and add to our scientific understanding of what influences microhabitat selection in tropical grazing stream fish more generally.

Characterizing the microhabitat use of juvenile *M. cephalus* is an important first step in understanding how streams and rivers in Hawai‘i and elsewhere serve as nursery habitat for this species. Juvenile *M. cephalus* in Hawaiian streams currently face many challenges such as changes in habitat availability due to channelization for flood control (Timbol and Maciolek 1978) and decreasing discharge (Oki 2004; Oki et al. 2010), as well as increased competition with invasive species for food resources. For example, Oki (2004) showed that long-term trends in base flow from a number of gauged streams across the Hawaiian Islands are trending downward, possibly due to decreases groundwater storage and recharge. In the meantime, armored catfish species (another benthic grazer) are spreading to many streams in Hawai‘i and other parts of world (Yamamoto and Tagawa 2000; Chaichana et al. 2011; Pound et al. 2011; Lienart et al. 2013), where they may compete with *M. cephalus* and other native grazers. Future research, on how the presence of armored catfish might affect *M. cephalus* feeding will be important to understanding the risk armored catfish pose to juvenile *M. cephalus* populations (and other native grazers) in Hawaiian streams. More generally, improved understanding of the *M. cephalus* habitat use will help resource managers better understand how changes in the habitat available and potential competitors and predators might impact the productivity and sustainability of native *M. cephalus* in tropical streams and rivers.

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## TABLES

Table 1. Chi-squared test results for each habitat parameter comparing habitat used with the habitat available. df = degrees of freedom.

<b>Parameter</b>	<b><math>\chi^2</math> value</b>	<b>df</b>	<b>P value</b>
Water Velocity (m/s)	97.5	8	<0.0001
Water Depth (m)	66.7	13	<0.0001
Dominant Substrate	80	7	<0.0001
% Canopy cover	61	7	<0.0001

## FIGURES

Figure 1. Habitat availability and use for juvenile *M. cephalus* in Waiāhole Stream, Hawai‘i. There were 286 total available habitats in our study reach, of which 54 were used by juvenile mullet.

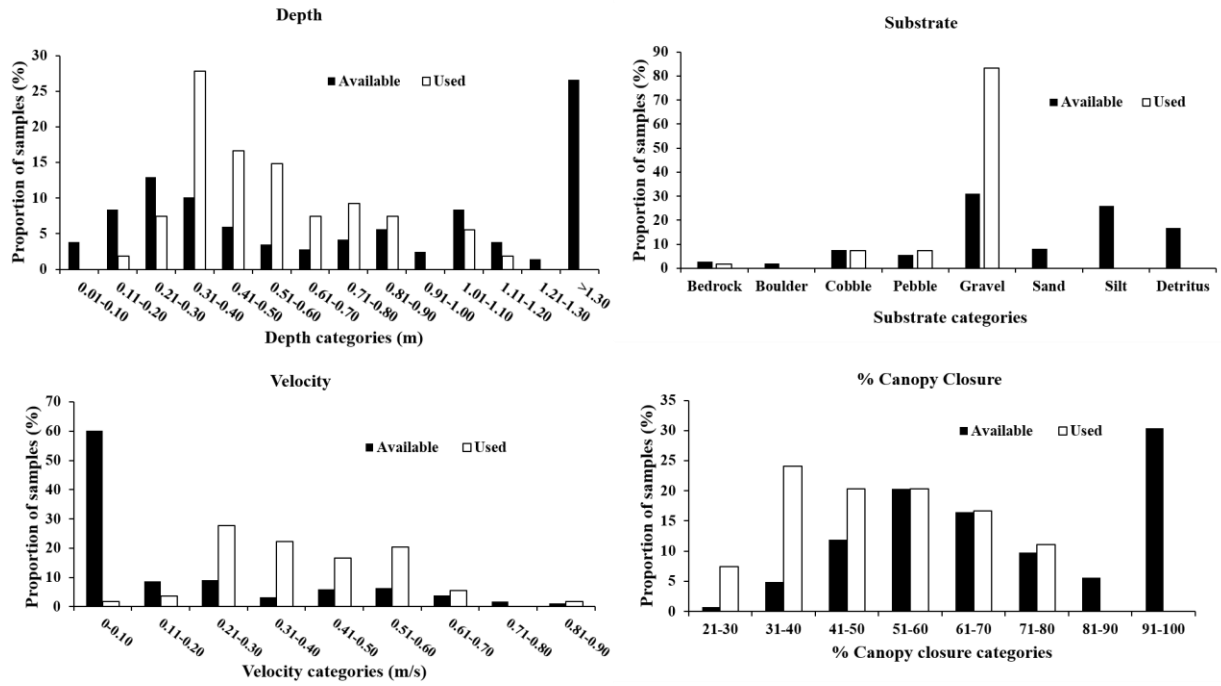
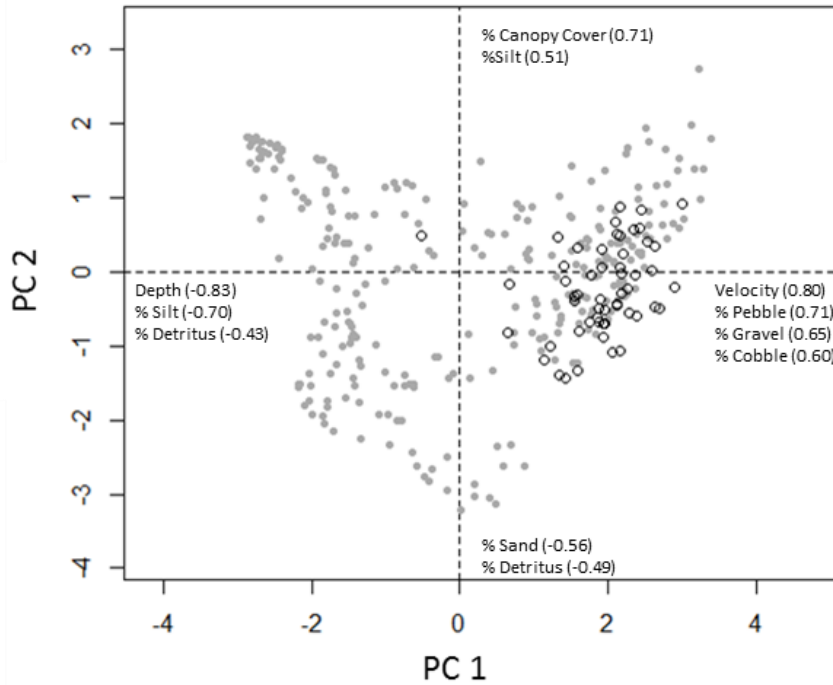




Figure 2. A graph showing the results of a Principle Components Analysis of the “available” microhabitat (gray circles) with the “used” microhabitat (open circles) superimposed on the ordination space of the available habitat. Microhabitat variables included were, depth, velocity, substrate size composition, and canopy cover. PC1 was interpreted as a gradient in canopy cover with more positive values being associated with higher canopy cover, while PC2 is interpreted as a gradient in depth, velocity, substrate composition – with deeper low velocity habitats that were dominated by fine sediments and detritus being more negative in value and shallower higher velocity habitats with more coarse substrate and less detritus having higher values.



