ASSESSING FISH COMMUNITIES IN DENSE SUBMERSED AQUATIC VEGETATION HABITATS USING UNDERWATER VIDEO CAMERAS

By

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To Mom, Dad, Dayna, Catlyn, and Grandpa Wilson

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LIST OF ABBREVIATIONS

DDHS	Density-dependent habitat selection: a theory in ecology whereby an individual animal selects for habitat to optimize their per-capita resource allocation which depends upon the intrinsic habitat quality at that location and the density of animals with which the individual may compete.
DO	Dissolved oxygen: the amount and availability of oxygen in water necessary for environmental and physiochemical processes in plants and animals.
MaxN	Maximum number of fish for specified taxa identified onscreen simultaneously as recorded by underwater video cameras.
SAV	Submersed aquatic vegetation: aquatic vegetation whose growth is limited at or below the air-water interface.
UVC	Underwater video camera: a camera designed to be submerged and used to quantify fish behavior, abundances, and/or occurrence in a variety of aquatic environments.

Abstract of Thesis Presented to the Graduate School
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There is concern that large expanses of dense hydrilla *Hydrilla verticillata* reduce fish habitat quality and thus affect fish communities. These processes have been understudied due to sampling difficulties leading to misinformed fish-habitat quality relationships. Underwater video cameras (UVC) provide a new sampling technique for fish in dense hydrilla. The objectives of this thesis were to: 1) develop methods for using a UVC-system to estimate fish abundance in dense hydrilla, and 2) determine the pattern for freshwater fish habitat selection across a gradient of physiochemical habitat qualities. I used a UVC-system to collect fish point counts in three 0.405 ha experimental ponds with dense, surface-matted hydrilla and stocked with varying densities of adult sunfish *Lepomis* spp. and largemouth bass *Micropterus salmoides*. Dissolved oxygen (DO), habitat complexity, and both fish occurrence and fish counts were measured at each point count. End of season collections confirmed that each pond held different fish densities. The UVC-system measured fish occurrence and fish counts that accurately reflected the differences in fish abundances among ponds.

Seasonal changes in fish abundance were reflected in fish occurrences and fish counts, suggesting that UVC counts captured proportional changes in fish abundance in dense hydrilla. Both fish occurrence and fish counts were positively influenced by DO and negatively influenced by habitat complexity ($p << 0.05$). Additionally, the fish-habitat quality relationship differed across fish-density treatments ($p << 0.05$). In high densities, fish showed increased use of marginal habitats but at lower rates and abundance than high quality habitats. In low densities, fish were not observed in marginal habitats and preferred high DO and locations with less habitat complexity. This work suggested that UVC can be an effective sampling method to assess fish communities in dense hydrilla.

CHAPTER 1
INTRODUCTION

Problem and Scope

Overview

The physical attributes of habitat shapes the structure of food webs. Habitat complexity can influence predator-prey interactions, provide key refuge for life-history stages and create a more diverse animal community through niche partitioning (Schoener 1974; Gorman and Karr 1978; Crowder and Cooper 1982). In freshwater habitats, macrophytes, particularly submersed aquatic vegetation (SAV), provide important and complex habitat structure for diverse assemblages of fish and their food (Crowder and Cooper 1982; Dibble et al. 1996; Dewey et al. 1997).

High quality habitat for many freshwater fish is rich in dissolved oxygen (DO) with low/intermediate SAV densities (Wiley et al. 1984; Allen and Tugend 2002). Submersed aquatic plants aid in juvenile fish survival by reducing predation risks and providing oxygen and structure for food resources, such as macroinvertebrates (Miranda and Hubbard 1994; Havens et al. 1996). Intermediate SAV densities hold high fish biomass composed primarily of small and juvenile fish, but dense SAV is often uninhabitable for many fish due to reduced accessibility (i.e., fish are “screened” out) and potentially reduced DO levels in the interior of large vegetation beds (Wiley et al. 1984; Engel 1985). Managing aquatic systems often includes manipulation of SAV to improve habitat quality for fish.

Understanding fish use of dense SAV has been difficult owing to issues associated with sampling complex habitats. Attributes of SAV including plant species, morphology and density influence capture efficiency of sampling gears, making fish

community assessments in dense SAV difficult (Gu and Swihart 2004; Kroll et al. 2008). Bayley and Austen (2002) found that inshore catch probabilities (here after referred to as catchabilities) for electrofishing samples were significantly reduced and highly variable in higher aquatic plant coverage reducing information (e.g., fish density or occurrence) obtained for fish assessments.

Our limited ability to sample fish in dense SAV habitats limits our understanding of how fish utilize these habitats (Barnett 1973; Bayley and Austen 2002; Bonvechio et al. 2008). Due to the difficulties (e.g., imprecision and variability) sampling in SAV with traditional methods, new methods of assessing fish populations should be developed. Cameras obtain count metrics on animal populations proving useful in population-level studies to estimate abundance (Royle and Nichols 2003; Murphy and Jenkins 2010). Increasingly, marine studies utilize underwater cameras to obtain point count data of fish populations as these methods present a viable, non-lethal alternative to traditional fish sampling techniques (Cappo et al. 2004; Sheehan et al. 2010). Harvey et al. (2004) noted that detection probability in many marine camera studies is poorly understood, making absolute abundance estimates difficult. Evaluating the efficacy for UVC to reliably quantify changes in fish populations can increase confidence in fish abundance estimates and community-level studies, however no previous studies have used UVC to study fish abundance in dense SAV habitats.

The benefit of SAV habitats to fish communities is not ubiquitous. Dense SAV habitat changes species composition, reduces growth rates, and changes foraging interactions (e.g., omnivores switch from piscivory to insectivory; Suthers and Gee 1986; Savino and Stein 1989; Valley and Bremigan 2002). Hydrilla *hydrilla verticillata*, a

freshwater invasive aquatic plant, provides quality fish habitat at low-intermediate densities (Barrientos and Allen 2008). Nonetheless, aquatic plant management commonly assumes that hydrilla reduces fish habitat quality and utilization when present in dense surface mats (FWC 2011). As sampling in dense hydrilla is difficult, fisheries managers rarely evaluate this assumption and the extent that fish utilize this habitat remains understudied.

Management typically considers stakeholder sentiments before acting and hydrilla is a particularly heated topic of concern for Florida's aquatic stakeholders. Typical stakeholder responses to dense hydrilla includes: flood-control coordinators, recreational boaters, crappie anglers, and some homeowners responding negatively to dense hydrilla, and duck-hunters, bass fishermen, and other homeowners responding positively (Hoyer et al. 2005). Specific to angling, there have been mixed impacts of dense SAV on recreational usage with some anglers (e.g., bass fishermen) preferring to fish near areas of dense vegetation due to perceived increases in catch rates while others (e.g., crappie fishermen) do not prefer hydrilla due to reduced boat-access or poor catch rates (Colle et al. 1987; Henderson et al. 2003). Anglers often base these perceptions on anecdotal evidence, thus more rigorous documentation of hydrilla's ecological impacts may better inform these perceptions. Underwater video cameras (UVC) offer a new method to address many of the ecological concerns for hydrilla management by evaluating fish habitat use of dense hydrilla beds. This sampling approach provides a unique opportunity to understand basic ecology in fish-plant interactions focusing on how littoral fish select for habitats and how they respond to

changes in habitat quality in an invasive aquatic plant commonly targeted for management.

Objectives and Chapter Structure

Chapter 1 introduces the problem, provides an overview on fish-plant relationships, and presents the research objectives for this thesis. The objectives in Chapter 2 were to develop methods for use of UVC to estimate fish abundance in dense SAV and evaluate the efficacy of these methods in estimating fish abundance with manipulations of fish populations in experimental ponds. Chapter 3 presents basic ecological findings for fish habitat use in aquatic plants, the objectives of which were to: 1) quantify fish habitat use in response to a gradient of habitat qualities; 2) assess whether habitat use varies with fish density; and 3) explore the impacts of fish habitat use for habitat restoration efforts. Chapter 4 summarizes the key findings from this research, provides concluding remarks on the utility of UVC-methods in recreational fishery management, and recommends research objectives for future consideration.

Three appendices provide auxiliary information on the utility of the UVC as a method to assess fish communities in dense aquatic vegetation. Appendix A shows preliminary information for incorporating heterogeneity in area sampled and detection probability relationships, Appendix B details potential size-based inferences on fish sampled with UVC, and Appendix C lists the taxa, with images, that have been currently sampled with the UVC-methods presented.

CHAPTER 2
EFFICACY IN USING UNDERWATER VIDEO CAMERAS TO ASSESS FISH COMMUNITIES IN DENSE AQUATIC VEGETATION

Submersed Aquatic Vegetation and Associated Impacts to Sampling

Habitat complexity shapes the structure of food webs, mediates predator-prey dynamics, provides refuge for young animals, and fosters increased biodiversity through niche partitioning (Schoener 1974; Gorman and Karr 1978; Crowder and Cooper 1982). Submersed aquatic vegetation (SAV) functions as both ecologically critical and structurally complex habitat (Rozas and Odum 1988; Dibble et al. 1996). Across many life-history stages, fish utilize SAV to forage on plants, algae, aquatic macroinvertebrates, and other fish and as refuge from predators (Crowder and Cooper 1982; Dewey et al. 1997). The variety of these uses makes SAV habitats highly important to fishery and conservation concerns.

The complex habitats that fish inhabit are often difficult to access creating unique challenges for sampling strategies aimed to inform conservation and management. The physical attributes of habitat including complexity and morphology influence the sampling efficiency for many gears, making quantitative assessments of animal occurrence and abundance difficult (Bayley and Austen 2002; Gu and Swihart 2004). Sampling difficulties often limit the understanding of how fish communities utilize these habitats (Bayley and Austen 2002). Many studies have used traditional or modified gears to sample fish communities in SAV habitats including pop nets, seines, electrofishing, Rotenone, and trawls (Dewey et al. 1989; Tate et al. 2003; Dembkowski et al. 2012). However, the catchabilities of these sampling gears are often influenced by habitat cover and it is important to understand how catchability varies with habitat composition and complexity. Sampling problems become particularly evident in dense

and/or invasive SAV species, such as hydrilla *Hydrilla verticillata*, that can form dense surface canopies and grow in high biomass densities that prevent effective sampling.

Dibble et al. (1996) argued that a consequence of impaired sampling in SAV habitats is obtaining only a macro-scale understanding of fish-plant interactions, such as the average fish use in large vegetation patches, with very little micro-scale inferences available, such as fish use on a scale exploited by individual or groups of fish. Conservation strategies often assume that dense SAV reduces dissolved oxygen owing to high respiration rates at night, therefore limiting fish utilization and available habitat (Miranda et al. 2000). Unfortunately, fisheries managers rarely know the extent that fish utilize dense SAV because of the aforementioned sampling difficulties. Aquatic plant management actions (e.g., herbicides) are often applied at least in part under the assumption that fish habitat will be improved, but there is a need to evaluate fish use of dense SAV habitats to evaluate this assumption. Such an evaluation can also inform stakeholder perceptions on the impacts of dense, invasive SAV and these perceptions are often used to make policy. For example, the perception of hydrilla amongst anglers in the US Southeast is often positive and based on anecdotal evidence, but increased documentation of fish-hydrilla interactions could reveal an impact on the target-fishery that reverses their perceptions. Given the pervasiveness and spread of problematic and invasive SAV plants affecting aquatic ecosystems globally, managers need statistically valid fish samples in these complex but ecologically important habitats.

Cameras have been used to estimate animal abundance in fish and wildlife studies (Karanth 1995; reviewed in Cappo et al. 2007). Increasingly, marine studies utilize underwater video cameras (UVC) to obtain count data of fish communities as

these methods present a viable, non-lethal alternative to traditional fish sampling techniques (Priede and Merrett 1998; Cappo et al. 2004; Sheehan et al. 2010). Currently, the use of UVC to sample fish in freshwater ecosystems has been limited to behavioral studies, not population-level analyses or evaluations of their utility in SAV habitats (Chidami et al. 2007; Martin and Irwin 2010). Our objectives were to (1) develop methods for use of UVC to estimate fish abundance in dense SAV and (2) evaluate how these methods performed with manipulations of fish populations in experimental ponds.

Methods

Study Site

To evaluate the efficacy of the UVC to sample fish in dense SAV, we used three 0.405 ha experimental ponds (maximum depth 2.4 m, average depth 1.7 m) located at a United States Geological Survey facility in Gainesville, Florida during summer 2011. We manipulated fish densities among ponds by varying stocking abundances. Stocking occurred in June 2011. Pond 1 was stocked with 75 adult (>140 mm TL total length) *Lepomis* spp. (bluegill *L. macrochirus* and redear sunfish *L. microlophus*), pond 2 was stocked with 150 adult *Lepomis* spp. and pond 3 had an established multi-year fish community assumed to be at carrying capacity with *Lepomis* spp. and largemouth bass *Micropterus salmoides*. The size distribution of fish was similar for fish stocked into ponds 1 and 2. All three ponds were aerated continuously to prevent fish kills, and all contained surface matted (i.e., ~100% coverage) hydrilla by mid-June. Sampling occurred from July to October 2011 and only during daylight hours (9:00 to 17:00) to ensure sufficient light for the UVC.

We drained all ponds in October 2011 to estimate total fish population size at the end of the pond manipulations. We collected all fish in the catch basin and randomly placed quadrats (0.25 m^2) to sample fish in the area outside of the catch basin. We identified fish by taxon (e.g., *Lepomis* spp., juvenile largemouth bass or adult largemouth bass) and analyzed quadrat samples with a negative binomial log-likelihood to obtain maximum likelihood estimates (MLE) of the average fish density per quadrat. We then scaled from fish density up to the total fish population size per taxa that was still remaining in each pond (i.e., they were not removed from the catch basin) by multiplying the MLE for fish density by the total area of the drained pond inhabited by fish. We added the total fish caught from the catch basin to the MLE of the number of fish outside the catch basin from quadrat sampling to obtain a final estimate of fish population size per species in each pond.

Materials

We used a sampling system composed of a UVC and a mini digital-video-recorder (DVR) powered by a 12 volt DC marine battery (Figure 2-1; Table 2-1). The single-lens UVC model SMM-50-C (Seaview, Inc.; Tampa, FL; www.seaview.com) are relatively inexpensive (Table 2-1), 85° wide-angle, color cameras powered by DC or AC current that provide high resolution (540 lines of resolution) video images in turbid and low-light habitats (up to 0.005 lux with internal infrared (IR) light emitting diodes (LED) turned off). The SMM-50-C automatically alternates between color (in abundant light) and black-and-white (in low light) signals to maintain optimal video imaging. Color video allowed fish to be more easily distinguished during fish identifications. We created a mount around the video cable and UVC to orient the camera horizontally (i.e., the camera view was parallel to lake-bottom; Figure 2-2). The UVC, DVR and video

monitor (liquid-crystal display [LCD]), were powered from the single 12-v battery using a four-way socket power adapters. All equipment (Table 2-1) was placed within a waterproof case inside a jonboat.

Field Procedures

Fish were counted using 10-20 point counts every two weeks at random locations at each pond. For each video point count, we lowered the UVC from the jonboat (Figure 2) and through the SAV canopy layer. The dense weight (1.8 kg) and small profile of the UVC allowed the camera to descend with few snags through dense SAV thereby reducing habitat disturbances. The SAV canopy thickness varied spatially, and therefore the depth of UVC deployment can also vary at each site. Our objective was to place the UVC below the dense SAV canopy. Before recording, we used the live-feed from the video display to ensure the UVC viewpoint was oriented away from the jonboat and was not overly obstructed by vegetation. We then used a clamp to hold the video cable against the side of the boat at that depth and began recording video for 10 minutes per point count on a 16 gigabyte secure digital memory card (maximum storage capacity of 4 hours of video recording). We used this sampling design to capture presence-absence (hereafter called occurrence) and count fish abundance beneath the SAV surface canopy layer in each pond (Figure 2-2). To quantify plant biomass, we used a boat-based vertical rake to sample plant biomass density ($kg \cdot m^{-2}$ in dryweight) from seven random sites in each pond every month (Johnson and Newman 2011).

Video Analysis

We measured fish occurrence and fish counted from video analysis at each point count. We used the video playback software GOM media player (available for free;

http://player.gomlab.com/eng/download/) and manually broke each ten minute video into 30 second intervals for analysis. This breakdown allowed longer video counts to be assessed quickly (playback speed ~1.5x) such that we could return to an interval without reanalyzing the entire ten-minute clip. We identified fish down to the lowest taxonomic level (e.g., functional group, family, or species). Video saturation, contrast, and brightness could be adjusted in the media player to optimize our ability to enumerate fish and aid in identifications.

Fish occurrence and abundance measures were adapted from previous video studies. If fish were observed at any time in the ten minutes we noted fish as present, otherwise fish were considered absent. When fish were present we counted the abundance of each fish type onscreen at any one time within every 30 second interval. To prevent double-counting of fish within that sampling site, we used the $MaxN_{species}$ statistic as the maximum number of individuals of a taxa onscreen at any one time during the point count (Figure 2-3; Cappo et al. 2004; alternatively called *mincount* in Gledhill et al. (2005) and *npeak* in Priede and Merrett (1998)). Table 2-2 shows an example video count for *Lepomis* spp. for a case where the interval would be recorded as having a $MaxN_{Lepomis}$ of two. We utilized generalized linear models (GLM) in Program R to evaluate whether occurrence and *MaxN* metrics varied between the ponds and through the sampling season with the sample week as the predictor covariate (R Development Core Team 2011). Analysis of variance (ANOVA) was used to test whether fish occurrence and counts varied among the ponds with pond number set as a predictor factor. We used logit transformations to analyze fish occurrence data

with a binomial GLM and analyzed fish count data with a negative-binomial GLM due to overdispersion in the count data.

