

**Developing a rapid fluorescence imaging tool for *in situ* estimation of
coral reef health in diverse environments**

Hagai Nativ

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE MASTER'S DEGREE

University of Haifa

Faculty of Natural Sciences

Leon H. Charney School of Marine Science

Department of Marine Biology

October 2021

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By: Hagai Nativ
Supervised by: Prof. Tali Mass

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Approved by: _____ Date: _____
(Supervisor)

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(Chairperson of Master's studies committee)

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Developing a rapid fluorescence imaging tool for *in situ* estimation of coral reef health in diverse environments

Hagai Nativ

Abstract

Coral recruitment represents a key element for coral reef persistence and resilience in the face of environmental disturbances. Studying coral recruitment patterns is fundamental for assessing the reef health and to implement appropriate management strategies in an era of climate change. *In situ* fluorescence census techniques have increasingly been used as they make highly cryptic coral recruits much easier and faster to detect than under standard white light census. The FluorIS system has been developed to acquire high resolution, wide field-of-view (FOV) *in situ* images of coral recruit's fluorescence, and it has been shown to be successful in shallow reef environments. However, up to now, its applicability to mesophotic coral ecosystems has been restrained by the time limits and technical constraints imposed by non-recreational deep diving. In this study we optimized the FluorIS system by utilizing a single infrared-converted camera instead of the bulkier regular dual-camera system, significantly reducing the system complexity and the time consumed for completing the underwater survey. With this faster, more flexible and easier to handle system we surveyed coral recruits under 2 cm in diameter using a 25x25 cm quadrant across shallow and mesophotic reefs of the northern Red Sea and Bermuda. Our single-camera system represents a valuable, non-invasive and rapid underwater tool to investigate *in situ* coral reproductive ecology over a broader range of depths and habitats than previous fluorescence methods that are mainly limited to shallow reefs. Our improved technique significantly increases the speed and accuracy of coral recruits count, ultimately allowing us to obtain a deeper

understanding of coral recruitment patterns, which is critical for developing suitable reef conservation and management strategies.

Abbreviations

FOV- Field-Of-View

GFP- Green Fluorescence Protein

FPS- Fluorescent Proteins

MCEs- Mesophotic Coral Ecosystems

ICF- Infrared Cut Filter

UV- Ultra Violet

FS- Full Spectrum

VF- Viewfinder

IUI- Interuniversity Institute

FPS- Frames Per Second

WB- White Balance

BIOS- Bermuda Institute of Ocean Science

DRRH- Deep Reef Refugia Hypothesis

Introduction

The Ocean is critical to humanity's well-being. It is a source for food, energy, resources, recreation and also acts as a regulator for global climate (Halpern et al. 2008). Global and local stressors are affecting the biodiversity and abundance of marine flora and fauna throughout the world. With over half of the world's population living in proximity to the coast, anthropogenic activities including pollution, overfishing, habitat destruction, introduced pests and global warming are eroding the ocean's ability to sustain these benefits in the future (Halpern et al. 2012). The coral reef ecosystems which are an important habitat for numerous species of marine life are suffering massive global declines (De'ath et al. 2012) and even though marine extinctions are not as prevalent as terrestrial extinctions, human activity has a direct effect on marine defaunation that may result in mass extinction if not managed properly (McCauley et al. 2015). In order to allow conservation managers and policymakers to direct their efforts more accurately, relevant data from regional and national sources are gathered into open databases that will facilitate macroecological research. Scientists are urgently trying to gather accurate, comparable data that will enable detection of global patterns, quantitative reference for changes over time and other covariates that emerge when broad-scale data is available (Edgar et al. 2016).

Unfortunately, recent year data indicates that climate related stressors particularly pronounced on shallow water reef systems, where corals and their dinoflagellate symbionts live close to their physiological thermal maximum and, as a result, warming of 1°C or more can reduce their fitness and cause tissue loss or whole-colony mortality (Ove Hoegh-Guldberg 1999; Jones et al. 2008; Jones 2008). However, coral reefs extend far beyond these well-studied shallow reefs zones into mesophotic coral ecosystems (MCEs) ranging from 30m to approximately 150m depth (Hinderstein et al. 2010). In fact, the conservation status of 704 species of scleractinian corals

worldwide revealed that only 40% are restricted to depths <20m, whereas the remaining 60% can survive at greater depths (Hinderstein et al. 2010).