## **Chapter 4**

## Chapter 4

### **Feasibility of using Passive Integrated Transponder technology for studying the ecology of juvenile striped mullet (*Mugil cephalus*) in streams.**

#### **ABSTRACT**

Passive Integrated Transponder (PIT) tags are an important technology used to study the ecology and behavior of fish. In this study we investigate the feasibility of using PIT technology to study the ecology of juvenile *Mugil cephalus*. First, we quantified the retention rates of PIT relative to VIE (visible implant elastomer) tags for juvenile *M. cephalus* through a laboratory study. Next, we conducted a preliminary field study of the movement patterns of individually-tagged juvenile individuals in a lowland Hawaiian stream (Waiāhole Stream) from early to mid-August 2014. For the lab portion of the study, 30 individuals (5-7 cm fork length) were randomly distributed among three treatments: PIT, VIE, and control (no tag). Tag retention was 100% for PIT and zero for VIE. For the field study, 19 individuals (14.6 - 18.7 cm fork length) collected from Waiāhole stream were tagged with PIT tags and the movement of individuals between the stream and estuary were inferred using a stationary PIT antenna array for 7.5 days. The antenna array registered 198 detection (97% during daylight hours, one missed detection) from 5 individuals, translating into 26 visits to the estuary. Trip lengths to the estuary ranged from 45 min to 22.25 hrs, with longer forays (>10 hrs) occurring primarily at night, while shorter forays occurred throughout the daylight hours. Together our results suggest that PIT tags are a feasible and effective marking technology to tag and track juvenile *M. cephalus* in streams, which paves the way for future research using this approach to study other aspects of their ecology.

## INTRODUCTION

Marking is an indispensable method for gathering data on the biology and ecology of fishes for research, conservation, and management (Nielsen 1992). By recapturing marked animals, researchers are able to measure growth rates, record movements, estimate population sizes, and identify the origins of individuals (e.g., wild versus captive bred). Of the wide variety of marking techniques available, only a few are suitable for very small individuals (*i.e.*, < 65 mm, (Skalski et al. 2009), and even fewer allow recovering information on the tag without physically recapturing or, in some cases, sacrificing the fish.

Marking small fish for the purposes of ecological research presents a unique set of challenges. Visible implant elastomers (VIE) tags and Passive Integrated Transponders (PIT) tags are two such marking techniques that allow for rapid identification of tagged fish without the need to sacrifice fish. Both have been successfully applied to small fish from a wide variety of taxa (Bruyndoncx et al. 2002; Younk et al. 2010; Burdick 2011; Hohn and Petrie-Hanson 2013). VIE marks are made by injecting a small amount of colored surgical grade silicone elastomer under the skin or in tissue that is translucent (Frederick 1997). PIT tags are small transponders surrounded by an inert coating (often glass) that are inserted into the body cavity or muscle (Gibbons and Andrews 2004). The lower cost, smaller volume and weight, and multiple color options of VIE tags makes them financially feasible for large tagging projects, allows for the marking of smaller fish, and offers a limited number of options for unique marks based on color and location of markings. While more expensive, PIT tags include unique identification number providing an opportunity to follow the fates of individual fish, can be combined with automated monitoring devices to study movements, and the tags have lifespans that are indefinite (Gibbons and Andrews 2004).

While marking animals creates an opportunity to learn much about their ecology, the process of marking and the marks themselves carry costs for the tagged organism. When considering various marking techniques for species that have not been the subject of earlier marking studies, it is critical to evaluate the efficacy of different marking options. *Mugil cephalus* L. (order: Mugiliformes) is one such fish species for which these tags have yet to be tested, and to which these tagging techniques might be used to address a number of interesting questions surrounding their ecology. *M. cephalus* is a culturally and economically important coastal fish species that is distributed globally, from latitudes 42 North to 42 South (Thomson 1963). Of the many mullet species *M. cephalus* is known for its strong affinity for freshwater habitats (McDowall 1988), and is often found in freshwater ecosystems, such as coastal streams and rivers (Thomson 1955; Wang et al. 2010). Research on the ecology of *M. cephalus* in freshwater ecosystems is limited, although some studies have revealed movement between freshwater and adjacent estuary and marine ecosystems over long time scales, e.g. across seasons and years (Thomson 1955; Wang et al. 2010). Mark and recapture studies could shed light on the ecology of *M. cephalus* populations with freshwater ecosystems, including addressing questions such residency times, growth rates, and movement patterns between the estuary and freshwater habitats. Moreover, answers to these questions would be helpful for resource managers working to ensure the persistence of *M. cephalus* populations in the face of increasing demands on freshwater resources and climate change.

The overall goal of this study is to investigate the feasibility of using PIT tag technology to study the ecology of juvenile *M. cephalus* in streams. We accomplished this goal through a two-pronged approach. First, we quantified retention rates of PIT tags for juvenile *M. cephalus* through a laboratory study. Second, we conducted a preliminary study of the movement patterns of individually-tagged juvenile *M. cephalus* in a lowland Hawaiian Stream using PIT tags. We were particularly interested in exploring potential movements between the freshwater rearing stream and downstream estuary.

## **MATERIALS AND METHODS**

### ***Study site and species***

This study took place in Waiāhole Stream (latitude: 21.48°, longitude: -157.85°) on the island of O‘ahu in Hawai‘i, U.S.A. that empties into Kāne‘ohe Bay. Waiāhole Stream is a second order stream (10.4 km<sup>2</sup>) with an average annual discharge of 1.2 m<sup>3</sup>/s (42 ft<sup>3</sup>/s, 2002 – 2012 water years, USGS 16294100). The watershed is dominated by scrub/shrub and evergreen forest and the stream ranks high in terms of native biodiversity (Parham et al. 2008).

In Waiāhole Stream, juvenile *M. cephalus* approximately 5 - 7 cm FL (fork length) begin entering the stream from the ocean in August and late September (K. Fraiola, personal observation). In this particular stream, juvenile *M. cephalus* occupy the lower 1.5 km of the stream, beginning immediately above the estuary. Juvenile *M. cephalus* tend to travel in schools or individuals, and spend most of their time feeding (grazing) on the surface of various substrates in erosional habitats with moderate depth, velocities, and substrate sizes ((Fraiola and Carlson in prep). However, when not feeding they can be found resting in low velocity habitats or cleaning themselves on sandy substrate. *M. cephalus* can move rapidly among adjacent mesohabitats (i.e., pools, riffles, and runs) and across entire reaches (multiple mesohabitats) of stream.