We measured relative stem count for each video count by counting the number of visible plant stems following a horizontal grid from a single-frame captured from the video. If fish were present, stem count was measured around the time interval where the maximum number of fish occurred; if fish were absent, stem count was measured at a time interval that best represented the 10 minute video. We used ANOVA to evaluate whether stem count and plant biomass (see above) varied between the ponds and a linear GLM to test whether they changed through the sampling season with sample week as the predictor covariate.

Fish length could not be estimated from video because the single-lens UVC lacks depth distance measurements meaning that a small fish closer to the screen can appear the same size as a larger fish further from the screen. However, we were able to qualify some individuals as either juveniles or adults based on morphological characteristics that vary across ages including: color, length to height ratios, and girth.

We tested whether the number of fish counted was influenced by time to evaluate potential gear or behavioral biases. If fish avoided the gear at the time of deployment we would expect fish counts observations increased at the end of the recording, if fish were attracted to the gear this would be biased to the beginning of the video sample. The number of fish counted per time interval was evaluated with a linear regression with time interval as the predictor covariate. In this model we removed all *MaxN* counts that had >15 fish (n=1) to reduce the bias of outlier samples. We also tested the significance of the null linear regression which would indicate that the slope

of the fish counts across time interval is flat (i.e., there is no bias over the video duration). We then evaluated these two models based on Akaike's Information Criterion (AIC; Akaike 1974). Models with ΔAIC value of < 8.0 have support and < 2.0 have substantial support; if models were equivalent (i.e., the two models are differentiated by < 2.0 ΔAIC) we selected the most parsimonious model among them (Burnham and Anderson 2004).

Results

We collected a total of 324 total point counts and >55 hours of video footage. For all sampling, we asked Seaview, Inc. to disable the internal, IR LEDs in the UVC to reduce backscattering on floating particulates and SAV stems. This backscattering substantially limited the field of view in our initial trials, and thus, we only used the camera without the LED for all point counts in this study. We note that the automated LEDs could prove useful in other low-light or turbid systems.

The UVC worked well identifying and capturing video counts on juvenile and adult *Lepomis* spp. and juvenile and adult largemouth bass *Micropterus salmoides* in dense, surface-matted SAV habitat (Figure 2-3). In total, fish were present in 179 of 324 point counts across all three ponds. We observed adult largemouth bass in 2 of 106 point counts (as largemouth bass were only in pond 3 which was sampled 106 times); juvenile largemouth bass in 9 of 106 point counts; *Lepomis* spp. in 175 of 324 point counts and unidentified fish in 4 of 324 point counts. Every largemouth bass observation also had *Lepomis* spp. co-occurring. Further, our highest *MaxN* was two for adult largemouth bass, two for juvenile largemouth bass, and 21 for *Lepomis* spp. indicating that UVC can distinguish between species and enumerate many individuals.

The UVC sampling gear functioned well in high plant biomass densities of surface-matted SAV. Surface coverage of hydrilla did not change during the study period remaining ~100% throughout the duration of the study. Plant biomass density was high and did not significantly differ among ponds (ANOVA p= 0.906) with pond 1 mean plant density at 2.11 kg·m^{-2} dwt (95% confidence interval (CI) 1.62-2.60 kg·m^{-2} dwt), pond 2 at 2.14 kg·m^{-2} (95% CI 1.55-2.74 kg·m^{-2} dwt), and pond 3 at 2.26 kg·m^{-2} (95% CI 1.82-2.71 kg·m^{-2} dwt). Among all ponds, plant biomass densities significantly decreased (linear GLM p= 0.04) over each month with mean plant density of 2.68 kg·m^{-2} dwt in July, 2.64 kg·m^{-2} in August, 2.00 kg·m^{-2} in September, and 1.54 kg·m^{-2} in October. Relative stem count from UVC counts differed significantly differed across ponds (ANOVA p<<0.05) with stem count highest in pond 1 (mean stem count 59.5, 95% CI 56.0-63.0), intermediate in pond 2 (51.8, 95% CI 47.8-55.9), and lowest in pond 3 (47.4, 95% CI 43.8-50.9), however stem counts did not significantly change over the sampling season (linear GLM p= 0.21).

Fish abundance varied among the ponds based on the pond draining. Fish abundance in pond 1 started at 75 adult *Lepomis* spp., and we recovered 21 fish with zero recruitment of young fish in this pond. Fish abundance in Pond 2 started at 150 adult *Lepomis* spp. and ended at an estimate of over 5,000 fish owing to large recruitment of young fish. Fish abundance in pond 3 (assumed to be at carrying capacity) ended with an estimate of over 15,000 *Lepomis* spp., three adult largemouth bass (fish >300 mm TL) and >700 juvenile largemouth bass (fish >80 mm TL).

The differences in population size in each pond were accurately reflected in UVC fish occurrence and *MaxN* between the ponds. Fish occurrence and *MaxN* were lowest

in pond 1, intermediate in pond 2, and highest in pond 3 (Table 2-3) and these differences were significant (ANOVA $p<<0.05$). Furthermore, mean *MaxN* increased with fish abundance among ponds, suggesting that video counts captured proportional changes in abundance (Figure 2-4). In ponds with stable fish densities (pond 1 and pond 3) the mean *MaxN* did not significantly change through the season (negative-binomial GLM $p= 0.17$ and 0.34 respectively); however mean *MaxN* in pond 2 increased through the season (negative-binomial GLM $p<<0.05$; Figure 2-4). Mean fish occurrence also increased with increases in fish abundance. The mean fish occurrence in pond 1 and pond 3 did not have significant changes through the season (binomial GLM $p= 0.15$ and 0.82 respectively; Table 2-3), but pond 2 had significant increases (binomial GLM $p<<0.05$; Figure 2-5). Thus, the UVC was able to detect changes in fish abundance among the three ponds and within pond 2 as fish abundance changed through the summer.

Fish counted did not significantly change over time during the ten-minute UVC point counts. Both the tested model (that fish counts changed through the ten-minute duration) and the null model significantly predicted fish counts (linear regression $p= 0.048$ and $<<2e-16$ respectively; Table 2-4). The slope of the tested model was very shallow (-0.013) predicting only a small decrease in fish abundance from the video start compared with the video end. The two models ΔAIC scores were less than 2.0 indicating that the models were equivalent in explaining the variation in fish count data over video duration (Table 2-4). This suggests that the time of arrival for fish is random and that UVC may not repel or attract fish to the sample location. This may allow UVC point counts to utilize shorter time durations per count and increase sample size.

Discussion

The UVC provided an innovative, non-lethal technique to sample fish communities in dense SAV habitats. To our knowledge, this is the first study of its kind to use UVC to quantify fish abundance in dense freshwater SAV. An advantage of UVC to other sampling techniques in SAV, such as electrofishing, is that it allows for direct observation of fish in structurally complex habitat, which addresses concerns listed by Dibble et al. (1996) regarding the lack of micro-scale inferences on fish-plant interactions. Furthermore, UVC can provide several different metrics at the population (e.g., presence-absence, abundance, species diversity) and behavioral level (e.g., co-occurrence, movement, foraging) and provide permanently captured data that can be reviewed repeatedly for accuracy. The method also captures charismatic images for public/outreach media and communication.

Fish behavior can be altered by the presence of sampling equipment (reviewed in Stoner et al. 2008). The number of fish counted was not influenced by the time of each point count, which indicates that sampling duration can be modified to improve efficiency. Shorter duration of point counts would allow for larger sample sizes, and thus, improved statistical power and efficiency. The time of arrival on video cameras and time of *MaxN* can be different among fish species and can be a predictor variable in fish density estimates influencing the duration of sampling events for slow-moving or cryptic species (Priede and Merrett 1998; Cappo et al. 2004). Because fish were not significantly affected by the presence of UVC in this study future work with this sampling design can infer that differences in time of arrival or time of *MaxN* are naturally occurring and not a result of fish response to the sample gear.

Video data of fish provide several different indicators of presence and/or abundance, including: 1) presence-absence or occurrence of fish; 2) maximum count or the total number of fish of each taxon observed over an entire video sample (Chidami et al. 2007); 3) maximum number of fish of each taxon onscreen at one time (*MaxN* in Cappo et al. 2004; *mincount* in Gledhill et al. 2005); and 4) a mean count (the average number of fish present in the video frame over the course of a video sample; a new video metric *meancount* provided in Conn 2011). Occurrence, *MaxN*, and *meancount* have advantages over maximum fish encountered to track true fish abundance by avoiding the potential of double counting individuals. Simulations in Conn (2011) has found that *meancount* may provide an unbiased index of true abundance, while *MaxN* may be slightly underestimating trends in true abundance but provides less variability. Furthermore, different software is available to aid in video analysis including EventMeasure™ (SeaGIS, http://www.seagis.com.au/event.html) designed specifically for analyzing video data with *MaxN* metrics on species-rich fish communities. There is no consensus on using *MaxN* or *meancount* to analyze video, although we recommend future experimental work in dense SAV should evaluate the use of both. The baited cameras used in marine ecosystems attract fish to the camera, encounter large schools of fish, and can observe high fish densities to the point where *MaxN* may be underestimating abundance and *meancount* should be used; however, this occurs more often in schooling marine fish (Cappo et al. 2007). In freshwater littoral ecosystems where species can be particularly cryptic, *MaxN* is a useful and easy metric to obtain for tracking changes to abundance across a wide gradient of population levels.

The sampling design for using UVC can be specialized to fit the needs for a variety of fishery and conservation objectives. Many studies have utilized baited camera traps to bring cryptic fish species closer to observable areas, while others have used non-baited cameras to avoid biasing behavior (reviewed in Cappo et al. 2007). For example, Chidami et al. (2007) focused their efforts on understanding day/night differences in behaviors of scavenger fish by sampling on lake-bottom and using IR lights for night time sampling; however our study focused on littoral fish populations in dense, invasive SAV habitats. Other UVC research has been useful for a variety of objectives including: using stereo-video techniques to measure length-frequencies of reef fish and quantify reef fish assemblages (Watson et al. 2005); tracking relative abundance of reef fish communities (Cappo et al. 2004); evaluating fish breeding behaviors in regulated rivers (Martin and Irwin 2010); and estimating demersal shark densities (Priede and Merrett 1998). We encountered design problems and limitations for using UVC in dense, surface-matted SAV habitats and decided upon using these particular cameras mounted from the boat. In preliminary trials, we attempted to use transect-surveys, fixed-placement UVC (i.e., not boat mounted), and a cone over the UVC to aid in canopy penetration, all of which snagged on plants and obstructed view. Currently, we believe that the use of stereo-video to aid in length-frequency analyses in dense SAV will be limited, but this has high potential if low-profile cameras (i.e., small and smoothed surface) can be obtained to create a stereo-video UVC system.

We showed that the UVC can be useful for assessing fish abundance in dense SAV habitats, which have traditionally been difficult to sample with standard gears such as electrofishing or fyke nets. The UVC effectively measured differences in fish

abundance among the ponds and detected temporal changes in abundance for the pond with high recruitment of young fish. Resource managers could use these methods to evaluate fish community responses to changes in habitat (e.g., sample for littoral fish community's response before/during/after aquatic plant removals) or to detect differences in fish abundance among lakes with different habitat characteristics. For example, freshwater fish frequently inhabit areas of low dissolved oxygen or impacted water quality; a sampling protocol that quantifies fish responses, taken from UVC metrics, and habitat/water quality metrics might be superior for time course sampling of fish communities in hypoxic environments compared with traditional methods. This method offers new opportunities to quantify fish population responses to changes in habitat quality and quantity.

The UVC could also be used to evaluate a variety of ecological and management objectives in freshwater ecosystems. Trophic interactions in the aquatic food web could be evaluated by sampling for multi-species occurrences across benthic, pelagic, and littoral fish communities. Video cameras could track spatio-temporal patterns in fish behavior and/or habitat utilization in a variety of habitats. Sampling designs could be focused on areas, depths, and seasons to evaluate a particular species of concern for conservation. The use of the UVC will require water to have adequate transparency, which limits its utility in highly turbid systems. However, many other systems would allow use of UVC to measure fish occurrence and abundance, and these methods could be explored relative to traditional sampling gears in a range of freshwater systems (rivers, ponds, etc.). We encourage future efforts to use UVC to assess fish communities in freshwater systems.

Tables

Table 2-1. Relative costs of equipment needed to build underwater video camera system. This setup is for one complete sampling unit with the number needed noted parentheses. Multiple cameras could be added given subsequent increases to power adapter, DVR capabilities and cables.

Equipment	Price ($, USD)
Camera	250
15.2m cable length[1]	25
LCD Video Monitor	50
12v Marine Battery	200
4-way Socket Adapter	10
mini-DVR	65
16GB SD memory card	30
RCA video cable (2)	5
Total	635

[1] 15.2m cable length is standard, additional lengths available.

Table 2-2. Example video count taken in October 2011 on pond 3 broken down into 30 second intervals (min:sec). The maximum number of fish (*MaxN*) is shown for each species in each time interval, the time interval where *MaxN* occurs is noted, and each fish at time of *MaxN* was classified as either adult or juvenile from morphological characteristics.

	Time Interval for 10 minute video sample							
Fish type	4:00	4:30	5:00	5:30	6:00	Time of MaxN	Juvenile	Adult
Lepomis spp.	2	1	1	1	1	4:00	1	1
M. salmoides	0	2	0	0	1	4:30	2	0

Table 2-3. Three ponds with dense SAV and different levels of fish abundance were sampled with a UVC system. The mean fish occurrence and the mean *MaxN* were taken for all fish taxons and all sampling periods in that pond. Trend refers to how those metrics responded over the course of the sampling season.

Pond	Fish Abundance	Abundance trend	Fish Occurrence	Occurrence trend	*MaxN*	*MaxN* trend
1	Low	Stable	0.222	Stable	0.433	Stable
2	Intermediate	Increased	0.495	Increased	1.61	Increased
3	High	Stable	0.95	Stable	4.34	Stable

Table 2-4. Evaluation of two linear regression models testing whether fish counts changed through the duration of video sampling ranked with the significance of the regression model (*p* –values) and with AIC model selection. If ΔAIC <2.0, models are considered equivalent and the most parsimonious model is selected.

Model	Intercept	Slope	*p*-value	Parameters	AIC	ΔAIC
Time	0.736	-0.013	0.0481	2	26026.28	0
Null	0.673	NA	<2e-16	1	26028.18	1.9

Figures

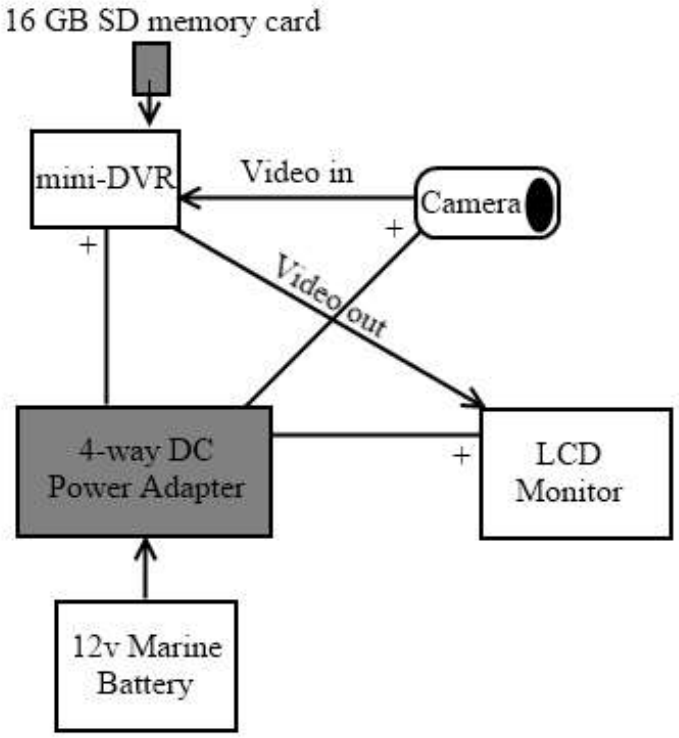

Figure 2-1. Schematic design for using the UVC system to sample fish in dense submersed vegetation. A 12v DC marine battery powers the system. Power adapter connections to the DVR, UVC, and video monitor are indicated with a + symbol. The 16 GB memory card is used to store video recordings from the DVR. Video cables connect the camera to the DVR, and connect the DVR to the LCD monitor.