MCEs are deep reef communities where low light is a dominant abiotic feature, and they are composed of highly diverse low light-adapted zooxanthellate scleractinian corals, macroalgae and sponge communities (Hinderstein et al. 2010). The physical environment of these deep reef communities includes strong gradients in downwelling solar irradiance and protection from high wave action compared to their shallow counterparts. Under normal circumstances there are modest changes in seawater temperature from the surface to deeper waters (up to 150m) on coral reefs and mesophotic reefs are not regularly affected by high temperature stress associated with shallow coral reefs (Lesser and Farrell 2004; Hoegh-Guldberg et al. 2007). Moreover, nutrient delivery from deeper depths via internal waves (Leichter et al. 1998; Leichter et al. 2007) can result in significant increases in productivity on MCEs (Leichter and Genovese 2006; Leichter et al. 2007; Leichter et al. 2008). Thus, in light of the large-scale degradation on shallow reef systems, MCEs have gained considerable interest as they appear to be protected from many of the local and global impacts affecting shallow-water coral reefs, such as storms, sedimentation, habitat fragmentation, etc., as well as impacts of global climate change, such as increased sea surface temperature and associated bleaching (Huston 1985; Hoegh-Guldberg et al. 2007; Lesser et al. 2009; Lesser 2010; Bongaerts et al. 2010; Kahng et al. 2010; Loya et al. 2016). While MCEs are not immune to the impacts of disturbance (Bongaerts et al. 2013; Appeldoorn et al. 2016; Smith et al. 2016), they have not experienced the same degree of decline as their shallow-water counterparts (Bak, et al. 2005). For example, work in the Caribbean suggests long-term community stability, as well as an associated transition from reliance on autotrophy to heterotrophy by symbiotic corals as particulate food increases (Bak et al. 2005; Lesser 2010; Lesser and Slattery 2013). It is hypothesized, therefore,

that coral populations at depths greater than 30m may serve as a refuge for coral reef species and provide a source for recruits, genetic diversity, and repopulation of shallow regions via larval exchange (termed the Deep Reef Refugia Hypothesis “DRRH”; (Bongaerts et al. 2010; Lesser et al. 2018).

If MCEs are in fact an important life boat for coral survival, it is critical to understand the various processes regulating community composition and ecosystem function. Resilience of ecosystems relies in part on the capacity of organisms to adapt and/or acclimatize to different conditions. Adaptation and acclimatization can occur via changes in morphology and/or physiology. For several scleractinian coral species, changes in skeletal morphology have been documented across depths (Bruno and Edmunds 1997; Goodbody-Gringley et al. 2015; Goodbody-Gringley and Waletich 2018). Likewise, changes in physiology such as nutrient acquisition, calcification and photosynthesis are reported to differ over increasing depth (Mass et al. 2007; Einbinder et al. 2009).

Resilience of ecosystems under rapid environmental change relies, in part, on successful recruitment of juvenile corals. Formation of new juvenile colonies indicates good conditions for development and growth of coral reefs. Thus, efforts to preserve these environments require an understanding of this important demographic process and have long been identified as a research priority (Glassom et al. 2004; Martinez and Abelson 2013). Coral recruits are very small and often cryptic at settlement which makes them difficult to detect with normal census techniques (Baird et al. 2006). To date, the main methods for determining coral recruitment are artificial settlement plates, small-scale macro photography or visual searches in the field (Edmunds et al. 1998; Martinez and Abelson 2013). These methods can be labor-intensive or time-consuming, as they require microscopic examination of the settlement surface or enough time for the coral to

grow large enough to be visible to the naked eye (Piniak et al. 2005). Corals and their symbiotic zooxanthellae both contain fluorescence pigments, including chlorophyll and Fluorescence Protein (FP) that emits under UV or visible light, with emission maxima at 420-620nm (Singh et al. 2004). Using fluorescence techniques to detect coral recruitment is a valuable tool for assessment of their success rates. It can potentially be a simple, sensitive and non-invasive tool that can increase the speed and accuracy of juvenile coral counts.

For this reason, a recently developed fluorescence imaging method (FlourIS) that enables *in situ*, high resolution, wide FOV daytime fluorescence imaging of corals, draws our attention (Treibitz et al. 2015). Modification of this system was further used for *in situ* images of coral recruits fluorescence during daytime (Zweifler et al. 2017), however, its applicability to Mesophotic ecosystems is highly challenging, due to the time limits and technical constraints imposed by non-recreational deep diving.