### ***Laboratory study of tag retention, growth, and survival***

Ninety juvenile *M. cephalus* between 7 – 10 cm FL were collected with bait cast nets (mesh size = 6.3 mm) from the mouth of Waiāhole Stream on January 4<sup>th</sup> and 5<sup>th</sup>, 2014. We chose this size range because it encompasses the smallest size observed in Waiāhole Stream, and represents the size of *M. cephalus* likely to be tagged in future studies of juvenile growth rate in this and other Hawai‘ian streams. Fish were collected from the stream mouth in order to avoid disturbing juvenile *M. cephalus* residing further upstream. Fish were then transported to the Anuenue Fisheries Research Center (Honolulu, Hawai‘i), which is operated by the State of Hawai‘i Department of Land and Natural Resources.

At the facility, we placed fish in a hypotonic bath to remove ectoparasites before transferring fish to one of three pens, each 1.2m diameter and 1.6m deep, held within a larger 5,940 gallon tank with a moderate salinity (15 ppt). The larger tank was an “open system” with new water (fresh well and filtered sea water) pumped in at a combined rate of ~ 8-9 gallons per minute. We then removed fish from the holding pens and anesthetized them with MS222. We then measured each fish’s fork length (FL, mm) and weight (nearest 0.1g), and randomly assigned individuals to one

of three treatments: visible implant elastomer (VIE), passive integrated transponder (PIT), or control (no tag). We assigned treatments randomly to one of three mesh pens, with a total of 30 fish per treatment (and hence per pen). Fish were then allowed two weeks to acclimate to their new surroundings before tagging commenced.

Following two weeks of acclimatization, we anesthetized all 90 study fish, and then weighed, measured, and tagged each individual in the VIE and PIT treatments. VIE marking entailed depositing a small bead of elastomer (Northwest Marine Technology, Inc.) into the postorbital adipose tissue of the eye with a 29-gauge needle while the fish was anesthetized. Fish in the PIT tag treatment were implanted with an 8 mm x 1.4 mm PIT tag (0.027 g, FDX-B "Skinny," Oregon RFID) in the peritoneal cavity. We chose to use the smallest tags available (i.e., 8 mm PIT tags) in order to minimize the potential negative effects of the tag on fish health and maximize the likelihood of tag retention. We then monitored the fish for 48 days. During the course of the experiment, fish were fed daily and the pens were cleaned three times a week. Fish that died were removed without replacement (n = 4, 5, 6 for the VIE, PIT, and control treatments, respectively). These data were used to evaluate the efficacy of VIE and PIT tags for longitudinal studies of *Mugil cephalus* (objective 1). At the end of the experiment, fish in the PIT tag treatment were re-measured for growth rates.

*Statistical analysis.* Differences among the initial length, weight, and condition factor of fish in each treatment were analyzed using single-factor analysis of variance (Zar 2010). Each fish's condition factor (K) was calculated as,  $K = \text{weight}/(\text{length})^3$  (Busacker et al. 1990). The growth rate of fish (difference in final and initial length or weight divided by 48 days) was calculated from fish in the PIT tag treatment only because it was not possible to identify individuals in the VIE treatment or the control. We did not estimate growth based on the change in mean size of the fish in the VIE and control because the mean size of the fish in each pen was also affected by the size of individuals who died (fish that died tended to be among the largest/smallest fish).

### ***Field study of movement behavior using PIT tags and a stationary PIT antenna***

Beyond the tag efficacy study described above, we were also interested in evaluating the effectiveness of PIT tags combined with a stationary antenna to document directional movements of mullet between their nursery stream and downstream estuary. To monitor movement between the estuary and Waiāhole Stream, we installed two HDX RFID stationary antennas (5.5 m x 1 m, spaced 2 m from each other) above the upstream extent of the saltwater wedge of the estuary. Two parallel antennas were necessary to detect direction of movement. The antennas were constructed of 12 gauge, 16 strand insulated copper wire put into two loops (1m x 4.5m) that spanned the width and depth of the stream. A 12V deep cell battery powered the reader and antennas (Oregon RFID) on deep cell 12V batteries. The reader was a multi-antenna HDX reader (Oregon RFID), with a scan rate of 5 scans per second. Maximum water velocity in the area of the readers was approximately 0.37 m/s. Batteries were changed every 36 hours, at which time the data from the reader was downloaded and the antennas tested. Missed detections were recognized as detections at the wrong antenna given the fish's last known location and the time between detections.

For the movement study, juvenile *M. cephalus* that ranged in size from 14.6 - 18.7cm fork length were collected with bait cast nets on August 6<sup>th</sup> 2014 from a 0.5 km stretch of Waiāhole Stream just above the antenna. These fish were larger than the fish tagged in the tag retention study; therefore, we tagged them with a larger tag with a greater range (12mm x 2.12mm HDX PIT tag, 0.1g). We anesthetized fish with MS222 and then measured their weight and length and then implanted each with a PIT tag. After PIT-tagging, we placed fish in flow-through pens in the river to recover for one hour, after which fish were released in the area where they were originally captured. 24 hours after the fish were tagged, the antennas had to be removed (i.e., on the afternoon of the August 7<sup>th</sup>) due to an impending storm. Two days later, the antennas were reinstalled and then operated for 10 consecutive days (August 9 – 18, 2014). To avoid mixing movement data due to flooding and those that occurred during base flow periods, we restricted our movement analyses to 08/11/2014 05:45 and 08/18/2014 10:54 (end of experiment), for a total of 7 days 5.1 hours.

To study the timing of movements into and out of the estuary region of Waiāhole Stream, we focused on forays that were > 30 minutes in duration, as anything shorter was considered feeding or resting in the vicinity of the antenna array. We define diel movements as movements that take place within the course of 24 hours, whereas diurnal movements are those movements that coincide with changes from day to night and vice versa. We divided forays into “long” (> 10 hrs.) and “short” ( $\leq 10$  hrs.) forays. These categories were based on the length of night (~10 hours) during a 24 hour period. Setting the boundary between long and short forays at 10 hours helped to improve the resolution of different diel behaviors by minimizing the mixing of potential diurnal movements from other shorter, diel movements.

*Statistical analysis.* For the purpose of investigating activity levels during the day versus the night, we compared the proportion of detections during the night with those expected based on proportion of night to 24 hours (i.e., 10/24 hrs) using Chi-square test following (Bentley et al. 2014). Visualization, estimates of modes, and tests of uniformity from data on the timing of movements were all conducted using the “circular” package in Program R (R Core Team 2014).

We also explored the time of the day (night or daylight) that the estuary region was used during diel forays by comparing the proportion of night time spent among all individuals in the estuary versus the null hypothesis that the proportion would equal the proportion of night time to total hours in a day (i.e.,  $p_0 = 10/24$ ) during the experiment (Bentley et al. 2014). Finally, we also explored the timing of fish movement in relation to tidal height using a two-tailed Student’s t-test (Zar 2010).