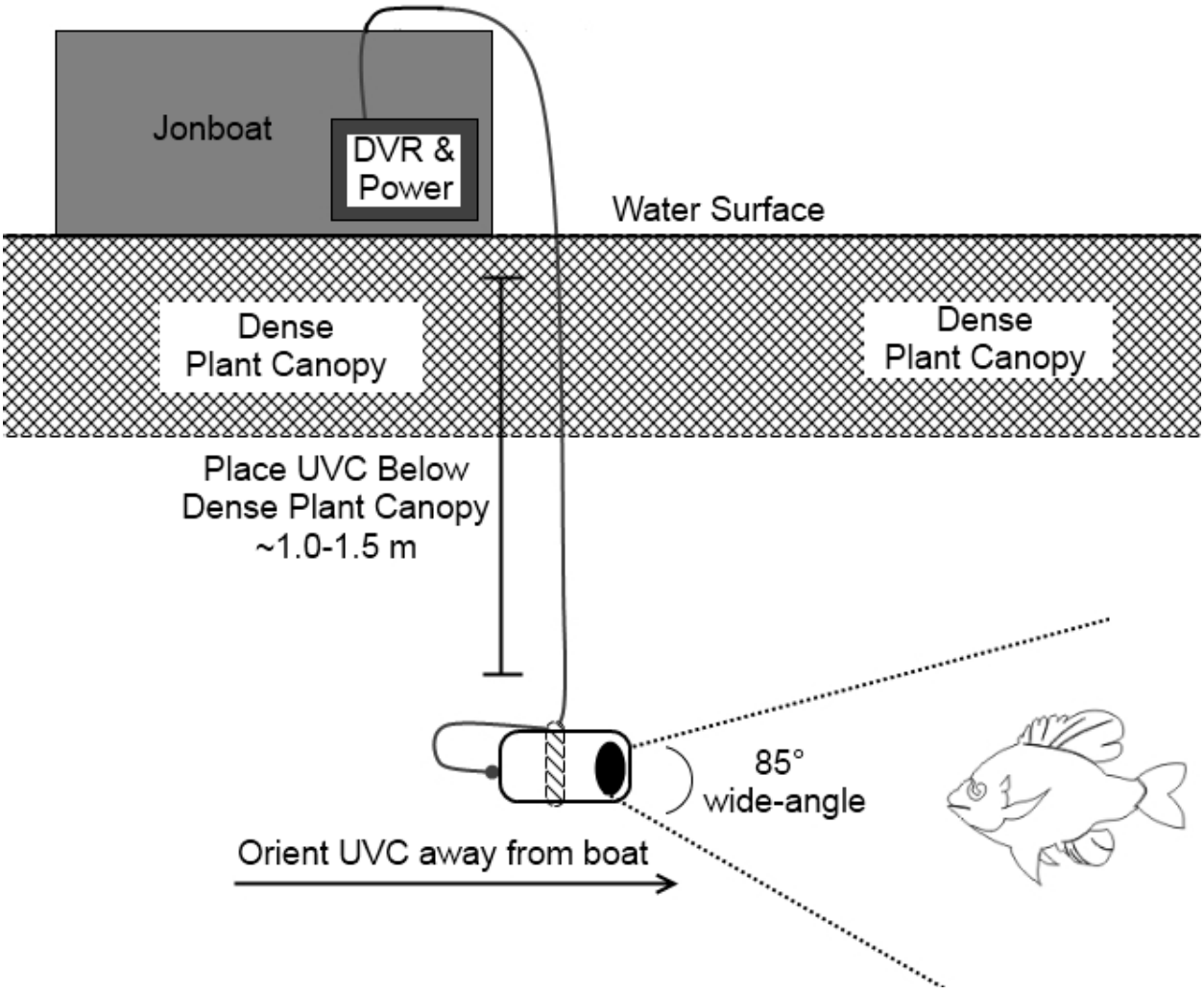

Figure 2-2. Diagram for underwater camera deployment to sample fish in dense aquatic plants.

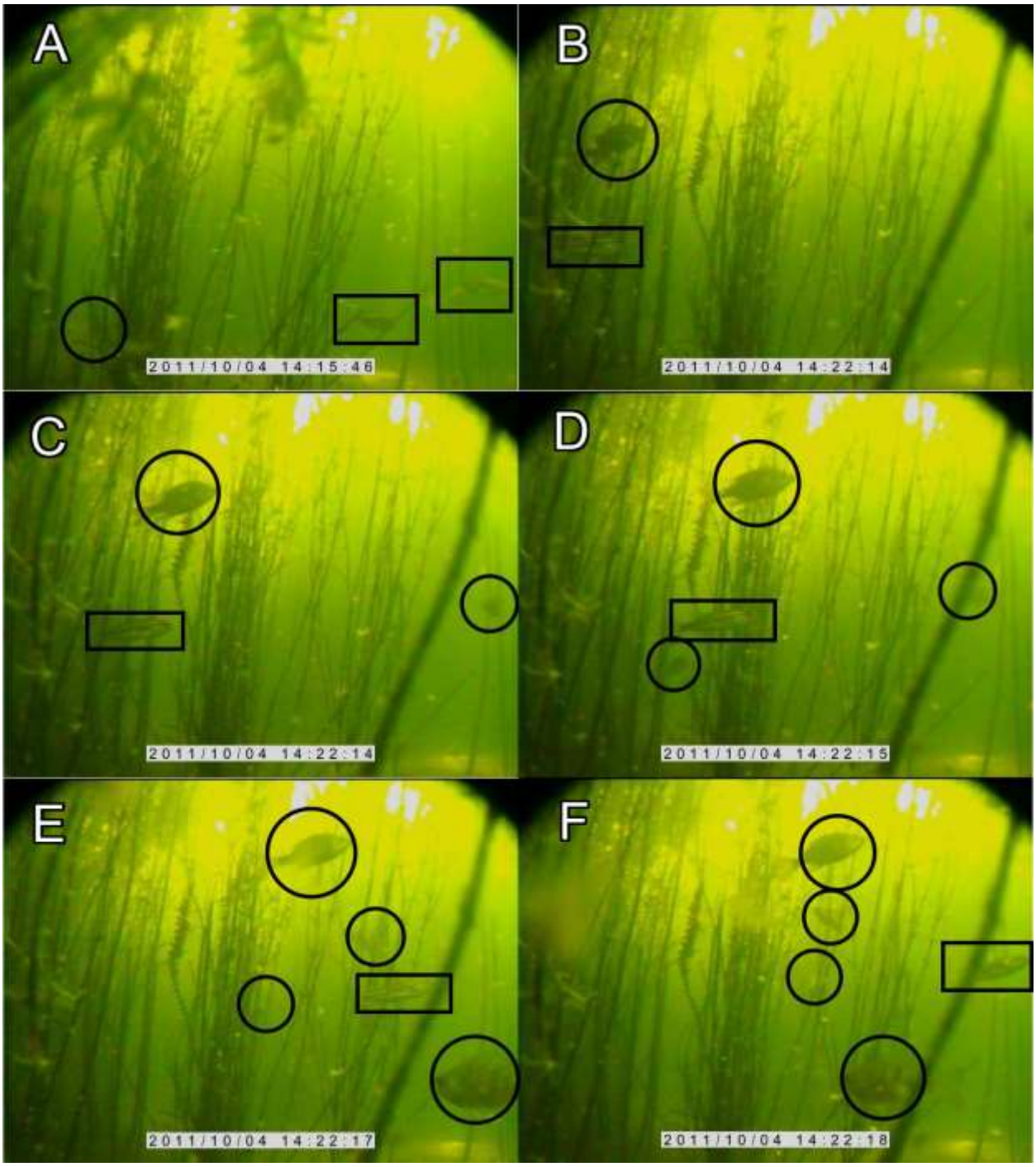

Figure 2-3. Example UVC point count taken underneath dense, surface-matted SAV showing the *MaxN* for juvenile largemouth bass (*MaxN*=2; in rectangles) and *Lepomis* individuals (*MaxN*= 4; in circles) occurring at different times. Two juvenile largemouth bass (panel A) were observed with one *Lepomis* individual in the beginning of the video. By the end of the video (panels E and F) only one juvenile largemouth bass was observed with up to four *Lepomis* spp.

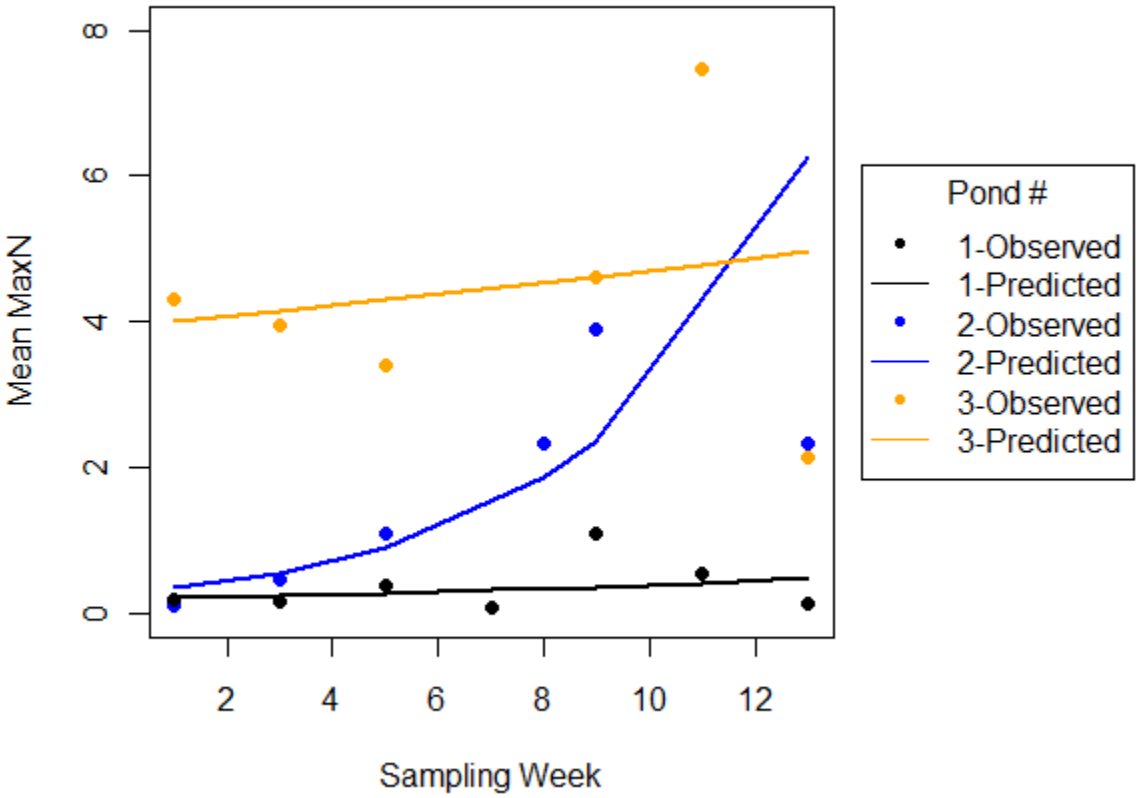

Figure 2-4. The observed mean *MaxN* (maximum number of fish simultaneously onscreen; points) and predicted mean *MaxN* (solid lines) for all fish sampled in dense SAV with UVC in each pond per sampling period.

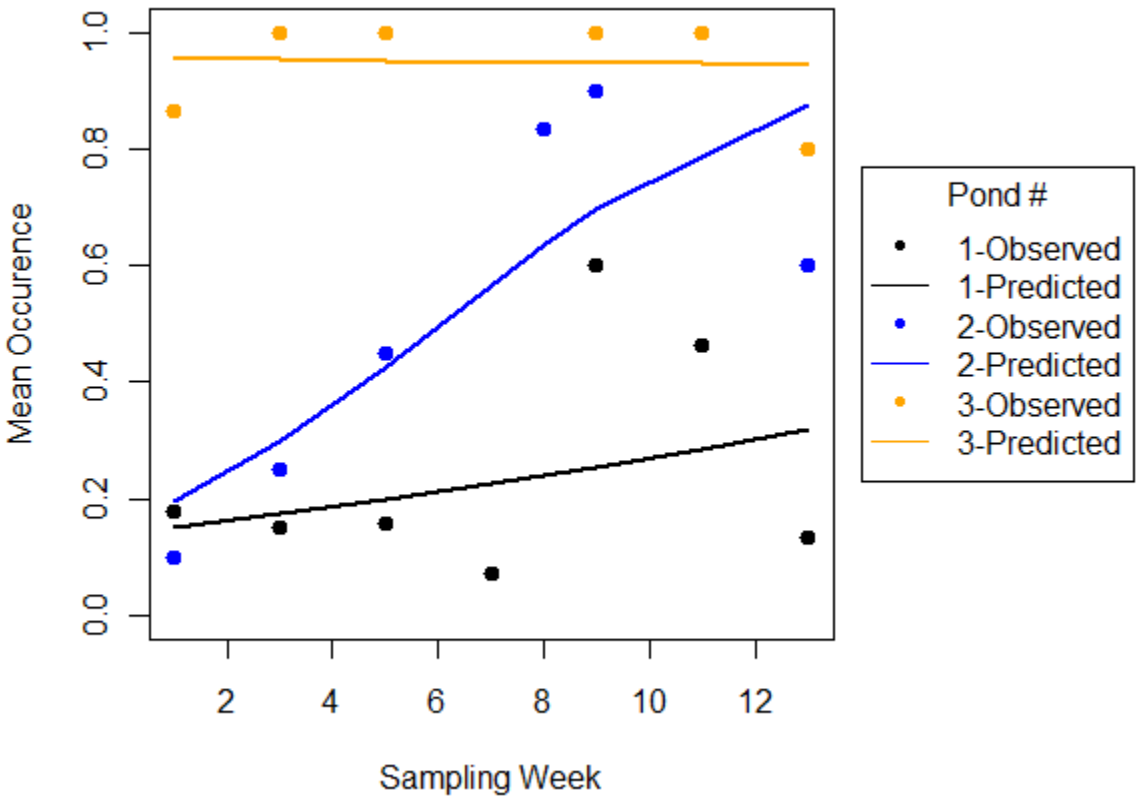

Figure 2-5. The observed mean occurrence (points) and predicted mean occurrence (solid lines) for all fish sampled in dense SAV with UVC in each pond per sampling period.

CHAPTER 3
DENSITY-DEPENDENT FISH HABITAT SELECTION ALONG PHYSIOCHEMICAL GRADIANTS WITH IMPLICATIONS TO HABITAT RESTORATION

Restoration and Fish Habitat Selection in Invasive Aquatic Plant Habitats

Ecosystem changes from climate change, invasive species, and habitat loss have demonstrably increased in recent decades, combining to alter habitat quality and quantity for freshwater fish (Vitousek 1994; Saunders et al. 2002). Such stressors are of serious concern for North American freshwater biodiversity where threatened and endangered freshwater fishes have risen by 92%, from 364 to 700 listed fishes, in the last 20 years and extinction rates are increasing rapidly (Ricciardi and Rasmussen 1999; Sala et al. 2000; Jelks et al. 2008). Invasive species (defined here as ecologically problematic native or nonnative organisms) drive large changes to aquatic ecosystems, including changes to fish habitat, affecting as many as 70% of listed or endangered North American freshwater fishes (Sala et al. 2000; Dextrase and Mandrak 2006). Relatively scarce freshwater ecosystems (0.009% of global waters) hold high levels of fish biodiversity (43% of all fish species reside in freshwater) and thus, increased changes to aquatic systems alter fish habitat quality and threaten freshwater fishes (Saunders et al. 2002).

Habitat restoration, including aquatic ecosystem restoration, is a common tool to combat habitat changes (Dobson et al. 1999). Restoration aims to reverse degraded ecosystems as well as increase habitat quality by restoring lost habitat or reversing the processes that changed those systems, such as removing dams, supplementing large woody debris, and replacing nutrients (Simenstad et al. 2006). Excluding stock enhancements, most restoration work alters habitat and thus indirectly influences animal densities. The success of this approach depends on the truth of a few

assumptions: 1) that habitat restoration can successfully improve habitat quality, 2) if habitats are restored or rehabilitated then animals will utilize those restored areas, and 3) that habitat quality and animal density is coupled.

Monitoring programs measure habitat quality from animal density or occupancy surveys assuming that animal density and habitat quality were correlated (Fielding and Bell 1997; Sergio and Newton 2003). Restoration work aims to increase the availability and prevalence of habitats that often afford high animal densities. Van Horne (1983) argued that in many instances habitat quality and animal densities are not coupled, and that density measures were observed at too coarse a scale to evaluate the true habitat-selection process. Given the prevalence of rapidly undertaken monitoring, such as site-occupancy surveys, if animal density and habitat quality become decoupled conservation programs may be misinformed or fail to produce the desired effect. Many models used in restoration ecology (e.g., population viability analyses) fail to consider the impact of density-dependence which can lead to misestimation of model parameters and potentially reduce the efficacy in restoration actions based on those models (Sabo et al. 2003). Thus, the rush to implement restoration projects outpaces scientific understanding causing a limited ability to evaluate successes and failures (Minns et al. 1996; Caro 1999).

Most animals must make decisions on what locations to inhabit based on a gradient of habitat qualities that optimize their individual resource allocation and minimize predation risk. This indicates a bidirectional relationship where an individual selects for habitat based upon the perceived habitat quality (i.e., available per-capita resources), but habitat quality responds to density-independent (e.g., temperature

change) and density-dependent processes. As animal density increases in a habitat the per-capita quality declines, resulting in potential for density-dependent habitat selection (DDHS; Fretwell and Lucas 1970; MacCall 1990). Animal-habitat relationships depend on the species and system, but DDHS theory assumes that animals evaluate habitat quality and freely distribute to those habitats that optimize individual fitness (Fretwell and Lucas 1970; Rosenzweig 1981; MacCall 1990; Shepherd and Litvak 2004). Specific instances of DDHS have been frequently observed in terrestrial animals but less so in fish populations (Lindberg et al. 2006).