Research objectives

1- To optimize the FluorIS system (Treibitz et al., 2015) for daylight survey in broad environmental conditions and to significantly reduce the system complexity and the time consumed for completing the underwater survey, while also broadening the types of topographic shapes and angles that can be surveyed.

2- To successfully survey in different locations and down to Mesophotic depth with enough data to make a statistical analysis in a practical number of dives.

Working hypothesis

The greater the ability of coral-planulae to adapt to conditions experienced at the time of metamorphosis, the greater the chance of recruits to survive across a wide depth distribution. My hypothesis is that the rates of coral recruitment will differ across the depth gradient.

Reference:

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Author contributions:

H.N., G.G.G and T.M. designed the research. H.N., G.G.G, S.M, S.E., A.C. and T.M. performed the underwater surveys. F.S. and H.N. carried out the image analysis and recruit counts. H.N., F.S., G.G.G and T.M. wrote the manuscript. All authors contributed to improving, revision and approval of the manuscript.



In situ Estimation of Coral Recruitment Patterns From Shallow to Mesophotic Reefs Using an Optimized Fluorescence Imaging System

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Coral recruitment represents a key element for coral reef persistence and resilience in the face of environmental disturbances. Studying coral recruitment patterns is fundamental for assessing reef health and implementing appropriate management strategies in an era of climate change. The FluorIS system has been developed to acquire high resolution, wide field-of-view (FOV) *in situ* images of coral recruits fluorescence and has proven successful in shallow reef environments. However, up to now, its applicability to mesophotic coral ecosystems remains unknown due to the complexity of the system and the limited time available when working at mesophotic depth. In this study we optimized the FluorIS system by utilizing a single infrared-converted camera instead of the bulkier regular dual-camera system, substantially reducing the system complexity and significantly decreasing the acquisition time to an average of 10 s for a set of 3 images. Moreover, the speed-FluorIS system is much more economical, decreasing the cost of the full set-up by roughly 40% compared to the original dual-camera system. We tested the utility of the speed-FluorIS by surveying coral recruits across shallow and mesophotic reefs of the Red Sea (Gulf of Eilat) and Bermuda, two of the most northerly reefs in the world with markedly different substrate and topography, and demonstrate that the modified system enables fast imaging of fluorescence to study coral recruitment patterns over a broader range of depths and reef topographies than previous fluorescence methods. Our single-camera system represents a valuable, non-invasive and rapid underwater tool which will help standardize surveys and long-term monitoring of coral recruits, contributing to our understanding of these vital and delicate early life stages of corals.

Keywords: coral recruitment, ecological monitoring, fluorescence, underwater imaging, underwater survey tool

INTRODUCTION

Over the last three decades, coral reef ecosystems have been suffering massive declines due to both local (e.g., pollution, nutrient enrichment, overfishing, and sedimentation) and global stressors (global warming, ocean acidification, and sea level rise) (Dustan and Halas, 1987; Hoegh-Guldberg and Bruno, 2010; D'Angelo and Wiedenmann, 2014). The resilience of these ecosystems under the pressure of rapid environmental change heavily relies on successful recruitment of juvenile corals. The presence of new coral recruits is a strong indicator of the health of the reef (Glassom et al., 2004; Baird et al., 2006). Thus, knowledge and monitoring of recruitment processes assist the implementation of conservation and management actions to preserve coral reef ecosystems (Glassom et al., 2004; Martinez and Abelson, 2013).

Coral recruitment patterns are commonly quantified by examination of artificial settlement tiles that are deployed on the reef and subsequently inspected in the lab using microscopes under UV light. This method, however, does not allow continuous monitoring of recruitment dynamics, as the tiles need to be taken out for inspection (Mundy, 2000; Soong et al., 2003; Field et al., 2007). As an alternative, *in situ* visual surveys are also conducted (Miller et al., 2000; Soong et al., 2003; Martinez and Abelson, 2013), but they can be very time-consuming. Coral recruits in fact are very small and often cryptic at settlement, which makes them very difficult to detect via normal census techniques (Baird et al., 2006). Fluorescence census techniques instead have increasingly been used as they make coral recruits much easier and faster to detect than under standard white light census (Piniak et al., 2005). Fluorescence techniques depend on the high abundance of fluorescent pigments in many coral species (Papina et al., 2002). Most scleractinian corals contain two primary groups of fluorophores in their tissues. The first major group is comprised of photosynthetic pigments, mainly chlorophyll-*a*, from the coral endosymbiotic dinoflagellate algae (Symbiodiniaceae family) (Warner et al., 2010; Lajeunesse et al., 2018), and the second group includes GFP-like fluorescent proteins (FPs) found in the coral host tissue (Alieva et al., 2008). Recently, the FluorIS system was developed to acquire high resolution, wide field-of-view (FOV) *in situ* images of coral recruits fluorescence during daytime (Zweifler et al., 2017), enabling simultaneous imaging of GFP and chlorophyll-*a* with a single excitation source. Such a system has been shown to be successful in shallow reef environments (Zweifler et al., 2017), however, its application to mesophotic ecosystems is highly challenging, due to the time limits and technical constraints imposed by non-recreational deep diving.