## RESULTS

### *Laboratory study of tag retention and survival*

Tag retention was 0% for VIE (visible implant elastomer) and 100% PIT (passive integrated transponder) treatments, with tag ratios (tag weight/body weight) in PIT tag treatment ranging from 0.18% - 0.57%. No fish died during the tagging process, and there were no signs of infection near the tag insertion points. Individuals included in the tagging experiment ranged in

length from 6.8 - 10.4 cm (mean = 8.7 cm, SD = 0.81), weight 4.2 - 16.5 g (mean = 8.6 g, SD = 2.6), and condition factor 0.83 - 1.63 (mean = 1.3, SD = 0.16), and there were no significant differences in these values at the start of the experiment among the three treatments (Table 1).

While no individuals died during the tagging process or immediately after (e.g., with 24 hours of tagging), some individuals in each treatment died over the course of the 48 day experiment. Survival rates among the treatments were very similar over the course of the experiment: 80%, 87%, 83% for control, VIE, and PIT, respectively (Table 1). Fish from the PIT treatment had an average weight increase of 0.1 g/day (SD = 0.05), average length increase of 0.03 g/day (SD = 0.02), and an average condition factor (K) increase of 0.001/day (SD = 0.009) over the course of the experiment. Because some of the largest and smallest fish died in the other two treatments, size-biased mortality limited our ability to examine growth in the VIE or control treatment.

### ***Field study of movement of individually-tagged mullet***

We tagged 19 individual mullet in the field that ranged in size from 14.6 - 18.7cm fork length (mean = 18.4 cm, SD = 1.1) and 49.7 - 97.7g (mean = 75g, SD = 12.5g). There was no mortality during collection or tagging. Five of 19 individuals were detected moving from the stream into the estuary during the seven consecutive days after the flood, and these 5 individuals yielded 189 tag detections at the antennas. During this period, one downstream (DS) detection was missed by our antenna (*i.e.*, a miss-rate of 0.5%).

Of the 189 tag detections, 97% occurred during day light hours (Figure 1) and 3% (5 detections) during night or twilight hours. The proportion of detections during the night was significantly lower ( $\chi_{0.025,1} = 5.024$ ,  $P < 0.001$ ) than would be expected based on the null hypothesis that the proportion of movements during the night should be proportional to the proportion of night to day (~43%). The median time of day at which detections occurred was 12:30. From the detection data, we identified 26 different forays into the estuary. The forays consisted of 10 long forays (>10 hours) and 18 short forays ( $\leq 10$  hours). The proportion of time spent in the stream by the five individuals detected over course of our field study was 68% - 86% (mean = 77, SD = 8%).

Movement patterns differed among individuals (Figure 2). Individuals spent 3 - 4 consecutive days in the stream before heading to the estuary after being tagged, suggesting a possible short-term effect of tagging on mullet behavior. Long forays on average comprised 80% (median = 88%, SD = 19%) of the time individuals spent in the estuary. For long forays, movement times into the estuary from the stream had a modal time of 19:00 (sunset = 19:30), while return movements from the estuary to the stream had a modal time of 06:48 (sunrise = 05:45, Figure 3a). The modal time for short foray movements into the estuary was 07:00, and 16:12 for movements from the estuary back into the stream (Figure 3b). In contrast, the distribution of times for short foray movement into the estuary ( $P > 0.1$ ) and out of the estuary ( $0.05 < P < 0.1$ ) were non-uniformly spread throughout the day. Over the course of seven consecutive days and across both foray types, 59% (SD = 9.5%) of the total time spent by individuals (summed across all five individual that moved) in the estuary region was at night, this was significantly greater ( $\chi_{0.025,1} = 5.024$ ,  $\chi = 21$ ,  $P < 0.001$ ) than would be expected based on the null hypothesis that the amount of time spent in the estuary at night should equal the proportion of night during a 24-hour period (43%). In contrast, the proportion of the time spent in the stream at night was much



closer 37% (SD = 3.1 %) to what would be expected based on 10 hours of night per 24 hour day, but this too differed statistically from the null hypothesis of proportion of  $(\chi_{0.025,1} = 5.024, \chi = 7.2, 0.01 < P < 0.005)$  (Figure 4).

## DISCUSSION

The results of our lab and field experiment suggest that juvenile *M. cephalus* as small as 7 cm (FL) can be safely and reliably tagged with 8 mm passive integrated transponders (PIT), and that stationary RFID antenna can be an effective and affordable tool for monitoring the movement of individuals tagged with 12mm PIT tags in freshwater stream ecosystems. In addition, our field study suggests that juvenile *M. cephalus* in Waiāhole Stream exhibit diurnal feeding behavior, as well as long and short forays into the downstream estuary, with individuals tending to spend night hours in the estuary.

### *A comparison of tag retention and fitness effects using PIT and VIE tags*

Our laboratory study suggests that juvenile *M. cephalus* have high retention rates for PIT tags. High retention rates and low mortality of small fish tagged with PIT tags has also been noted in other juvenile fish taxa. For example, Boland et al. (Bolland et al. 2009) reported results on retention rates using 12 mm PIT tags over the course of 180 days with juvenile chub (*Leuciscus cephalus*), dace (*Leuciscus leuciscus*), and roach (*Rutilus rutilus*) (tag:body weight ratio = 0.4 – 1.4%). were between 96.6 and 100%, with no direct effect of the tags on survival. In some instances PIT tag retention rates in small fish have been very low, an issue which suturing of the wound has been found to significantly improve (Baras et al. 2000). Given that we observed 100% retention of PIT tags over the course of 48 days in juvenile mullet, suturing does not seem necessary in this species. This is important as suturing is another source of stress for small fish and, in some cases, has been found to decrease survival and growth of smaller individuals due to an increased incidence of infection at the site of the suture ((Skov et al. 2005).

The inability to effectively tag juvenile *M. cephalus* with visible implant elastomer (VIE) in postorbital adipose tissue (PAT) suggests that this tissue is unsuitable for short-term and long-term studies. Low tag retention of VIE in PAT has also been noted in smaller individuals of several other fish, such as coho salmon smolts (*Oncorhynchus kisutch*, (Bailey et al. 1998), common bully (*Gobimorphus cotidianus*, (Goldsmith et al. 2003), and barbel (*Barbus barbus*, (Farooqi and Morgan 1996). Researchers have hypothesized that low rates of VIE tag retention in PAT is related to the size of a fish, with smaller individuals having smaller PAT, which results in low retention rates due to shallower insertion depths and tearing of the adipose tissue (Hale and Gray 1998; Close 2000). For example, adult brown trout and rainbow trout (*Onchorhynchus mykiss*, (Hale and Gray 1998) were found to have very high retention rates- between 94-98%-of VIE tags in postorbital adipose tissue. In contrast, (Olsen and Vøllestad 2001) “found it too difficult” to tag small juvenile brown trout with VIE in PAT. Based on this work, these authors recommend that fish less than 40 – 50 mm length be tagged elsewhere on the body and that positions near vital organs or in fins should be avoided, and this recommendation seems to apply beyond brown trout. In addition, long-term studies have found that retention rates of VIE tend to gradually decline over time (Haines and Modde 1996; Summers et al. 2006; Weston and Johnson

2008), which adds to the challenge to applying VIE tags to fish species that are long lived. Based on the results of this study the use of VIE tags in postorbital adipose tissue of juvenile *M. cephalus* is not recommended. Future laboratory studies investigating the effectiveness of VIE tags with juvenile *M. cephalus* in other locations, such as fin rays and the base of fins over long periods of time would help us better understand the potential of applying VIE tags to the study of *M. cephalus* populations.