Behavior plays a key role in mediating demographic rates and local animal distributions. Animals often have a spatially-limited perception of all potential habitat patches limiting their ability to compare habitat qualities and distribute towards improved habitat quality. Within patches, animals make trade-off decisions between forage and refuge behaviors based on incomplete information within specific volumes, or “arenas”, which has strong influences on animal vulnerabilities in predator-prey dynamics (Ahrens et al. 2011). Furthermore, changes to patch quality influences the foraging gain-predation risk tradeoff whereby animals begin to sacrifice growth in order to survive due to limited optimal foraging habitats, often called the “minimize μ/f hypothesis” (Gilliam and Fraser 1987). Consequently, limitations in high quality habitats often caused by invasive aquatic plants influence growth, foraging, survival and productivity in animal populations.

A common example of habitat restoration is the management for invasive aquatic plants. Globally, invasive aquatic plants drive substantial changes to aquatic ecosystems by changing habitat structure and affecting physiochemical processes

(Canfield et al. 1984; Langeland 1996; Hershner and Havens 2008; Coetzee et al. 2009). For example, native and nonnative canopy-forming plant species strongly influence dissolved oxygen (DO) dynamics generally reducing DO underneath the surface canopy (Miranda et al. 2000; reviewed in Schultz and Dibble 2012). Most fish species are sensitive to low DO levels, thus canopy forming invasive aquatic plants often reduces fish habitat quality (Suthers and Gee 1986; Miranda and Hodges 2000; reviewed in Schultz and Dibble 2012). Invasive aquatic plants often increase habitat structure and complexity which tends to increase habitat available for some fishes. However, high habitat structure and complexity reduces foraging efficiency for predators and prey and a gradient of habitat complexity provides useful inferences of the foraging gain-predation risk tradeoff when sampling prey species (Crowder and Cooper 1982; Schramm et al. 1987; Lillie and Budd 1992; Kelly and Hawes 2005).

Spread of invasive aquatic plants often reduces fish habitat quality and alters fish habitat selection and fishery production. Habitat restoration attempts to ameliorate these impacts, but restoration efforts often fail to unite fine-scale processes that govern local population distribution and growth (e.g., fish behavior) with landscape-scale patterns (e.g., habitat loss) used to identify the problem (Van Horne 1983; Caro 1999; Simenstad et al. 2006). As such, conceptual approaches based on DDHS allow us to evaluate whether changes in fish habitat use might be caused by changes in habitat quality, changes in animal density, or both. This approach provides testable hypotheses helpful for evaluating restoration objectives (Table 3-1). For example, in DO-limited ecosystems, fish need minimum oxygen levels to satisfy physiological demands but might select low DO habitats if their per-capita benefits of growth and

survival are maximized. As the availability of high-quality habitats limit fishery productivity, failing to consider DDHS in restoration has cascading impacts to achieving goals.

Demonstrating whether fish habitat use is influenced by fish density and habitat quality in invasive aquatic plant habitats can aid in restoration efforts. The objectives of this study were to: 1) quantify fish habitat use in response to a gradient of habitat quality, 2) assess whether habitat use varied with fish density, and 3) explore the impacts of fish habitat use for habitat restoration efforts. Habitat quality gradients tested included physiochemical (e.g., dissolved oxygen) and complexity (e.g., stem density), which are often considered to influence both predation risk and foraging gain.

Methods

We used three 0.405 ha experimental ponds located at a United States Geological Survey facility in Gainesville, Florida during summer 2011. All three ponds had ~100% coverage of surface-matted hydrilla *Hydrilla verticillata* by mid-June and were aerated continuously at one end of the pond in order to prevent fish kills thereby also creating a gradient for DO levels independent of hydrilla habitat complexity. We manipulated fish densities among ponds by varying stocking abundances. Stocking occurred in June 2011. Pond 1 was stocked with 75 adult (>140 mm TL total length) *Lepomis* spp. (bluegill *L. macrochirus* and redear sunfish *L. microlophus*), pond 2 was stocked with 150 adult *Lepomis* spp., and pond 3 had an established multi-year fish community assumed to be at carrying capacity with *Lepomis* spp. and largemouth bass *Micropterus salmoides*. The size distribution of fish was similar for fish stocked into ponds 1 and 2.

We used a UVC system to obtain 10-20 point counts every two weeks for 13 weeks at random locations in each pond (for further details on the UVC system see Wilson et al. *In review*). Sampling occurred from July to October 2011, and only during daylight hours (9:00 to 17:00) to ensure sufficient light for the underwater video camera system (UVC). For each video point count, we lowered the UVC from the jon boat and through the hydrilla canopy layer. Before recording, we ensured the UVC viewpoint was not overly obstructed by vegetation and subsequently recorded video footage for ten minutes per point count. We used this sampling design to collect presence-absence (hereafter called occurrence) and count fish abundance beneath the SAV surface canopy layer in each pond. Immediately after recording we collected dissolved oxygen data with a handheld YSI 556MPS dissolved oxygen meter.

We measured fish occurrence, abundance, and habitat complexity from video analysis of each point count. If fish were observed at any time in the ten minutes we noted fish as present, otherwise fish were considered absent. When fish were present we counted abundance with the *MaxN* metric, the maximum number of individual fish onscreen at any one time during the ten minute point count (for further details on video analysis see Wilson et al. *In review*). For each UVC point count, we measured habitat complexity as the relative stem count, the number of distinguishable plant stems following a horizontal grid across a single-frame captured from video. If fish were present, habitat complexity was measured at the time interval where the maximum number of fish occurred; if fish were absent, stem count was measured at a time interval that best represented the ten minute video.

Fish habitat selection was measured across a gradient of habitat qualities using dissolved oxygen (DO) and habitat complexity. Generally, DO is a useful metric for fish habitat quality in freshwater as these ecosystems are often DO-limited due to stresses from biotic (e.g., algal blooms or canopy-forming plants) and abiotic (e.g., eutrophication or high water temperatures) changes. Furthermore, DO levels strongly influence the presence and abundance of aquatic animals, and many fish and macroinvertebrates require adequate oxygen for direct (e.g., physiological demands) and indirect reasons (e.g., increased food and prey items in high DO areas; Killgore and Hoover 2001). Some fishes exhibit morphological changes to inhabit hypoxic waters, suggesting shifts of fish assemblages or habitat use during hypoxic periods (Miranda et al. 2000). Habitat complexity is another metric for fish habitat quality as fish behavior, growth, and foraging strategies are influenced by habitat complexity or aquatic plant biomass (Crowder and Cooper 1982).

We drained all ponds in October 2011 to estimate total fish population size at the end of the manipulations. All fish in the catch basin were collected and we randomly placed quadrats (0.25 m^2) to sample fish in the sediments outside of the catch basin. We identified fish by taxon (e.g., *Lepomis* spp., juvenile largemouth bass or adult largemouth bass) and analyzed quadrat samples with a negative binomial log-likelihood to obtain maximum likelihood estimates (MLE) of the average fish density per quadrat. We then scaled from fish density up to the total fish population size per taxa that was still remaining in each pond (i.e., they were not removed from the catch basin) by multiplying the MLE for fish density by the total area of the drained pond inhabited by fish. We added the total fish caught from the catch basin to the MLE of the number of

fish outside the catch basin from quadrat sampling to obtain a final estimate of fish population size per species in each pond. We calculated 95% confidence intervals for the MLE for each population using +/- 1.96 times the standard error.

We used an analysis of variance (ANOVA) in Program R (R Development Core Team 2011) to assess whether dissolved oxygen and habitat complexity differed among the ponds. We also used a generalized linear model (GLM) to evaluate if fish occurrence and fish abundance within ponds were influenced by DO and habitat complexity. Fish occurrence was modeled with a logit-linked binomial GLM and fish counts were modeled with a negative-binomial GLM due to overdispersion in the data. We used a second series of GLMs to test whether the relationships between each fish metric (fish occurrence and fish counts) and DO and habitat complexity varied across the different fish densities by using pond number as a block factor and a logit-linked binomial GLM for fish occurrence and a negative-binomial GLM for the fish count data.

Results

Dissolved oxygen and habitat complexity varied among the three ponds. While DO conditions in pond 1 (mean DO 2.49 mg/L, 95% confidence intervals (CI) 2.18-2.81 mg/L) and pond 2 (2.46 mg/L, 95% CI 2.14-2.78 mg/L) were not significantly different (ANOVA p= 0.898), pond 3 (3.20 mg/L, 95% CI 2.81-3.58 mg/L) had significantly higher average DO (ANOVA $p << 0.05$). Further, the habitat complexity from UVC counts significantly differed across ponds (all ANOVA $p<0.05$) with stem count highest in pond 1 (mean stem count 59.5, 95% CI 56.0-63.0), intermediate in pond 2 (51.8, 95% CI 47.8-55.9), and lowest in pond 3 (47.4, 95% CI 43.8-50.9). The number of distinguishable stems was occasionally saturated onscreen whereby video analysis

could not further identify individual stems; this occurred most often at ~80 stems counted and represented very high habitat complexity.

Fish abundance varied among the ponds based on results from the pond draining. Fish abundance in pond 1 started at 75 adult *Lepomis* spp., and we recovered 21 fish with zero recruitment of young fish in this pond. Fish abundance in Pond 2 started at 150 adult *Lepomis* spp. and ended with a maximum likelihood estimate (MLE) of over 5,000 fish (95% confidence intervals [CI] 4,027-6,100 fish) owing to large recruitment of young fish. Fish abundance in pond 3, assumed to start at carrying capacity, ended with an MLE of over 15,000 *Lepomis* spp. (95% CI 11,874-17,354 fish), three adult largemouth bass (fish >300 mm total length [TL]) and an estimate of >700 juvenile largemouth bass (fish >80 mm TL; 95% CI 67-1478 fish) for a total of over 15,000 fish. For subsequent analyses, we combined all fish species together since largemouth bass, particularly adults, were not abundant and were only present in pond 3.

Our results showed that both DO and habitat complexity significantly influenced fish habitat use. Fish occurrence was positively related to increased DO and negatively influenced by increased habitat complexity (Figure 3-1; binomial GLM $p<<0.05$). Similarly, fish counts were positively influenced by DO and negatively influenced by habitat complexity across ponds (Figure 3-1; negative-binomial GLM $p<<0.05$).

Our results indicated density-dependent fish habitat selection across the DO and habitat complexity gradient. Fish occurrence was significantly influenced by fish density, DO, and habitat complexity (Figure 3-2; binomial GLM <<0.05) indicating that fish occurrences were influenced both by fish population size and habitat quality. Fish

counts were also significantly influenced by fish density, DO, and habitat complexity (Figure 3-2; negative-binomial $p<<0.05$) suggesting that fish density and habitat quality influenced local fish habitat use. In the high fish density treatment (Pond 3), fish occurrence was stable across the DO and habitat complexity gradients (Figure 3-2). Additionally, when fish density was high, fish counts were highest in high DO locations and highest in low complexity locations. In the low fish density treatment (Pond 1), fish utilized areas of high DO and avoided low DO habitat and utilized areas of low habitat complexity and avoided high habitat complexity (Figure 3-2).

Discussion

We found strong evidence for density-dependent habitat selection for fish across a physiochemical gradient generated by a commonly managed invasive aquatic plant. Overall, fish habitat use was significantly influenced by the combination of a location's intrinsic habitat quality (DO and habitat complexity) and the fish density in the population. Fish use of marginal habitats was density-dependent. At high fish densities species-occurrence would not indicate habitat quality. Conversely, in low fish densities, the flat relationship between fish counts and habitat quality showed that the use of fish counts did not provide enough signal to indicate habitat quality but fish occurrence did. Our results suggest that fish made decisions for habitat selection to maximize fitness based in part on 1) total fish density, 2) the limitation of physiochemical resources, and 3) the availability of high quality forage habitats as measured by the gradient of complexity.

Fish choose habitats based on factors such as food, refuge, and oxygen and the distribution of these habitats is patchy. Eby et al. (2005) found that marine fish in DO-limited habitats overcrowd in high DO habitats while avoiding hypoxic areas; this

overcrowding had density-dependent impacts to growth and survival. Habitat manipulations for Gag grouper *Mycteroperca microlepis* found that density-dependent habitat selection and limited high quality habitat regulates growth, survival, and foraging dynamics thus governing fishery production (Lindberg et al. 2006). We found that individual *Lepomis* spp. and largemouth bass preferred patches of low habitat complexity and high DO but these habitats were not always available. High habitat complexity negatively influences visibility and foraging efficiency for fish and their predators. Complex refuge habitats have limited food resources and individuals compete to maximize food intake in risky-areas or minimize predation-risk by foregoing food (Crowder and Cooper 1982; Gilliam and Fraser 1987; Biesinger et al. 2011). This has implications for fish vulnerability to predation as the proportion of risk-averse behavior in high density populations should decrease with increased competition for limited resources (Gilliam and Fraser 1987; Biesinger et al. 2011). Therefore, limitations of high-quality habitat in invasive aquatic plants negatively impact fish growth and survival.

The results from this study are consistent with hypotheses generated in the DDHS framework. This infers that animals in low density populations occupy areas of high habitat quality and can avoid areas of low quality, while animals in high density populations are forced into utilizing low quality habitats but at lower occurrences and abundances than areas of high quality. Habitat selection theory predicts that as local animal densities increase in a patch, the per-capita habitat quality is lowered. Ultimately, this results in individuals facing choices between habitats of different qualities but also different levels of competition for those resources (MacCall 1990).

The ideal free distribution (IFD) assumes that individuals of a population ultimately distribute among patches of habitat and that their fitness within those habitats is maximized (Fretwell and Lucas 1970). Therefore, we might assume that fish responding to invasive aquatic plants will eventually distribute in a manner that maximizes their individual which presumably improves total population size. Our results indicated that fish will use marginal habitats when faced with increased competition in high quality locations, consistent with IFD theory. This infers that increased fish density reduced the realized habitat quality in occupied patches forcing fish to distribute into marginal and unoccupied patches. Thus, fish occurrence does not provide a metric for habitat quality across all fish densities.

Our results showed that invasive hydrilla had significant impacts to fish habitat quality, and fish habitat use. Hydrilla increased the prevalence of hypoxia in these ecosystems as our ponds averaged DO levels of 2-3 mg/L for four months which is at or below the levels considered hypoxic for most fish species (Caraco et al. 2006). We found that fish will utilize marginal habitats (i.e., highly complex habitats or low DO), but they do not prefer to do so. Increased stressors from invasive aquatic plants may drive selective pressures across fish populations potentially impacting ecosystem function (e.g., aquatic plants providing nursery habitat for young fish) and ecosystem services (e.g., aquatic plant habitats producing trophy largemouth bass for recreational fisheries). In lake ecosystems infested with hydrilla, we anticipate that fish will avoid areas of low DO and increased complexity and expand their spatial range to seek out patches that have higher DO and lower habitat complexity, dependent on fish density and species-specific habitat needs. These changes to fish habitat use may contradict some

stakeholder perceptions of the impact that hydrilla has on their species of concern. Specifically, we showed that some recreationally-targeted centrarchid species avoided habitat characteristics (low DO and high habitat complexity) typically associated with areas of expansive surface-matted hydrilla while recreational anglers often hold a favorable perception of these areas of hydrilla.

We do not argue that fish select for habitat solely in a density-dependent manner. MacCall (1990) explains that per-capita optimal habitat changes as changes occur to population size, environmental conditions, and the population's spatial range. For example, spatio-temporal patterns in DO dynamics are density-independent, responding to both biotic (e.g., plant canopy-depth or microbial consumption) and abiotic (e.g., temperature) changes (Kaller et al. 2011). Thus, the intrinsic quality of a location changes with regard to environmental conditions, while realized quality changes with density-independent and dependent mechanisms. Long-term changes to habitat quality influences fish population dynamics regardless of density-dependent processes, and restoration efforts can mediate these problems (Shepherd and Litvak 2004).

Ultimately, DDHS could influence the methods required for evaluation of restoration efforts. Van Horne (1983) cautions against relying solely on one type of simplified animal surveys as a measure of habitat quality. We showed that fish use marginal habitats when found at high densities by contrasting fish occurrence with fish count metrics. In this case, fish use of low-quality habitats was indicative of a robust and productive fish population persisting in other habitat patches. Occurrence of fish in low DO habitats could indicate high population sizes reducing the need for management. In contrast, when fish populations do not utilize marginal habitat (e.g.,

adult largemouth bass not utilizing patches of dense hydrilla), management needs to consider alternative ecosystem pressures, such as overfishing (Post et al. 2002). Overfishing removes competitors from the population, continually opening up patches of high quality habitats and results in unoccupied marginal habitat patches. In such a case, stakeholders may falsely identify the invasive aquatic plant as a "problematic area" due to prolific hypoxia levels and demand restoration, while overfishing was the true mechanism for the low fish population.