Ranging from 30 m to approximately 150 m depth, mesophotic coral ecosystems (MCEs) comprise a diverse abundance of habitat-building taxa including corals, crustose coralline algae, macroalgae, and sponges (Kahng et al., 2016). In view of the large-scale degradation affecting shallow reef systems, MCEs have gained substantial interest as they appear to be protected from many of the local and global impacts affecting shallow-water coral reefs (Hoegh-Guldberg et al., 2007;

Lesser et al., 2009; Bongaerts et al., 2010; Kahng et al., 2010). Even though MCEs are not immune to the impacts of disturbance (Bongaerts et al., 2013; Appeldoorn et al., 2016; Smith et al., 2016), they have not experienced the same degree of decline as their shallow-water counterparts (Bak et al., 2005). Therefore, it is hypothesized that coral populations at depths greater than 30 m may serve as a refuge for coral reef species and provide a source for recruits, genetic diversity, and repopulation of shallow regions via larval exchange (termed the Deep Reef Refuges Hypothesis “DRRH”; Bongaerts et al., 2010; Lesser et al., 2018). Despite their inferred importance, data on recruitment patterns of MCEs are sparse, mainly due to the difficulties in accessing these habitats that lie beyond recreational SCUBA diving limits (Pyle, 2019), which poses increased logistical challenges. MCEs have been studied in only few areas around the world, making it difficult to obtain generalizable knowledge of the processes regulating their structure and dynamics, such as recruitment (Turner et al., 2017). If MCEs are in fact an important life-boat for coral survival, it is critical to develop and adopt broad, ecosystem-wide approaches that allow to have better understanding of recruitment dynamics in these deeper reefs worldwide. Some technological advances have enabled access to study MCEs, such as technical SCUBA diving, but pose significant challenges in terms of the amount of time that can be spent underwater.

To overcome these challenges and facilitate the assessment of coral recruitment dynamics at mesophotic depths, we optimized the FluorIS system by utilizing a single infrared-converted camera instead of the bulkier regular dual-camera system (Treibitz et al., 2015). We thus significantly reduced the system complexity and the time consumed for completing the underwater survey, while also broadening the types of terrain shapes and slopes that can be surveyed. With this faster, more flexible and easier to handle system we surveyed coral recruits across shallow and mesophotic reefs of the Red Sea (Gulf of Eilat) and Bermuda, two of the northern-most coral reefs in the world. These two locations are characterized by different substrate topographies and reef structures (Kahng et al., 2010; Murdoch and Murdoch, 2016). In Bermuda, cover of reef-building scleractinian corals declines with depth, although both shallow and mesophotic reefs maintain similar topographic complexity (Murdoch and Murdoch, 2016). In Eilat, branching structures in shallow waters become encrusting or plate-like at mesophotic depths, and this flattening creates a relatively low complexity and low rugosity of reef structure on MCEs (Kahng et al., 2010). The existence of such variations in reef structure across depths, and in general between reefs located in different geographical areas, represents a potential challenge for the development of an underwater survey system capable of efficiently detecting coral recruits with a standardized methodology.

With our improved system we provide a valuable, non-invasive and rapid underwater tool to investigate coral reproductive ecology *in situ* across wide depth ranges and different terrain shapes, significantly increasing the speed of coral recruit counts underwater. This new system ultimately allows us to obtain a deeper understanding of coral recruitment patterns across depths, which is of utmost importance for developing suitable reef conservation and management strategies

aiming at protecting these ecosystems in the face of increasing local and global stressors.