### ***Feasibility of PIT tags combined with stationary PIT-antennas for studying mullet movement***

Our results from Waiāhole Stream show that PIT tags combined with a stationary antenna can be successfully applied to study the movements of juvenile *M. cephalus* in streams. Our high overall detection efficiency is comparable to those found in other studies (Nunnallee et al. 1998; Axel et al. 2005; Connolly et al. 2008; Aymes and Rives 2009). Several factors can affect the efficiency of fish detections including the travel time through the antenna's detection field, the orientation of the tag relative to the antenna, and the number of tags in the antenna's detection field at any one moment (Castro-Santos et al. 1996; Aymes and Rives 2009). Regarding travel time, faster movement past the antenna translates to fewer opportunities the tag can be read given a certain scan rate (number of scans per second). Consequently, the direction a fish is moving (up-stream or down-stream) can significantly affect its detection, as fish moving downstream with the flow (as opposed to upstream, against the flow) may be moving at faster speeds. For example, (Aymes and Rives 2009) found a stationary antenna had lower detection efficiencies for downstream movements compared to upstream movements and attributed this discrepancy to faster fish speeds and changes in tag orientation due to turning. These are potential sources of error when studying *M. cephalus* movements as well, as these fish tend to move faster downstream than upstream because they tend to swim rapidly in the downstream direction with the current and to feed slowly as they swim upstream (K. Fraiola, personal observation). Such error can be ameliorated by increasing the scan rate of the reader, but only to a limited extent. Interestingly, our one missed detection was for a fish moving in the downstream direction, but with only one missed detection out of 198, we feel confident that this issue did not overly influence our results. Another potential issue that has been highlighted in earlier studies, is that detection rates go down with more tags in the vicinity of the antenna (Castro-Santos et al. 1996). We tagged a small number of fish in this preliminary study, so do not expect this was an issue in our study. However, it is worth mentioning that this could be a serious concern in studies of schooling species, such as *M. cephalus* and other mullet species. Perhaps by tagging individuals on different days and from different reaches of the stream, it may be possible to reduce the likelihood of multiple tagged individuals traveling together in the same school.

### ***Movement results***

Our preliminary movement study revealed that juvenile *M. cephalus* in Waiāhole Stream exhibited a diurnal pattern of stream habitat use, with movement activity occurring almost exclusively during day light hours. Although we did not measure feeding directly, juvenile *M. cephalus* activity in the stream and estuary region of Waiāhole Stream most likely reflects feeding activity, as juvenile *M. cephalus* in this system have been observed to move throughout the stream for feeding over the course of the day (Fraiola and Carlson 2015) and not at night (K. Fraiola pers. Obs.). Similar studies examining diel activity patterns, primarily with a focus on

feeding, from other areas of the world have found activity mainly occurs during daylight hours. For example, (De Silva and Wijeyaratne 1977) found evidence that fingerling *M. cephalus* (20 – 50 mm) in a Sri Lankan estuary fed mainly during the day, with feeding peaking around midday. Similarly, in the estuarine lake system of Saint Lucia in South Africa, Indian Ocean, juvenile *M. cephalus* (>5cm standard length) were found to feed predominantly during the day, although they noted a few individuals were collected with full guts at 02:00 hours. This diurnal feeding behavior has also been noted for larger *M. cephalus* (210 – 410 mm standard fork length) ((Collins 1981). Future research into nighttime microhabitat use of juvenile *M. cephalus* in Waiāhole Stream and its estuary would improve our understanding of nocturnal habitat needs and use, which can vary significantly from diurnal habitat use (Muhlfeld et al. 2003).

Diurnal behavior has been documented for a number of fish in stream/river habitats, and is often linked to minimizing predation risk ((Booth et al. 2013; Bentley et al. 2014) by moving into a habitat during nighttime hours that minimizes detection by visual hunters. Diurnal movements can also have beneficial effects on the metabolism of fish consumers by allowing individuals to better match their metabolic rates with their feeding times (Armstrong et al. 2013). Future research on differences in diurnal predation risk between Waiāhole Stream region and its downstream estuary, as well as the differences in thermal characteristics between these two regions, would greatly increase our understanding of the underlying causes of these patterns.

### ***Conclusions***

In this study, we show that PIT tags have the potential to be a useful method of marking juvenile *M. cephalus* and that PIT tags in conjunction with stationary antenna arrays are feasible for studying the movements of juvenile mullet between freshwater and downstream estuarine environments. Whereas, VIE tags in the post orbital adipose tissue would not work well for studying juvenile *M. cephalus*. Our preliminary movement observations suggest diel behavior of juvenile *M. cephalus* from a lowland Hawaiian stream that relate to their activity (i.e., more active during the day) and movement patterns (i.e., diel forays into the estuary region) between the stream and its estuary. Future research into the diel patterns of migration of individual *M. cephalus* from stream habitats to the estuary across a range of stream sizes would help us better understand the extent of this behavior in different stream systems and if there is a point at which the cost of migrating outweighs the benefits. Future research on how growth rates of individuals in the stream compare to those of juveniles outside the stream, and how growth rate of individuals relate to the degree to which they use the estuary would help us understand the net benefit of utilizing the estuary (as opposed to the stream) for resting at night. Knowing how juvenile *M. cephalus* move between freshwater and adjacent estuarine and marine habitats at short time scales, such as diel periods, is important to understanding the potential habitat requirements or preferences of this species and thus how to better anticipate how changes in streams or estuaries might affect the productivity and resilience of *M. cephalus* populations in freshwater habitats in Hawai‘i and other parts of the world.

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## TABLES

Table 1. Average ( $\pm$  SD) weight, length and condition factor of fish from both treatments and the control at the start of the experiment, along with the percent survival and tag retention at the end of the experiment.

Treatment	Weight (g)	Length (cm)	k	Survival (%)	Mark Retention (%)
Control	8.4 $\pm$ 2.4	8.6 $\pm$ 0.7	1.3 $\pm$ 0.16	80	NA
PIT	9.2 $\pm$ 2.7	8.8 $\pm$ 0.8	1.3 $\pm$ 0.16	83	100
VIE	8.3 $\pm$ 2.7	8.6 $\pm$ 0.9	1.3 $\pm$ 0.14	90	0

## FIGURES

Figure 1. The percentage of the total number of tag detection per each hour of the day over the course of the seven days analyzed. Shaded areas represent night hours and white areas represent day hours.