Difficulties in sampling design and the speed with which we undertake restoration efforts reduces our ability to evaluate restoration achieving targeted goals (Minns et al. 1996; Fielding and Bell 1997; Simenstad et al. 2006). The efficacy of restoration strongly depends upon the habitat quality-animal relationship and how that relationship responds to expected changes in habitat quality. Our work showed that, independently, neither animal occupancy nor count surveys indicated habitat quality across all population densities. In low fish densities, occurrence changed across changes to habitat quality signaling the availability of high quality habitat and thus, low fish density. However, fish counts from the low fish density treatment exhibited relatively little change across habitat quality gradients due to low abundance and zero-inflated counts. Use of both abundance and occurrence can identify the animal-habitat quality relationship and allow inference on population density changes with habitat. Management must consider that the sampling tactics used to monitor restored habitats has consequences for the interpretation of that data. Data interpretation in high animal densities breaks down when relying solely on occupancy surveys. Interestingly, restoration efforts often seek to improve animal density. Therefore, monitoring programs can evaluate both animal-

habitat quality relationships and potential consequences of density-dependent growth/survival by contrasting data from cost-effective occupancy surveys with growth and diet data from their species of interest. This alleviates programs from quantifying both occurrence and abundance patterns while still obtaining most of their data needs for understanding the drivers in population dynamics.

We recognize that this study could be criticized on limited replication and a low range of habitat complexity. Our design only had three fish-density levels in three ponds. Other ecological studies used three density-treatments in three lakes to evaluate density-dependent processes in fish populations (Osenberg et al. 1992). Furthermore, most field-based DDHS research used natural experiments or models, and generally did not manipulate large populations. In our study, we were able to manipulate the levels of large fish populations for a four month experiment and evaluated fish occurrence and relative abundances along two independent gradients of habitat quality. Habitat complexity forms an interesting metric that influences both animal habitat use and the detection probability of an animal (Savino and Stein 1982, Savino and Stein 1989; Gu and Swihart 2004). Elucidating whether changes in fish counted and occurrence across a gradient of complexities were due to changes in detection or changes in local fish density proved difficult because complexity influences both biological and observational processes. Our experimental ponds held only high levels of aquatic plant biomass and did not offer a full gradient of non-vegetated, low, and high habitat complexity. Thus, our metric for habitat complexity represented the higher portions of available complexity offered in natural ecosystems. Future work

should manipulate the spatial complexity of invasive aquatic plant habitat within experimental ponds to offer an increased gradient of complexities.

These results provide more specific hypotheses to evaluate restoration efforts that aim to improve freshwater fisheries. Sampling programs need to consider DDHS processes because fish occupancy and fish counts do not indicate habitat quality across all fish densities. Restoration plans can address whether invasive aquatic plants influence native fish communities by contrasting the fish counts and occurrence in habitats that typically provide high quality fish habitat (e.g., native aquatic plants, edge of vegetation) with areas of marginal habitat. Such an evaluation can determine whether restoration actions that reduce invasive aquatic plant coverage can aid native fish populations and, ultimately, if restoration is even needed.

Tables

Table 3-1. Hypotheses for fish habitat use under the framework of density-dependent habitat selection assuming the ideal free distribution (Fretwell and Lucas 1970; MacCall 1990)

Population Size	Hypothesis
High	Individual fish will use lower quality habitat as higher quality habitats become unavailable
High	Local fish densities at high quality habitats should be higher than local fish densities at low quality habitats
Low	Individual fish will not use lower quality habitat as high quality habitat is available

Figures

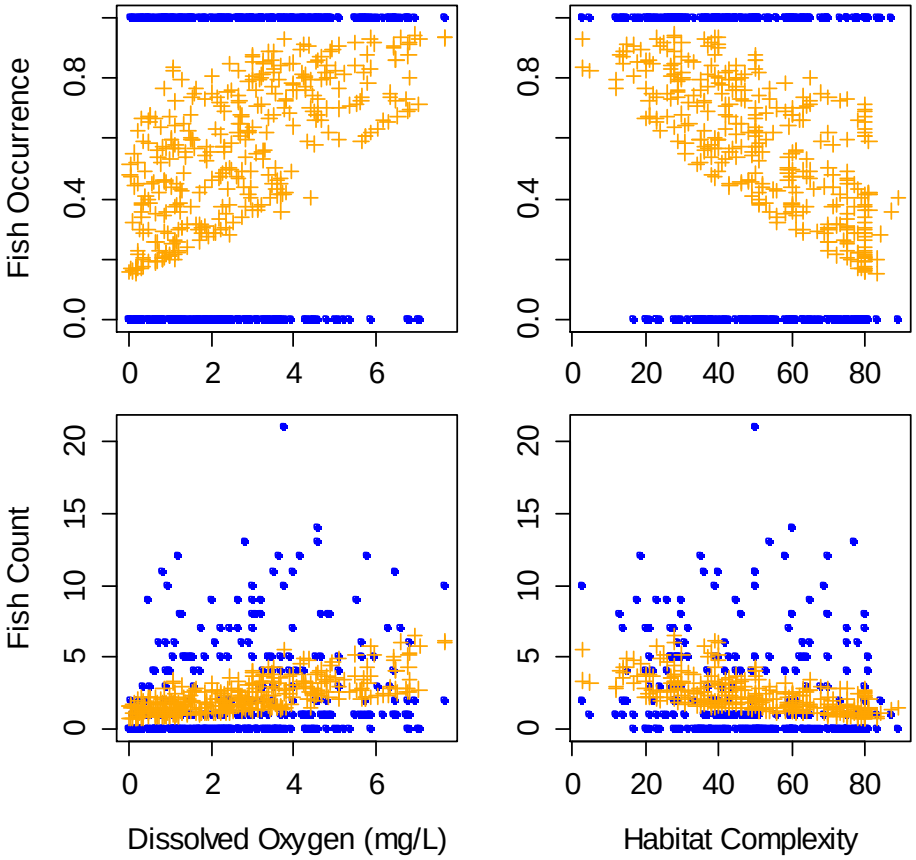

Figure 3-1. Observed fish occurrence (upper panels; blue solid dots) and predicted fish occurrence (upper panels; orange plus marks) and observed fish counts (lower panels; blue solid dots) and predicted fish counts (lower panels; orange plus marks) regressed onto dissolved oxygen (mg/L; left panels) and habitat complexity (right panels) for all fish sampled in dense hydrilla with underwater video cameras in each pond.

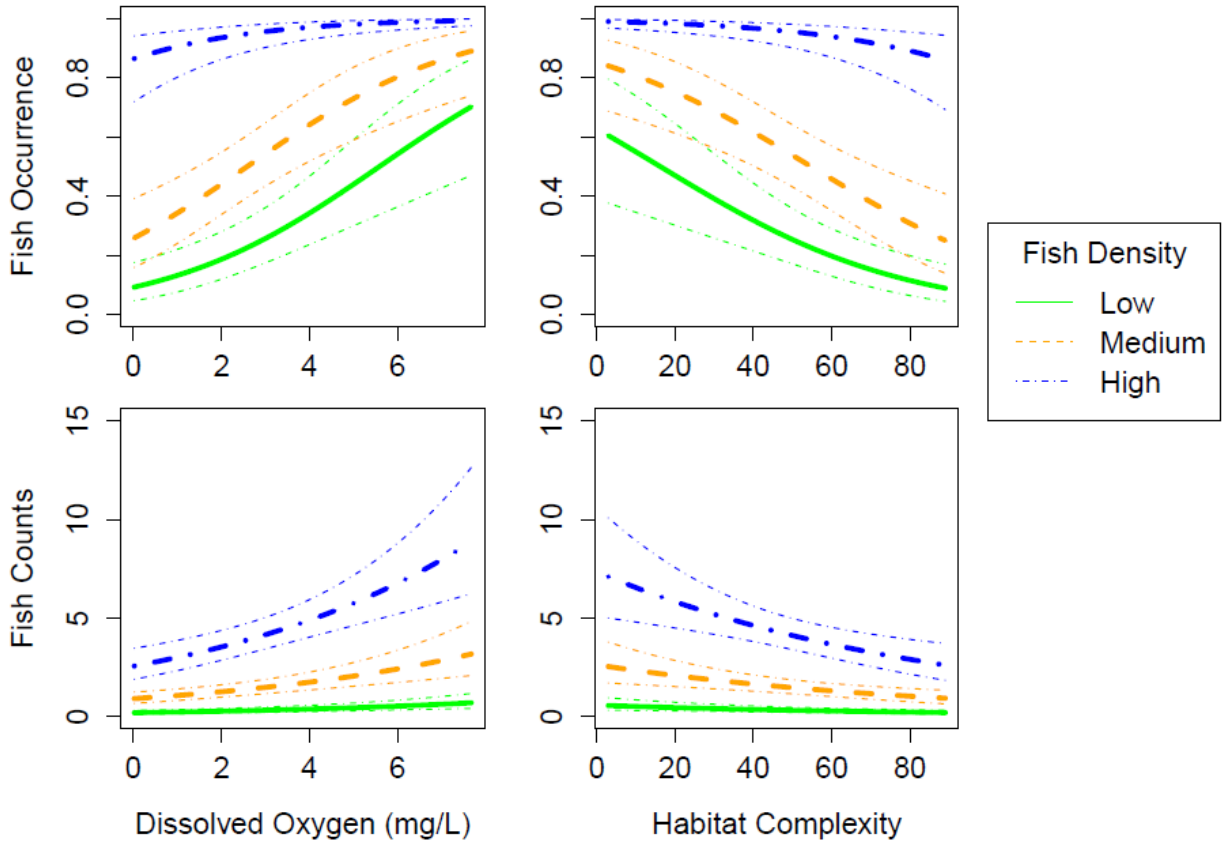

Figure 3-2. Predicted fish occurrence (upper panels) and fish counts (lower panels) across high (blue dashed-dotted line), intermediate (orange, dashed line), and low (green, solid line) fish densities regressed onto dissolved oxygen (mg/L; left panels) and habitat complexity (stem counts; right panels). For graphical portrayals, the depiction of the fish-dissolved oxygen relationships holds the opposing influence of habitat complexity at 52.71, the mean stem count for the sampling season. The depiction of the fish-habitat complexity relationship holds the opposing influence of dissolved oxygen at 2.74 mg/L, the mean value for the sampling season.

CHAPTER 4
CONCLUSION

Key Findings

Underwater video cameras (UVC) worked well as a tool to assess fish communities in dense submersed aquatic vegetation (SAV) habitats. The UVC method accurately quantified spatio-temporal trends in occurrence and relative abundances of fish populations in dense aquatic plants (Chapter 2). This may present the best non-lethal alternative to Rotenone for sampling fish communities in such habitats. The UVC gear successfully sampled fish important to Florida's recreational fisheries such as *Lepomis* spp. (bluegill and redear sunfish) and largemouth bass. Alongside this work, preliminary results indicate a wide range of fish and wildlife taxa successfully sampled with UVC (Appendix C). The presence of UVC gear did not seem to impact the presence and abundance of fish allowing future research to optimize sampling time for the system and species (Chapter 2). This also suggests that fish behavior was not significantly altered by the presence of the sampling equipment. The UVC system can potentially estimate fish total lengths for some species, and categorize adult vs. juvenile for others (Chapter 2 and Appendix B). Lastly, the detection probability for fish in sampled areas was estimated to be relatively high (>50%; Appendix A). This suggests that UVC has potential to estimate true fish densities if detection probability and area sampled models are developed further.

Additionally, my results found and reaffirmed basic ecological relationships in fish-plant interactions. Fish habitat selection was driven by physiochemical gradients and fish density (Chapter 3). I anticipated that fish selected for habitat based on dissolved oxygen but less so for the habitat complexity gradient. These relationships

have direct application to the management of Florida lakes as the response of fish habitat use across physiochemical gradients has rarely been quantified on a small scale in dense aquatic plants. Finding evidence for density-dependent habitat selection was not fully anticipated (Chapter 3). These findings present new context for fishery researchers to evaluate the performance of their fisheries and how populations are impacted by invasive aquatic plant management. Fish utilized low dissolved oxygen (DO) habitats and highly complex habitats but preferred high DO and low complexity (Chapter 3). These findings suggest that the management-assumption that fish are excluded from dense beds of hydrilla is not true in all ecological situations. Application of these findings allow for aquatic plant managers to quantify the need for aquatic plant management to target fishery improvements or if they can focus efforts elsewhere.

Future Directions

Modifications to habitat directly and indirectly (e.g., trophic cascades) influence animal populations and community composition (Gorman and Karr 1978; Halaj et al. 2000; Warfe and Barmuta 2006). In particular, hydrilla habitats undergo frequent management actions which rapidly change plant communities in a short duration and affect fish communities (Maceina et al. 1991; Hoyer et al. 2005). The impacts that plant management has upon fish are species-specific and mixed (i.e., some fish respond favorably, others do not; Colle et al. 1987; Maceina et al. 1991; Bettoli et al. 1993). However, much of the prior research on fish-plant relationships utilized coarse trawls, electrofishing transects, or other sampling tools which may not capture fine-scale ecological processes. Thus, hydrilla management might not quantitatively benefit all recreationally important fisheries in the US Southeast, where hydrilla is prevalent.

New herbicide management techniques or spatial arrangements may maximize beneficial habitat characteristics (e.g., reduced plant density to increase dissolved oxygen and habitat edge) for fish. In ecosystem simulations, increases in edge habitat increases foraging efficiency of top predators and increases growth rates of both largemouth bass and bluegill (Trebitz et al. 1997). Fisheries managers will need to adequately monitor fish habitat use before and after implementing these proposed actions to quantify the net improvement for fisheries. The UVC-method provides an opportunity to conduct fine-scale measurements across large lake-patches and vegetation gradients. I recommend that future research quantify spatio-temporal changes in fish habitat selection with UVC following an applied treatment of herbicides. This level of habitat alteration can form large macrocosm experiments and can increase management's cognizance of fish community impacts from invasive aquatic plant treatments.

Fish abundances are higher in SAV habitats than in non-vegetated habitats, with the highest fish abundances associated with intermediate SAV densities (Killgore et al. 1989; Wyda et al. 2002). Furthermore, hydrilla can hold higher fish biomass compared with native SAV species, indicating that fish can prefer invasive aquatic plants as habitat (Barrientos and Allen 2008). I recommend future research use UVC across wider diversities of aquatic plant species to evaluate differences in fish communities inhabiting non-native/invasive compared with native plants, and vegetated compared with non-vegetated habitats. These comparative studies will allow fisheries managers to form species-specific management plans related to aquatic plants.

I recommend the implementation of UVC technologies into existing aquatic ecosystem management plans, particularly in dense invasive aquatic plants. This includes using simulation models to evaluate the necessary sampling program for UVC to track fish community changes. Dependent on research objectives, detection probability and area sampled relationships probably require further refinement for applied use. However, the UVC can already document changes in relative abundances and estimate some fish densities given assumptions about the area sampled (e.g., area sampled occurs between 0.5-1.0 m^2).

Managers, researchers, and anglers often disagree on the quality of fisheries in dense plants, particularly in hydrilla. Colle et al. (1987) documented a steep dropoff in Orange Lake's angler use and expenditures when dense hydrilla coverages in the 1970's reduced water access. However, Henderson et al. (2003) hypothesize through creel surveys that increased patches of dense vegetation would increase angler use and revenue in two South Carolina reservoirs. There are both positive and negative effects to dense vegetation on recreational usage. Subsequently, many recreational users require managers to create navigable pathways through hydrilla beds to access the lake, while others desire hydrilla for quality fishing opportunities (Hoyer et al. 2005).

For management purposes, I foresee UVC providing potential to address discrepancies in recreational fisheries in dense hydrilla. Anglers often have contradictory evaluations of dense hydrilla, and many prefer fishing near hydrilla while others do not. Either way, aquatic plant management often treats patches to improve physiochemical processes, boat access, and other stakeholder needs. There is a need to simultaneously evaluate the ecological-state of recreational fisheries in comparison

with recreational anglers' perceptions of these environments. For example, ecological findings may suggest that adult largemouth bass do not utilize dense hydrilla whereas anglers report that adult largemouth bass are utilizing dense hydrilla. Whether anglers can perceive differences in fish habitat use resulting from aquatic plant management, and whether UVC can quantify the benefits of management is need for invasive aquatic plant management.

Further research programs using UVC can bridge key knowledge gaps in fish-plant interactions with examples provided below. First, is to conduct size-based evaluations for fish habitat use across physiochemical gradients. This work can compare juvenile versus adult fish habitat needs using occurrence, fish counts, and sizing techniques. Second, is to use UVC to quantify behavioral differences across environmental conditions. This includes predator-prey interactions, intraspecific interactions, and breathing rates (e.g., number of pumped gills per minute). Combining these research objectives in the context of density-dependent habitat selection allow for interpretation of density-dependent impacts to despotic fish habitat use (e.g., large fish chase small fish out of preferred habitat), foraging, and growth dynamics.

Underwater video camera systems are cost-effective and equipped on many recreational anglers' boats along with global positioning system (GPS). This presents high potential to develop citizen-science programs for anglers to monitor their own fisheries. Anglers equipped with UVC systems can sample fish communities, analyze video themselves, and document/image the habitat conditions. Such a program could provide a robust dataset composed of fish, habitat, and spatial information useful in management. Successful citizen-science programs in place across the country include:

the University of Florida's Lakewatch and the University of Washington's C.O.A.S.S.T programs, which monitors Pacific coast shorebirds. The benefit of such a program is increased stakeholder participation and increased trust between management and stakeholders.