MATERIALS AND METHODS

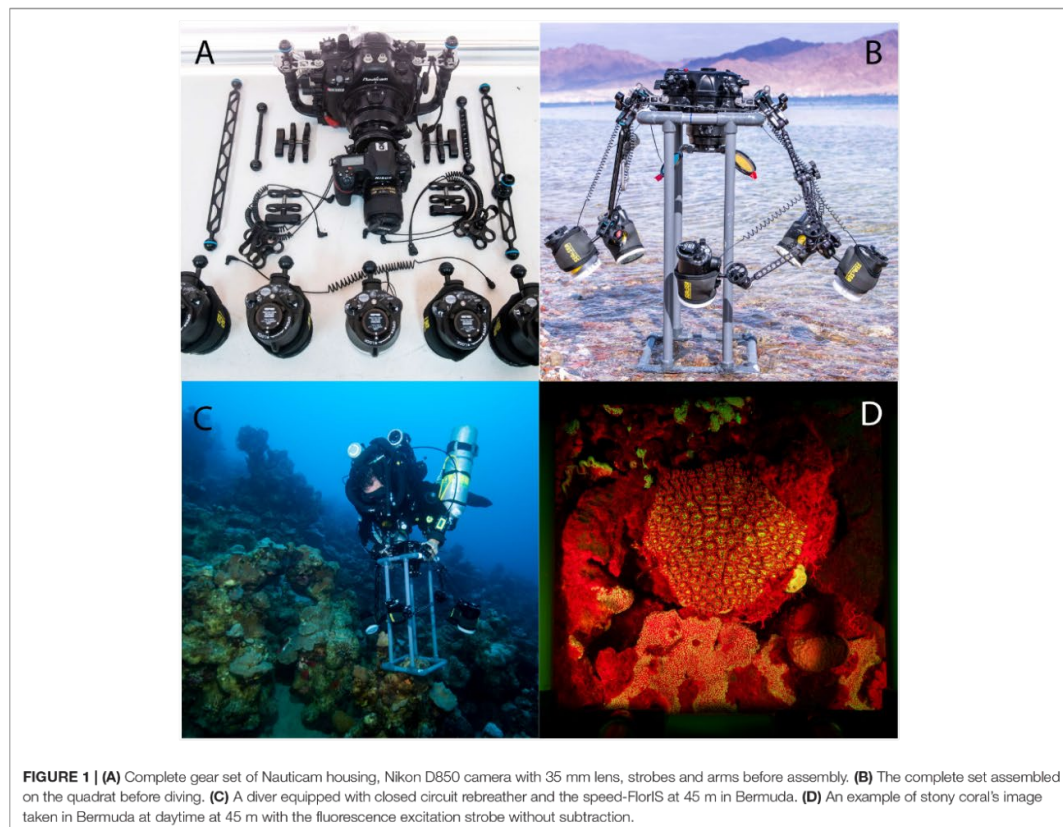
Imaging System

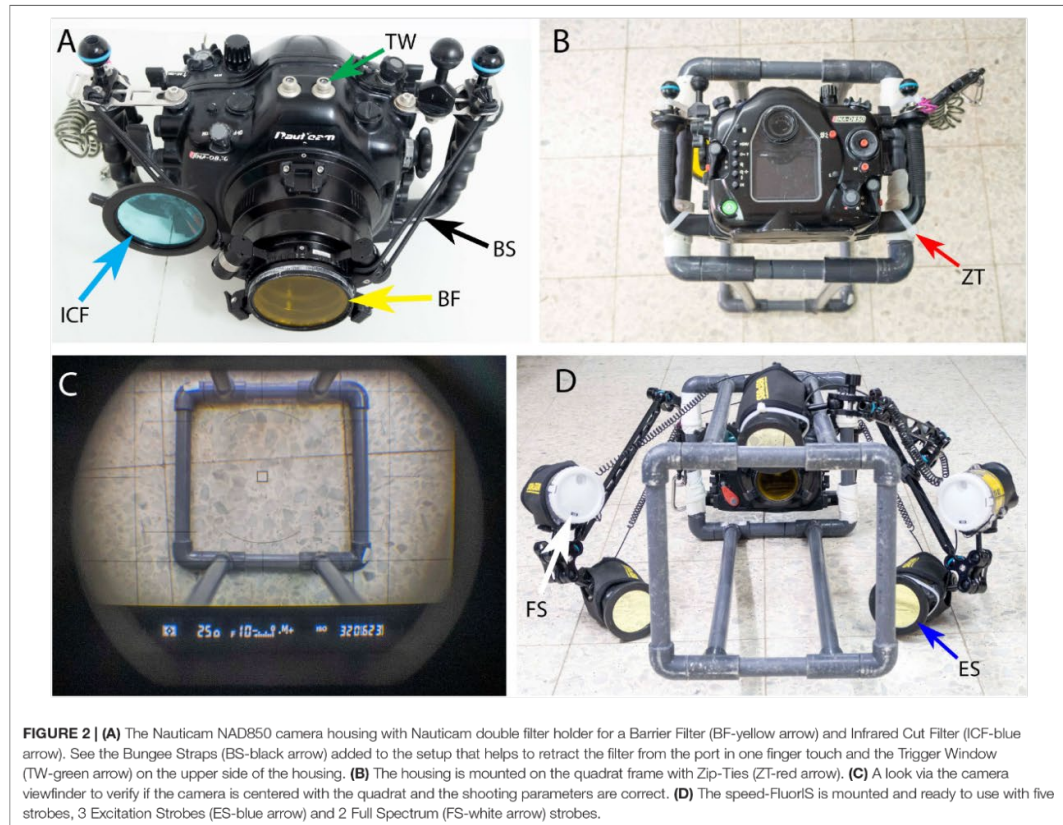
We modified the two cameras FluorIS system (Zweifler et al., 2017) to enable a wider spectrum capability and faster working volume in a one camera system, that we named speed-FluorIS (Figure 1). Specifically, similar to Treibitz et al. (2015), we removed the Infrared Cut filter (ICF), which is regularly located on top of the camera sensor, by applying an infrared conversion to the camera (Life Pixel, United States). The converted camera (we used Nikon D850) can capture a spectrum of 200–1200 nm, which improves the ability to record red fluorescence of chlorophyll-a, while giving the surveyor another layer of information that can be used for identification purposes, compared to a non-converted camera that can record only 200–700 nm. However, we replaced the use of a dome port with a flat port which allowed us to place the barrier filter out of the housing, this modification allowed us

to attach or detach the different filters rapidly underwater and to use a single camera system. A barrier filter is needed to block the blue UV light from the strobes and to let only the emission of fluorescence light to be seen by the sensor. When the barrier filter is removed, the camera acts as a wide spectrum camera, and can record ambient or white light (reflectance, or strobe light).

The camera was fitted with a Nikon 35 mm 1.8 lens and housed within a Nauticam NAD850 housing with a set of five Sea & Sea YS-D2 underwater strobes with a guide number of 32 (Figure 2). The original FluorIS used the same type of lens, as it is considered to be a very sharp prime lens with wide FOV and relatively no aberrations or optical distortion. In addition, this is the widest FOV lens that is suitable to use with a flat port, which is necessary for the ability to change filters underwater and is the fundamental condition of using a one camera system.