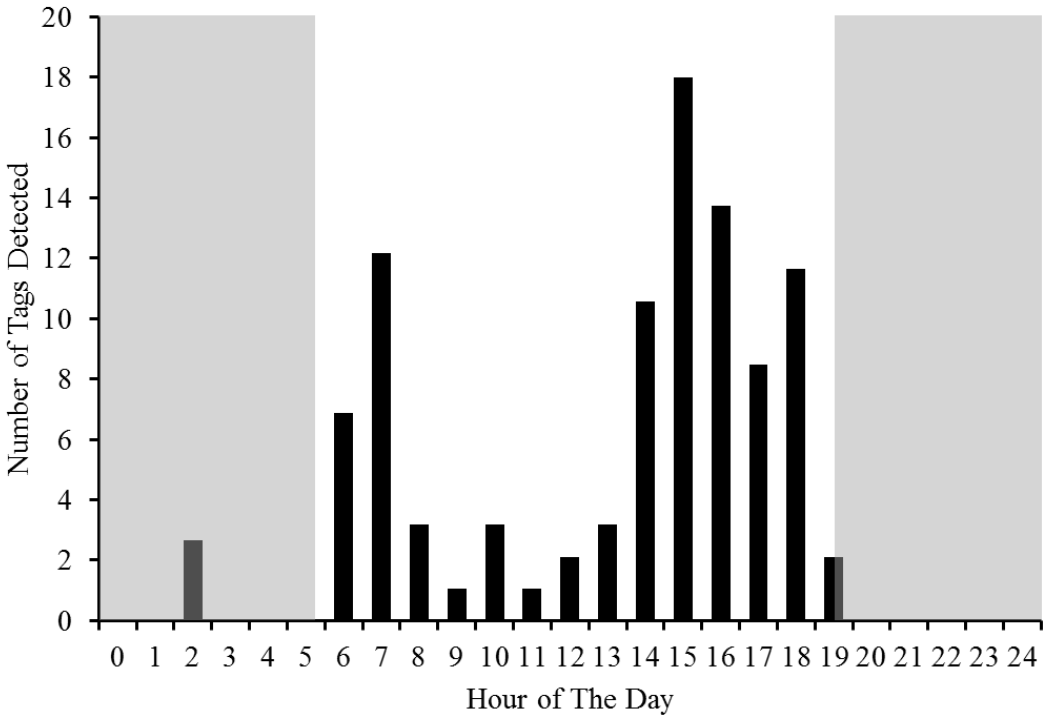


Figure 2. Graphical example of various movement behaviors between the “stream region” (solid line) and the “estuary region” (dashed line) for all five individuals monitored over the course of seven days in Waiāhole Stream, starting on August 11, 2014 at 05:45 and ending August 18 10:54. “a” = long foray ( $\geq 12$  hrs), “b” = short foray ( $< 12$  hrs), into the estuary from the stream (left) and back to into the river (right). “\*” = missed detection (2). Dashed vertical lines (gray) mark midnight (0:00).

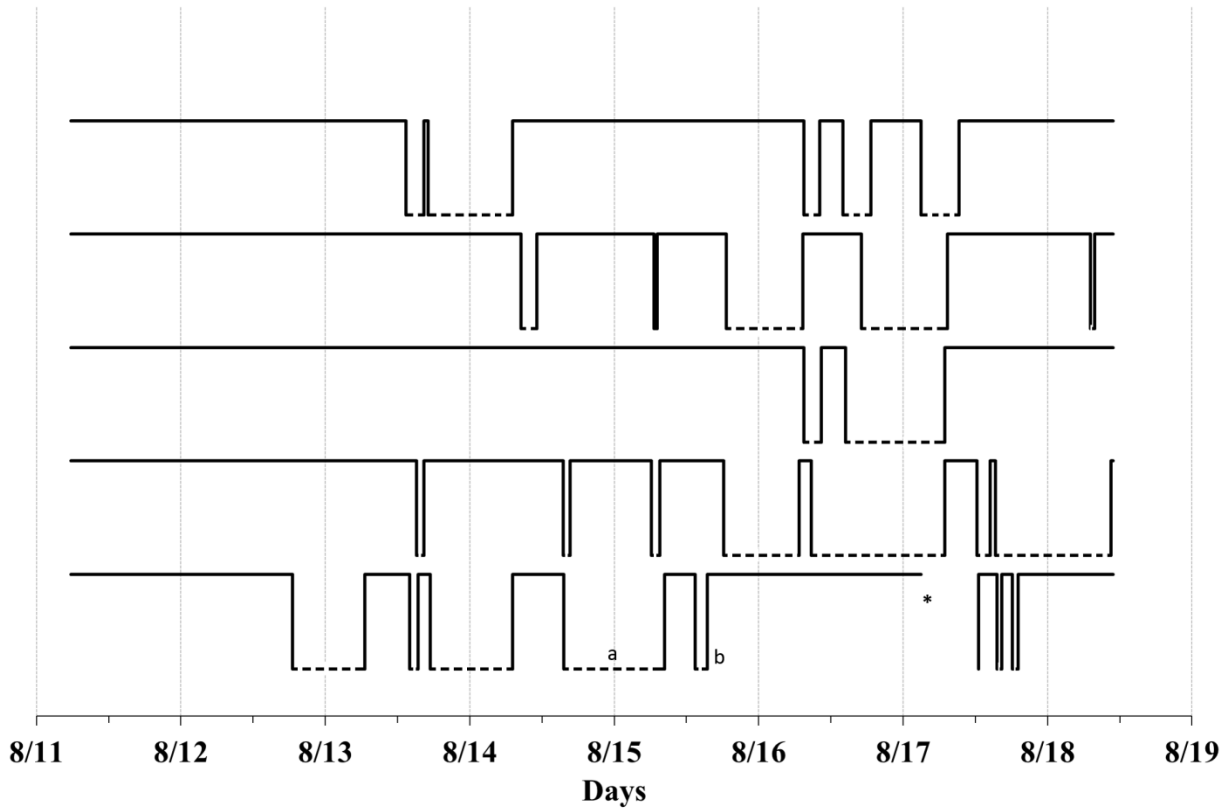


Figure 3. Rose diagrams showing the frequency and timing of movements into the estuary from the stream (“a”) and *vice versa* (“b”) for diel forays over the course of the seven days analyzed. The dashed line represents the mode and the darkened perimeter of the clocks represents the night.