APPENDIX A
SAMPLEABLE AREA AND FISH DETECTON PROBABILITY WITH UNDERWATER VIDEO CAMERAS

The Importance in Understanding Detection Probability

Point counts are commonly used to estimate animal abundance and density (Royle and Nichols 2003). Point count samples vary with changes to both animal abundance and detection probability. However, detection probability is often heterogeneous across many factors such as: local animal abundance (or density), habitat covariates, and distance from observation (Royle et al. 2005; Gu and Swihart 2004). Thus, reliable estimates of abundance require a thorough understanding of habitat-detection probability relationships for any sampling gear (Gu and Swihart 2004).

Many studies employ video cameras to obtain point counts to estimate population abundances in both terrestrial and aquatic ecosystems (Karanth 1995; Cappo et al. 2007). However, Cappo et al. (2007) notes that fish detection probabilities collected from underwater video cameras (UVC) are often not known, which limits the utility of point count observations for estimating abundance. Hankin and Reeves (1988) used visual techniques to estimate freshwater fish abundance but had problems calibrating detection probability to a known true abundance. Underwater cameras require adequate visibility which is a function of available light, turbidity and structural density of habitat (Watson et al. 2010). The habitat below a hydrilla canopy can be open and low in turbidity, potentially allowing use of low-light cameras to assess fish communities.

Studies utilizing UVC often make several broad assumptions on the habitat-detection or habitat-area sampled relationships to make inferences on fish communities. Off-shore reef UVC surveys conducted by Watson et al. (2005) indicated that visibility

was high during the study period and, without any formal documentation, capped the upper boundary of the area sampled by camera at 10 m from the lens and calculated the area sampled as 150 m^2 and standardized UVC samples to compare with diver-transect samples. McCauley et al. (2012) quantified the area sampled via UVC in a concrete pool which is a much different aquatic system than the marine off-shore reef where they conducted fish-density UVC surveys. Cappo et al. (2004) makes four main working, untested approximations in order to make inference on reef-fish assemblages:

> [F]ish were not counted on more than one baited remote underwater video surveys (BRUVS) in a transect; the BRUVS and trawl transects were independent and did not influence each other; the total area sampled by each BRUVS was the same; and, the total area sampled by five BRUVS was similar to the area swept by the trawl. These untested approximations provided the statistic used to compare the BRUVS transect with the associated trawl transect N as the sum of *MaxN* pooled across the five BRUVS for each fish species.

Habitat characteristics in vegetated areas are heterogenous. This heterogeneity can influence both detection probability and area sampled measurements ultimately influencing fish density estimates. However, directly measuring area sampled in vegetated habitats has proven difficult and the dimensions of the UVC sampled area change unknowingly across sites. Our objectives were to provide preliminary analyses and information on the relationship between habitat characteristics and the1) area sampled and 2) fish detection probability for UVC technologies by using point counts on replica-fish in dense submersed aquatic vegetation. Such findings can provide useful parameters to estimate fish density and evaluate aquatic plant ecosystems.

Methods

Field Collections

To evaluate the area sampled and fish detection probability of the UVC we used three 0.405 ha experimental ponds (maximum depth 2.4 m, mean depth 1.7 m) located at a United States Geological Survey facility in Gainesville, Florida during fall 2011 and fall 2012. Conditions in the pond were favorable to UVC sampling, light levels were high (for UVC visuals) and water turbidity was clear. Replica-fish built from particle wood (100 mm length x 25 mm height) were painted black with attached 170 g weights to simulate typical littoral fish such as bluegill *Lepomis macrochirus*. Replica-fish were placed at known distances from the UVC at depths between 1.3-1.9 m beneath the hydrilla canopy layer to simulate natural fish inhabitance and were suspended from fixed points on surface-floating boards attached by clear monofilament fishing line. The surface-floating board (180 cm length x 30 cm wide) was divided into a grid with attachment points spaced ~15 cm apart in length, and ~4 cm apart in width (Figure A-1). Each video trial consisted of randomly assigned numbers of replica-fish (ranging from 0-6 fish), at randomly assigned attachment points from the camera (distances ranged 15-175 cm from the camera). Trials could have fish located at the same distance, but could not have fish occupying the same attachment point, thus no two fish occupied the exact same space.

The fish and camera system were deployed at spatially-random sites across all three ponds. The UVC was attached to a plastic pipe and placed in the middle, end of the board. We used the pole to position the camera viewing angle directly towards the trial's sampling area. We verified that the camera was positioned towards the area with a removable target that was placed at 15 cm away from the camera in the middle of the

board prior to each trial that we could observe from the video monitor. After positioning the camera, we recorded video trials for 20-30 seconds on the sampling area.

Video Analysis

Recordings of the UVC trials were analyzed with video playback software by an observer with no prior knowledge of the true number of replica-fish. The observer recorded the presence or absence of each unique replica-fish observed at each trial. When multiple replica-fish were deployed the observers used the size ratio of the replica-fish to infer the relative spatial location/distances to help identify which specific replica-fish was observed (e.g., larger size-ratios for replica-fish onscreen were closer than smaller replica-fish). We verified which specific replica-fish were detected by then comparing the observer's detection/location to the known distances of each replica-fish deployed at each site. The fish detected at each site *i* were used as the count metric C_i to derive detection probabilities p_i using the formula:

$$p_i = C_i / N_i \tag{1}$$

where N_i is equal to 1 if a fish was deployed at the trial.

We quantified habitat complexity in two manners: the relative stem count and the percent area coverage (PAC) for habitat complexity. We measured the relative stem count for each video count by counting the number of visible plant stems following a horizontal grid from a single-frame captured from the video. The observer selected a video frame that best represented the trial. We estimated the PAC by dividing the video frame into a grid composed of a total of 55 squares, 48 equal sized squares and 13 partial squares (Figure A-2). Within each square, an observer qualified the square as

capable of being sampled (<50% habitat/structural complexity) or not able to sampled (>50% habitat/structural complexity).

Estimating Area Sampled

On any given trial between 0-100% of all deployed objects may be detected. Trials with 0% and trials with 100% detection give no information for area sampled because the potential area sampled occurred at distances further than the furthest deployed object, or closer than the closest non-detected object. However, when there is incomplete detection, which we define as a trial where at least one object is detected but not all objects are detected, we can gain information on how habitat structure influenced the area sampled. We first made a few assumptions: (1) water turbidity and light conditions are threshold parameters wherein they are either sufficient, or insufficient to sample for fish, (2) if sufficient did not influence area sampled, and (3) that the area sampled occurs at some point between the distance of the furthest detected object, and the closest non-detected object. For assumption 3, we will estimate this distance sampled for that trial to occur at the mean between those two distances (Figure A-1). We can then model the area sampled at each trial as an isosceles triangle with an 85° viewing angle (from the technical specifications of the camera) with a height equal to the estimated distance for that trial, and a base calculated from the tangent of the viewing angle and the height for that trial.

Statistical Analyses

We utilized a binomial generalized linear models (GLM) to generate a predictive model for detection probability as a function of habitat covariates (distance to fish and habitat complexity; R Development Core Team 2011). We used logit transformations to

analyze the binomial distribution of count data in a GLM that will predict detection probability (p) using the formula:

$$p(\text{detection}) = \text{logit}^{-1}(\alpha) = \frac{\exp(\alpha)}{1+\exp(\alpha)} \quad (2)$$

where α takes the form of a linear model:

$$\text{logit}\,(p) = \beta_0 + \beta_1 * x_1 \quad (3)$$

with $\beta_0$as the intercept and $\beta_1$as the weight given to the covariate x_1. Based on the significance of the habitat variables we created several regression models to estimate detection probability and evaluated these models based on Akaike's Information Criterion (AIC; Akaike 1974). Models with ΔAIC value of < 8.0 had support and < 2.0 had substantial support; if models were equivalent (e.g., 2 or more models with < 2.0 ΔAIC) we selected the most parsimonious model among them (Burnham and Anderson 2004). We calculated 95% confidence intervals for the selected models for using +/- 1.96 times the standard error for the parameter values.

We evaluated the relationships between the area sampled and both measures of habitat complexity with linear regressions, with complexity (stem counts and PAC) as predictor variables (R Development Core Team 2011). We used linear regression to test the relationship between stem counts and PAC to evaluate if these two measures of habitat complexity are related.

Preliminary Results and Comments

Overall, the underwater cameras detected 44% of all replica-fish inside our maximum sampled distance of 1.75 m. At distances 1 meter and closer, we detected 69% of all replicates, and past 1 meter only 12% (Table A-1). We identified that detection probability was negatively influenced by habitat covariates. Both distance to

the replica-fish (Figure A-3) and habitat complexity (Figure A-4) significantly, negatively influenced the detection probability of replica-fish (both P<<0.05). Distance and habitat complexity (stem counts) were the only habitat covariates to significantly influence detection probability. We tested a few different models (Table A-2) and ranked them by their ΔAIC score. Two distance and stem density models tested had ΔAIC levels <2.0 indicating they were nearly equivalent; however we selected the most parsimonious model which was 'Distance + Stem Density' (Table A-2). The results from the GLM analysis generated the Distance + Stem Density model's coefficients β_i. Simplified, the linear α logit transformation takes the form:

$$\text{logit}\,(\alpha) = 5.158 - 0.0308 * Stem\ Density\ - 4.7792 * Distance \qquad (4)$$

Estimated detection probabilities and associated uncertainty across the logit-transformed habitat covariates showed reasonable model precision (Figure A-5).

The mean area sampled with UVC in this study was 0.62 m^2 (95% CI 0.13-1.52 m^2). Area sampled was significantly negatively influenced by habitat complexity (percent area coverage [PAC]) in that location (*P*= 0.007, R^2=0.119; Figure A-6). However, the slope of this relationship is shallow and much of the variance in the data remains unexplained by this relationship. Future work should further refine the distance/area sampled and habitat complexity relationships, and include alternative habitat covariates significant to the study site (e.g., habitat bottom type, turbidity).

There was significant positive correlation between stem counts and PAC suggesting that either measure is suitable for quantifying habitat complexity (*P* <<0.05, R^2=0.282; Figure A-7). Habitat complexity, in the form of stem counts, significantly influenced detection probability and PAC significantly influenced area sampled. Both of

these measures are taken easily from UVC. However, PAC may be the superior measure of complexity in estimating both detection and area sampled as it is easier to quantify and seems less variable across different video analyzers by using a standardized sampling grid. However, I recommend the consideration of both habitat complexity metrics in future analyses of habitat-detection probability relationships until one metric shows superior for data needs.

We provided an innovative method for estimating heterogeneous detection probabilities for fish in dense submersed vegetation. Using known replica fish densities at each point count addressed whether zero-counts results from animal absence or non-detection (Gu and Swihart 2004). Alldredge et al. (2008) noted that replica-animals can provide a useful tool for estimating detection probability within varying habitats. Replica-animals' true abundances are known, thus sampling avoids problems with calibrating detection probabilities on varying abundances in wild animals. I recommend researchers further refine the area sampled-habitat complexity model before full application of UVC methods to estimate fish density. However, these results showed high potential to use UVC to estimate fish detection probability in sampled areas, thus estimating fish density from point count surveys.

Future work can use these models to link heterogeneity in habitat complexity to heterogeneous estimates of detection probability and area sampled. Specifically, area sampled can be estimated from habitat complexity using the regression model. Alternatively, we can set an assumption for area sampled at some certain value, such as the mean area sampled (Cappo et al. 2004). For example, we could use the mean sampled area in this study (0.62 m^2) to inform a Bayesian prior (with or without

uncertainty) on future estimates of area sampled. We could also estimate detection probability across categories of habitat complexity to create estimates of detection in low, intermediate, and dense vegetation.

Distance to individual fish remains un-quantified via UVC in dense aquatic plants. Thus, application of UVC methods needs to estimate the average detection for all fish in the sampled area in order to estimate true fish density. UVC can sample several individuals combining closer individuals with high detection and individuals at further distances with lower detection. This presents a source of potential bias in detection estimates if fish are not at distances from the UVC with even distributions. Assuming this even distribution, we can use the mean of the probability density function across the distance sampled to estimate the mean detection probability (equation 4) for all fish sampled in that area. The distance sampled by UVC can be estimated from back-calculating from the estimated area sampled. This implies that, though individuals have their own detection probability, all sampled fish within a single UVC point count experience one mean detection probability. This approach accumulates variance in parameter estimation, a major drawback of linking several models in this manner.

Tables

Table A-1. Replica-fish were deployed at known abundance (trials) and distances (m) to obtain detection probabilities (p) across a range of habitat parameters: light (PAR), turbidity and stem density. Note: standard devations in (parentheses).

Pond	Fish Deployed	Fish Detected	*P*	Stem Density	Distance to Fish
All Ponds	272	120	0.44	43.5 (17.45)	0.90 (0.46)
Pond 1	84	31	0.37	59.5 (14.6)	0.88 (0.46)
Pond 2	102	51	0.50	33.7 (9.9)	0.87 (0.46)
Pond 3	86	38	0.44	39.5 (15.9)	0.94 (0.44)

Table A-2. Three different logistic regression models were tested with AIC model selection. The models' log-likelihoods were calculated and the most parsimonious model selected from the ΔAIC score.

Model	Parameters	Log-Likelihood	AIC	ΔAIC
Distance+Stem Density	3	-109.2463	-224.493	0
Distance+Stem Density+ Stem Density*Distance	4	-108.689	-225.378	0.8854
Distance	2	-114.3741	-232.748	8.2556

Figures

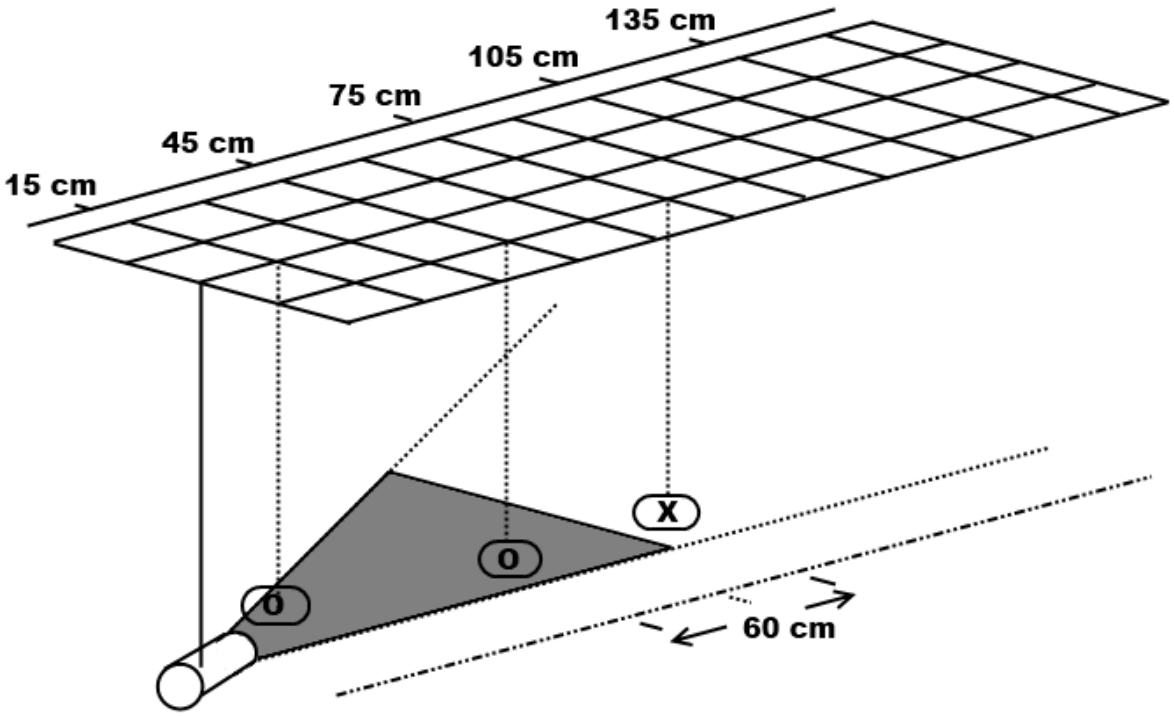

Figure A-1. Trial setup to estimate area sampled using underwater video cameras in dense submersed aquatic vegetation. Replica-fish were placed at known distances and observed with the camera. In this trial “O” designate replica-fish that were detected, and “X” represents replica-fish that were not detected. The mean distance, occurring in this trial at 60 cm, between the furthest detected replica-fish and the closest undetected replica-fish is used to estimate the area sampled (grey area).

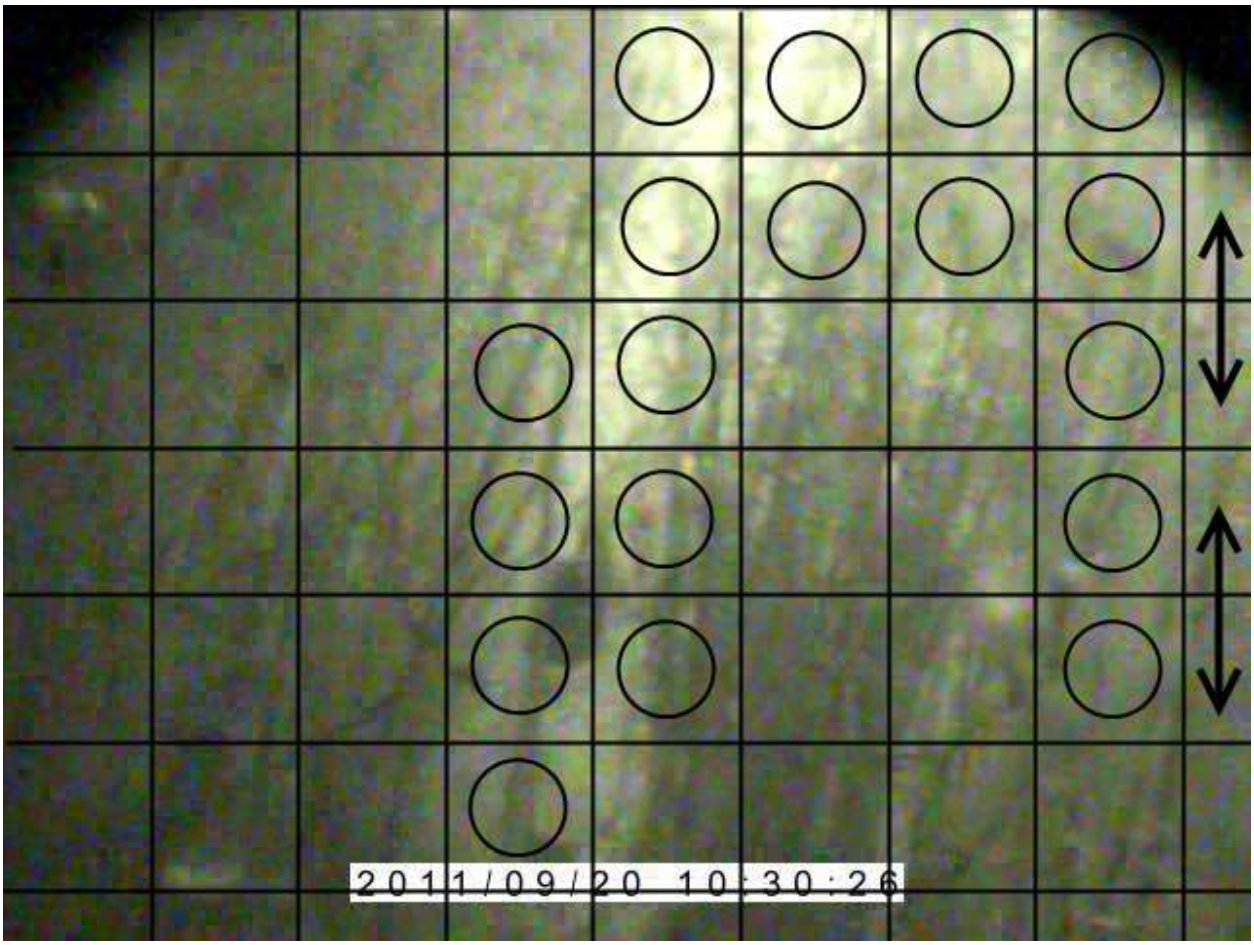

Figure A-2. Example of the 55-square grid (including partial squares) utilized to estimate habitat complexity. Open circles and the arrows along the side dictate areas of the screen-captured image that are qualified as areas that can be sampled (defined as squares that have less ~50% structure coverage). In this example, 49 individual stems were counted and the habitat complexity was 36 out of 55 squares covered for percent area coverage of 65%.

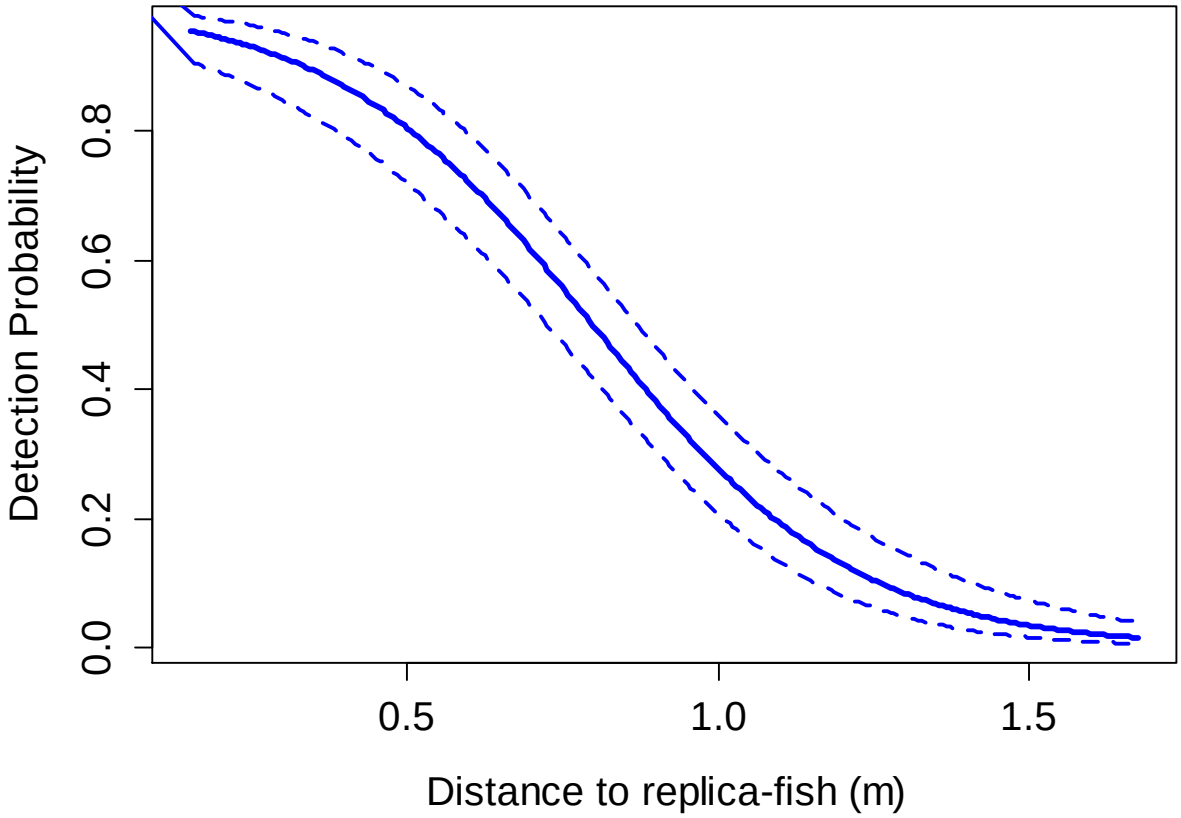

Figure A-3. AIC-selected model (distance + stem count) estimates of replica-fish detection probability across a gradient of distances. To portray this graph, stem count was held constant at the mean value in the sampling season (43.49).

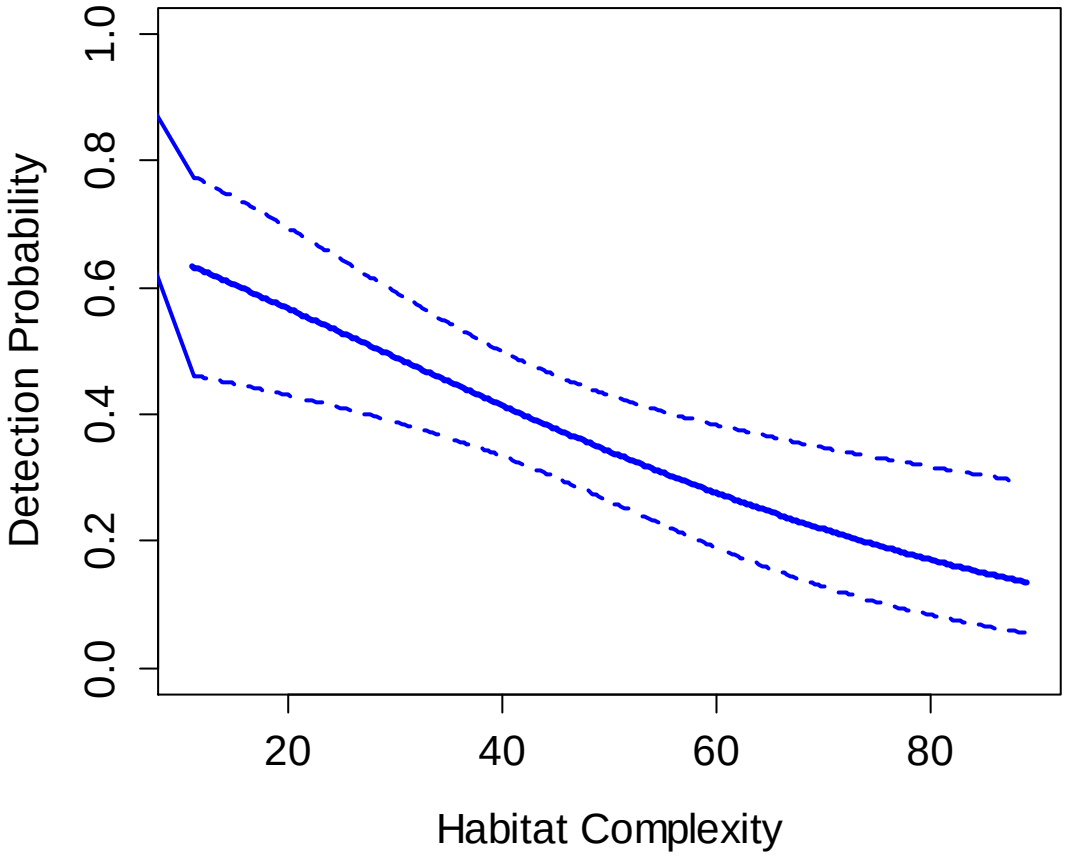

Figure A-4. AIC-selected model (distance + stem count) estimates of detection probability across a gradient of habitat complexity (stem counts). To portray this graph, distance was held constant at the mean value in the sampling season (0.89 m).

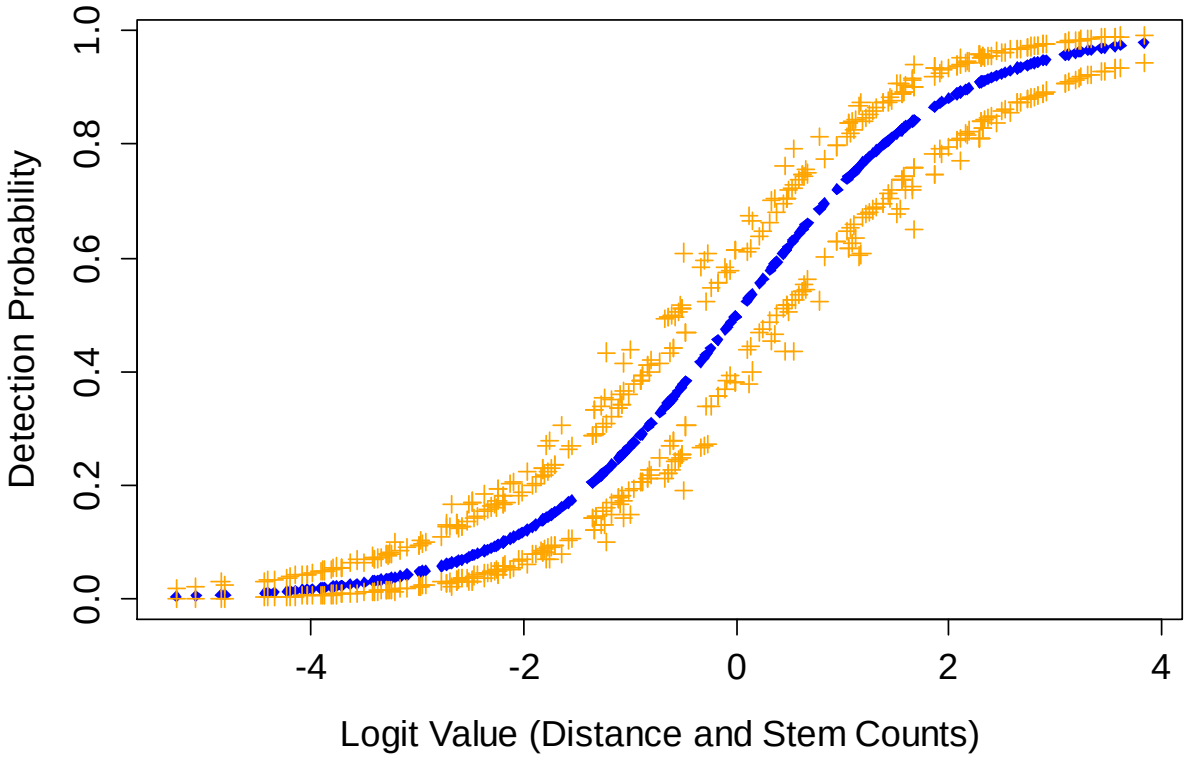

Figure A-5. AIC-selected model estimates of detection probability (blue dots) with 95% confidence intervals (orange plus signs) across the logit-transformation of two field-sampled habitat covariates (distance to fish sampled [m] and stem counts).

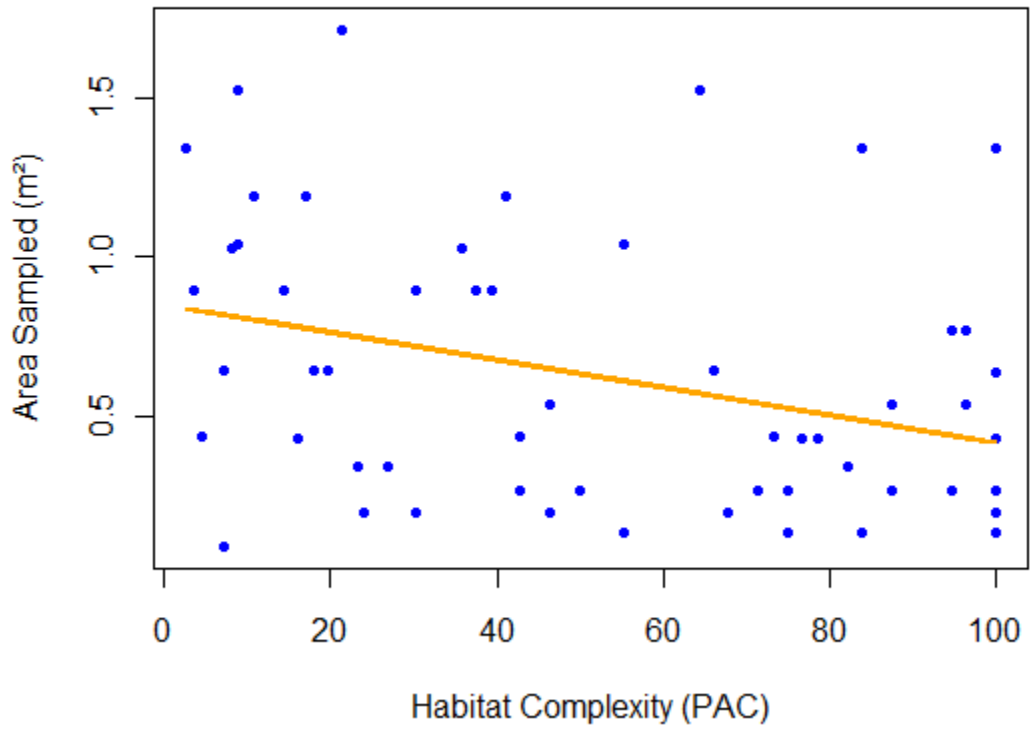

Figure A-6. Observed (dots) and predicted (line) relationships between area sampled and habitat complexity (percent area covered; PAC) from underwater video camera point counts (R^2=0.119).

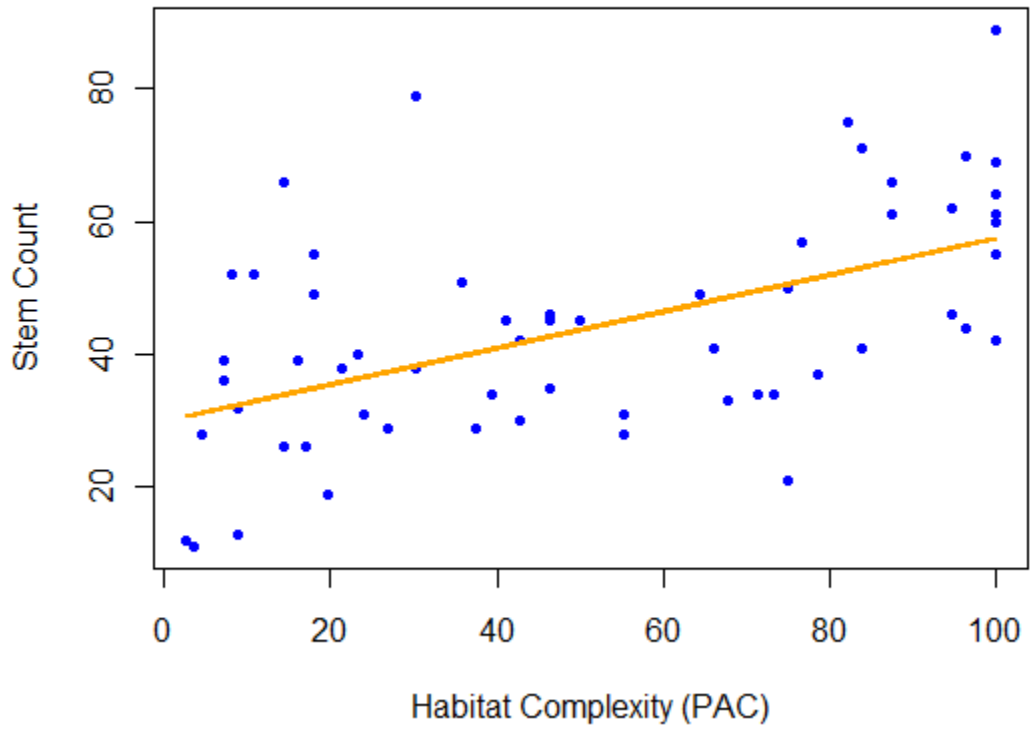

Figure A-7. Observed (dots) and predicted (line) relationships between stem counts and percent area covered (PAC), two measures of habitat complexity sampled with underwater video cameras (R^2=0.282).