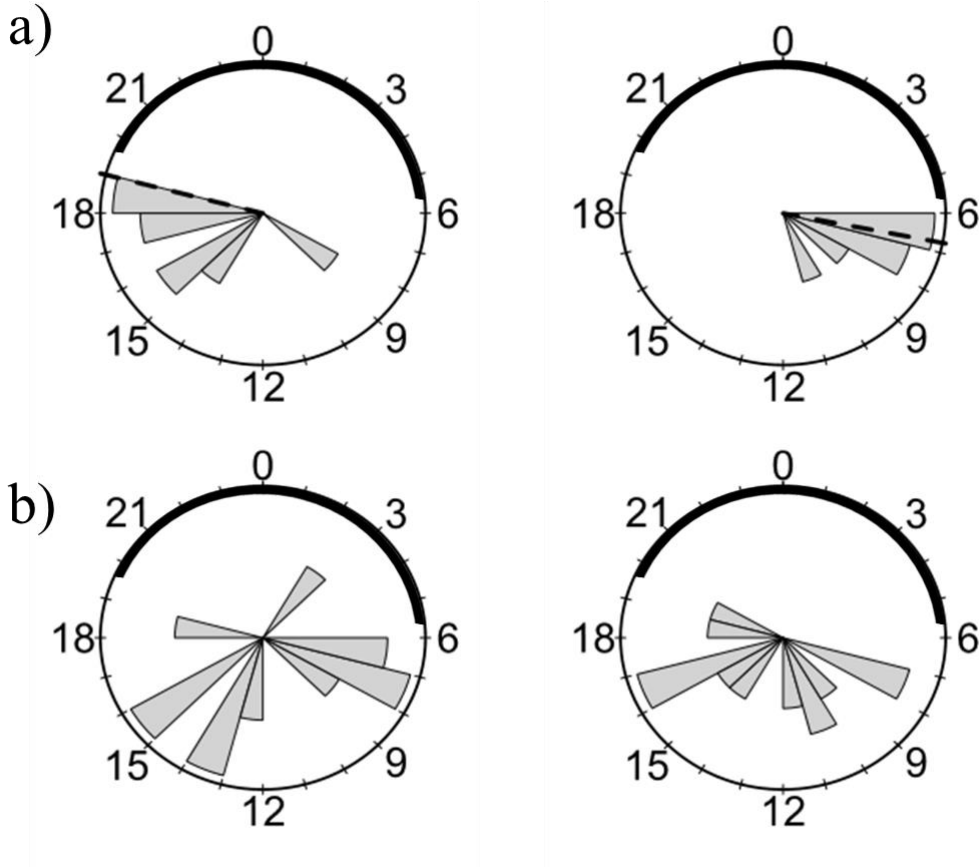
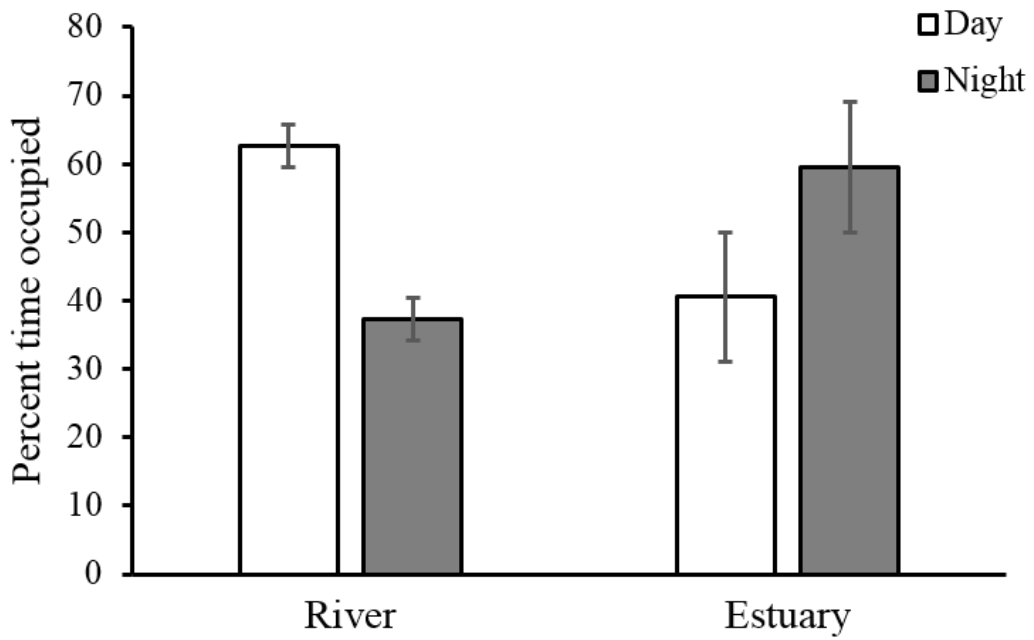




Figure 4. Graph depicting the average proportion of time in the river and estuary spent during the day and night across all five 'movers' over seven days of study. Error bars represent one standard deviation.



## **Chapter 5**

## Chapter 5

### Conclusions

In this dissertation, I investigated the ecology and behavior of striped mullet, *Mugil cephalus*, in Hawaiian streams. Despite mullet being an important coastal fish species that support fisheries around the globe, we know surprisingly little about its ecology when it moves into freshwater streams to feed and grow. My dissertation research sheds light on the freshwater ecology of mullet in Hawaiian streams at both large and small spatial scales, and on the movements of mullet between the stream and adjacent estuarine habitat. My research included a focus on the influence of a suite of large scale (i.e., watershed- and reach-scale) factors on the distribution of *M. cephalus* in Hawaiian streams, a field study documenting microhabitat selection of feeding *M. cephalus* in a lowland Hawaiian stream, and a combined laboratory-field study investigating the feasibility of using Passive Integrated Transponder technology to tag and track the movements of individually-marked mullet between the stream and adjacent estuary.

#### *Summary of key findings*

In my first study (described in chapter 2), I investigated the influence of a select set of large-scale environmental variables on the probability of *M. cephalus* occurrence in 33 watersheds distributed around the Hawaiian Islands. To do this, I developed 11 candidate generalized linear models, including combinations of the variables channel slope, watershed area, watershed health, and discharge to explain *M. cephalus* presence-absence among watersheds. My results suggest that slope and discharge are influential in explaining the distribution of *M. cephalus* among Hawaiian streams. Slope was the most influential variable and was negatively related to the probability of *M. cephalus* occurrence in a watershed, that is, mullet were more likely to be present where slope was low in the vicinity of the stream mouth. Discharge was also important, but to a lesser degree, and was positively related to *M. cephalus* occurrence. In addition, I compared how reach-scale physical habitat variables (i.e., depth, velocity, wetted width, and canopy cover) differed between a “mullet stream” and three neighboring “non-mullet streams” that were relatively lower in discharge but that all drained into the same bay. The results of our reach-scale comparisons suggest that the mullet stream tended to be wider and deeper than the neighboring non-mullet streams.

One way in which large-scale habitat variables can influence the distribution of aquatic organisms in streams is by constraining the types of habitats available within the stream, and ultimately the distribution of populations that interact with those habitats. In my next study (described in chapter 3), I investigated the microhabitat use of juvenile *M. cephalus* feeding in a lowland Hawaiian stream, Waiāhole Stream on O‘ahu. To do this, I measured the depth, velocity, substrate size, and canopy cover where mullet were actively feeding and compared the characteristics of feeding sites to the full suite of microhabitats available to the fish. My results suggest that *M. cephalus* are highly selective, and tend to feed in erosional habitats characterized by moderate depths and velocities, larger substrate sizes, and open canopies while tending to avoid more depositional habitats that were deeper, slower, and dominated by fine sediment and detritus. These results were surprising given that *M. cephalus* is often described as being

detritivorous. Instead, *M. cephalus* in Waiāhole Stream avoided depositional areas where detritus was abundant, and instead displayed a more herbivorous feeding strategy.