APPENDIX B
SIZING FISH FROM UNDERWATER VIDEO SAMPLES

Estimating Fish Size

Single-lens cameras have difficulty in providing size-based inferences of fish length due to reduced depth perception and distance-to-fish information. Smaller objects appear large when close to the camera. However, fisheries research is frequently interested in size information to inform population models, growth patterns, and size-based ecological processes. Much technology is available to help with distance-to-fish and fish-length sampling in un-obscured systems (e.g., marine reefs, pelagic waters, oligotrophic lakes) including stereo-video and paired lasers (Cappo et al. 2007). However, in highly vegetated habitats, sampling with extra-equipment is unrealistic due to vegetation snags and habitat disturbances.

I found that cameras with extra equipment (e.g., paired-lights) generally snagged and would not descend through hydrilla *Hydrilla verticillata*. This indicated that underwater video camera (UVC) samples in Florida lakes may not easily obtain fish length estimates. However, video can provide unit-less information in the form of morphometric ratios that could be used to estimate fish total lengths. Some fish species, such as bluegill *Lepomis macrochirus*, grow in height (i.e., dorsoventral axis) as well as in length (i.e., anterior-posterior axis) with age in order to become gape-limiting to predation. Thus, old/large *Lepomis* spp. were hypothesized to be proportionally taller onscreen than young/small *Lepomis* spp., and length- height ratios of larger *Lepomis* spp. would be lower compared with smaller *Lepomis* spp. when measured after field collections.

This preliminary work used samples of fish length (mm) and height (mm) to determine whether length-height ratios, inherently unit-less, could be used to estimate fish total lengths (TL). Thus, body ratios taken from video analyses could estimate total lengths by back-calculating from the regression model.

Methods

The sampled population consisted of adult *Lepomis* spp. (TL >149 mm; bluegill and redear sunfish *Lepomis microlophus*) electrofished from Lake Wauberg, Florida and young-of-year (YOY) *Lepomis* spp. Adults were stocked into three 0.405 ha experimental ponds in June 2011 and YOY were naturally spawned from the stocked adults. The ponds were drained and fish were collected with nets and quadrat sampling in October 2011. All adult *Lepomis* spp. were sampled for total length and body height (mm) and a subsample of YOY total lengths and body heights were measured. Total lengths were then regressed onto total length/body height ratios using linear regression in program R (R Development Core Team 2011).

Preliminary Results and Discussion

The length-height ratio for 154 sampled fish was significantly and negatively related to total length ($P << 0.05$, R^2 0.737; Figure B-1). *Lepomis* spp. with larger total lengths were taller in body height compared with smaller total length *Lepomis* spp. Thus, the ratio of length-height was smaller for larger *Lepomis* spp. These results indicate that length-height ratios measured from UVC analyses (i.e., measure the pixelated lengths of lines across the various body axes) can estimate fish total lengths for some species (Figure B-2). The regression model provides estimates of total length:

$$TL = 492.7 - (131.2 * (TL\ axis/Height\ axis))) \quad (1)$$

where TL is total length (mm) and the TL and height axes are the length-height ratios as measured with pixel lengths in an image software program. For example, a bluegill sampled with UVC on Lake Tohopekaliga, Florida had an anterior-posterior axis length of 393 pixels and a dorsoventral axis height of 206 pixels (L-H ratio=1.90) for an estimated total length of 242 mm (Figure B-2).

These results indicate that single-lens UVC can estimate some fish species' total lengths. This provides fisheries managers a tool to sample lengths for littoral fish without physically capturing fish specimens. However, due to constraints in the size-frequency in the population sampled, which has two modes (one for adult fish, one for YOY fish), not many fish of intermediate length were sampled. The significance of this model may not extend across all fish lengths and some length-classes may have high variability in total length estimates. A subsequent step to evaluate the efficacy of estimating fish lengths with UVC is to compare size-frequency distributions of *Lepomis* spp. between UVC and an alternative sampling gear.

Figures

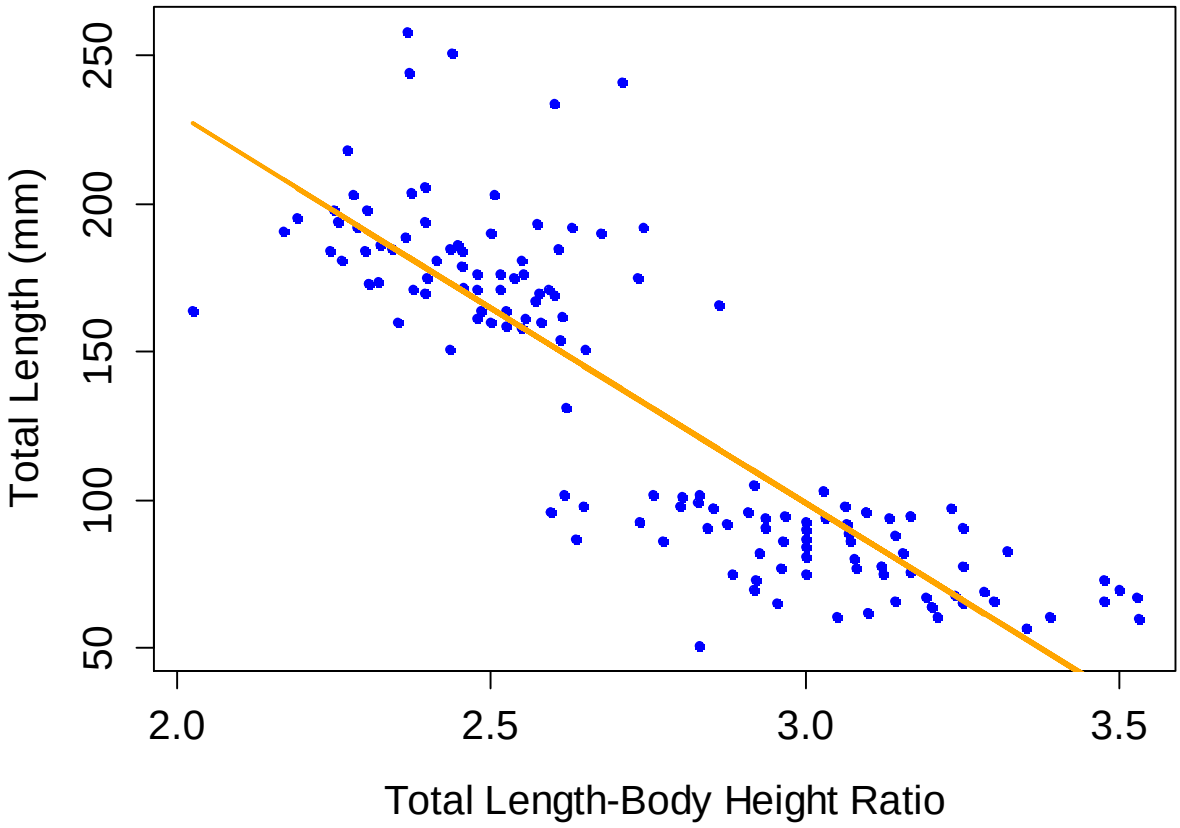

Figure B-1. Observed (dots) and predicted (line) relationship between length-height ratios and total lengths (mm) for adult and young-of-year *Lepomis* spp. (n=154).

Figure B-2. The length-height ratio (393/206 pixels) for a bluegill inhabiting hydrilla bed captured on video in Lake Tohopekaliga, Florida with estimated total length of 242 mm.

APPENDIX C
TAXA SAMPLED WITH UVC IN DENSE AQUATIC PLANTS

Table C-1. List of taxa sampled with underwater video cameras in dense hydrilla, whether adults (A), juveniles (J), or unknown (U) were sampled, and the panels in Figures C-1 and C-2 for example images.

Taxa	Species	Adult/Juvenile	Image Panel
Lepomis spp.	Bluegill *L. macrochirus*	A, J	E, F
	Redear sunfish *L. microlophus*	-	-
Largemouth bass	*Micropterus salmoides*	A, J	D, C
Centrachidae spp.	Unknown	U	J
Bluefin killifish	*Lucania goodei*	U	H
Chain pickerel	*Esox niger*	A	A
Lake chubsucker	*Erimyzon sucetta*	A	B
Prey fish	Small fish, Unknown	U	L
	Young-of-year, Unknown	J	K
American alligator	*Alligator mississippiensis*	U	G
Florida redbelly turtle	*Pseudemys nelsoni*	U	I

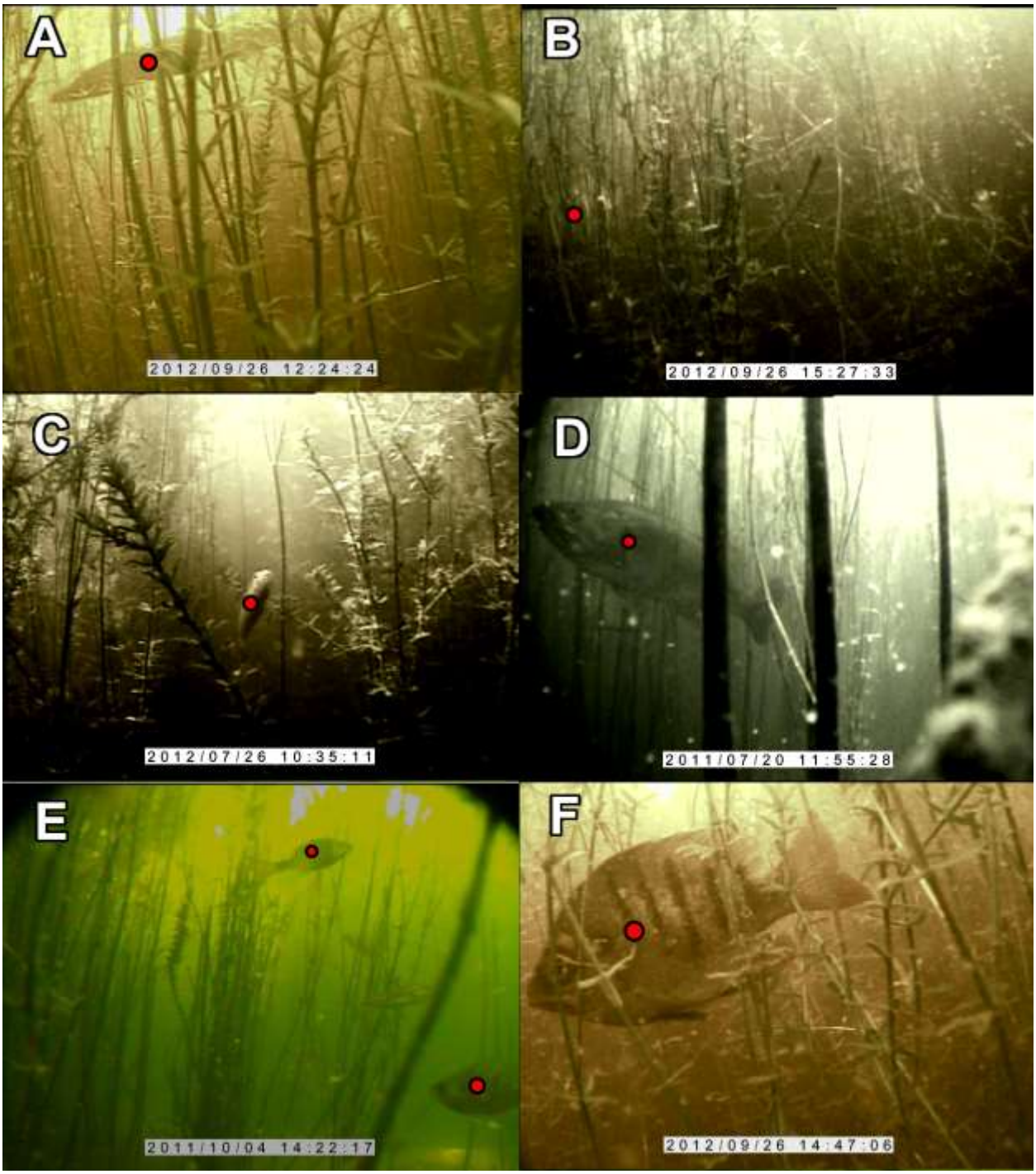

Figure C-1. Example images of taxa sampled with underwater video cameras in dense hydrilla habitats. Red dots indicate the location of individuals onscreen per taxanomic group. Table C-1 lists the taxa sampled including species name when identified.

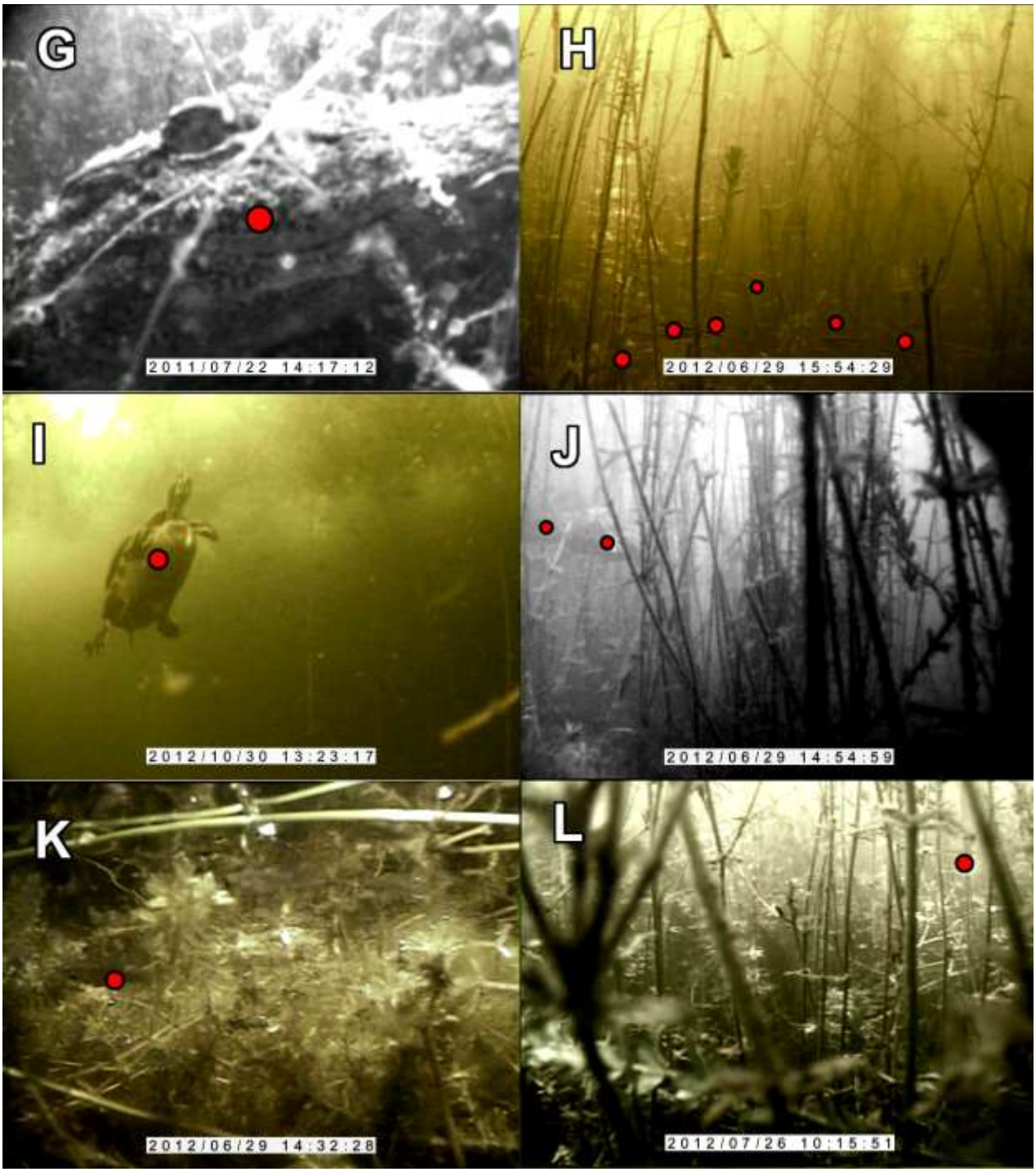

Figure C-2. Additional example images of taxa sampled with underwater video cameras in dense hydrilla habitats. Red dots indicate the location of individuals onscreen per taxanomic group. Table C-1 lists the taxa sampled including species name when identified.

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BIOGRAPHICAL SKETCH

Kyle Wilson received his Bachelor of Science cum laude in biology from San Diego State University in 2009 and is receiving his Master of Science in fisheries and aquatic sciences from the University of Florida in 2013. He worked on several coastal, marine, and climate ecology projects from 2007-09 while at San Diego State University. Kyle was hired by Utah State University in 2010 to help assess native fish responses to non-native fauna and degraded habitat qualities. He moved to work for the University of Florida in fall of 2010 to begin evaluating fish habitat quality and habitat use in hydrilla, a prolific invasive aquatic plant in the southeastern United States.