Through my observations of *M. cephalus* in Waiāhole Stream, I came to appreciate that mullet are highly mobile, moving constantly among feeding and resting sites and feeding as far as 1.3 km upstream from the estuary. However, whether or not mullet were making movements in the downstream direction, to the estuary, was a complete mystery to me. In my final study (described in chapter 4), I investigated the feasibility of using PIT tag technology in juvenile *M. cephalus* to study their movement ecology within streams. To do this, I first conducted a laboratory experiment where I quantified tag retention and survival rates of PIT tags and VIE (Visible Implant Elastomer) tags relative to untagged controls. PIT tags (8 mm x 1.4 mm) were placed in the peritoneal cavity and VIE tags were placed in post orbital adipose tissue for juvenile *M. cephalus* that I had collected from the wild and these organisms were held in a laboratory environment for 48 days. Results of the lab study showed that PIT tag retention was excellent (100%) but poor for VIE tags (0%). Lab results also suggest that juvenile *M. cephalus* as small as 7 cm can be tagged safely and effectively with PIT tags. With this information in hand, I next conducted a field study on the movement of juvenile *M. cephalus* in Waiāhole Stream in which I tagged mullet from the stream with PIT tags (12 mm x 2.12 mm) and monitored their movements between the stream and estuary using a stationary Radio Frequency Identification (RFID) antenna array for two weeks during August 2014. The results of my field studies suggest that PIT tags are an effective method for monitoring the movements of individually-tagged juvenile *M. cephalus* in streams. Moreover, my field study revealed that individual mullet are making diel forays into the estuary for varying lengths of time, with longer forays taking place primarily during the night time hours.

### ***Conservation implications***

Islands are often associated with both great beauty/biodiversity as well as extinction and ecosystem collapse. On developed islands, freshwater ecosystems are often highly impacted. Common threats include water withdrawals, habitat alteration (e.g., channelization, dam construction, etc.), invasive species, and pollution. The distribution of these impacts within watersheds is often uneven, with lower reaches of stream networks receiving the brunt of human impacts due to human settlement in these areas. Probably once a productive habitat for euryhaline or catadromous fish, such as *Mugil cephalus*, many Hawaiian lowland streams are now channelized or have had their discharge greatly reduced through diversions to meet societal demands for freshwater. One goal of my research was to bring attention to the role that these lowland streams and rivers play as nursery habitat for juvenile fishes, like *M. cephalus*, and to hopefully spawn interest in conservation of lowland stream habitat and studies on the life history and ecology of fish moving into and out of these systems.

*Mugil cephalus* is a species that bridges stream and ocean ecosystems through its movements, a relationship which exposes them to anthropogenic impacts affecting stream ecosystems. Results from my research have implications for how streams and rivers in Hawai‘i are managed for habitat and discharge. The higher probability of finding mullet in streams that are low gradient in their lower portions puts mullet at risk of being negatively impacted by human activities in these regions, such as channel engineering for flood control. For example, the

shallow homogeneous habitat of flood control channels could act as a barrier or deterrent to juvenile *M. cephalus* entering the stream from the estuary. These channels also offer little to no shelter from high flows or predators. Future research on the impacts of channels on the movement of juvenile *M. cephalus* into Hawaiian streams could help inform the design of future channels or help guide the retrofitting of the existing channels to make these more fish friendly.

The high degree of specificity in the type of habitat where *M. cephalus* feeds in Waiāhole Stream could have implications for how *M. cephalus* populations in streams respond to continued decreases in flow of Hawaiian streams. Decreases in discharge could result in decreases in deeper, faster flowing habitats and shifts toward ones that are shallower and slower moving, which could result in less feeding habitat for *M. cephalus* (e.g., see chapter 3). Future research investigating microhabitat preferences in a broader range of stream types in Hawai‘i will help us understand the degree to which selection of feeding habitats might differ among streams. Moreover, replicating the microhabitat preference study across seasons would help elucidate how microhabitat use patterns might depend on fish size because fish size increases over the course of a year and this study took place over a narrow range of dates (in July) and thus considered a narrow range of sizes as well.

### ***Future research directions***

Through my dissertation research, I explored how mullet ecology and behavior is related to abiotic variables and physical habitat. An important next step in this research is to examine how *biotic* factors may be influencing *M. cephalus* distributions, behavior, and ecology in streams. For example, predators (native or non-native) could be a significant force influencing the ecology of *M. cephalus* in streams at large-scales (i.e., among watershed) and small-scales (i.e., feeding microhabitat use). Mullet are fairly conspicuous in streams due to their flashing while swimming, which is a consequence of the sunlight reflecting off their bodies as they scrub the surface of rocks and feed. This flashiness may increase their risk of being predated upon by, for example, the black-crowned night heron (*Nycticorax nycticorax*), which would be the main native predator in Waiāhole Stream where much of my research was conducted. Future research on the predation risk that juvenile *M. cephalus* face in streams and adjacent estuaries would be a valuable addition to our understanding of the risks and rewards of spending time in these different habitats. Competition (e.g. food, grazing locations, etc.) could also be a potentially significant biotic factor that could affect the ecology of *M. cephalus* in streams and river ecosystems. For instance, *M. cephalus* are dedicated grazers in Waiāhole Stream, however they are not specialists in this feeding style, and can utilize a wide variety of food resources (e.g., sediments, small invertebrates, and phytoplankton, etc.) (Odum 1968; Odum 1970). If armored catfish (i.e., *Loricariidae*), a highly specialized benthic grazer invading streams in Hawai‘i, were to invade a mullet stream, could *M. cephalus* populations persist? This scenario is a real possibility on Oahu given that many of its streams have been invaded by armored catfish. Future research into these and other biotic factors would significantly advance our understanding of the full suite of factors influencing *M. cephalus* behavior and ecology in streams.

In my dissertation, I explored the behavior of *M. cephalus* in one stream, including through a focus on their microhabitat use and movement. Future research exploring how *M. cephalus* behave in a wide range of environments would be a useful and interesting extension to

my research. For example, streams where *M. cephalus* occur in Hawai‘i include those with a wide range of discharges. These differences in discharge offer a greater range of depths and velocities than are found in my focal stream (Waiāhole Stream). Understanding how differences in stream size influence *M. cephalus* ecology in freshwater systems would help identify how plastic are their behaviors, including with regards to movement and foraging behavior, which is important for understanding and predicting their ability to adapt to changing environmental conditions.

Building off this last research direction, a very exciting future direction is to explore the population genetic structure of *M. cephalus* among its widely distributed global population. Recent research has revealed significant genetic structure among populations from different regions of the world. Understanding the degree to which *M. cephalus* is one species with a global distribution or a species complex will help researchers make sense of the sometimes conflicting reports on life history and ecology for this species, which could be a result of cryptic species or evolutionary relationships that are not recognized presently given the current treatment of *M. cephalus* as a single, widespread species. More research in this area would greatly improve our understanding of the links between life history, ecology, and evolutionary relationships across the range of *M. cephalus* across the globe.

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