Three of the strobes were covered with excitation filters (Schott-BG39 and Night-Sea filters on top of each other) to give an excitation light of 385–500 nm, and the other two strobes, with 120° diffusers on, were unfiltered for white light (full spectrum). When shooting a fluorescence and reflectance image, the Full Spectrum (FS) set is disabled (turned off) and the barrier filter





is on, and when shooting a FS image, the excitation strobes are off, and the barrier filter is removed from the camera port. The camera triggers the strobes through two sets of dual fiber optic cables (Nauticam, United States). One set is triggering the excitation strobes, and the other set is triggering the FS strobes. To trigger the 3rd excitation strobe, we connect it with a single fiber optic cable to one of the excitation strobes triggered directly by the camera. By alternating between the two fiber optic cables and holding them in front of the housing synchronization window we can trigger the different strobe sets. Such modification enables the system to record the fluorescence, reflectance and FS image using one camera. The camera housing is equipped with a Nauticam Macro Port 60 in front of the camera lens and with a Nauticam double filter holder. We used two filters: one barrier filter (Tiffen yellow 12) to block the blue excitation light from the filtered strobes (all wavelengths below 510 nm), and a second external ICF filter, replacing the one removed from the camera sensor, for FS reflectance images (see Table 1 for a full list of the equipment used). The ICF blocks any wavelength above 750 nm. However, we found that the difference between images taken with or without the ICF was minor and, if needed, could be easily

adjusted in a post-processing software with the white balance tool as demonstrated in Figure 3 and Supplementary Movie 1.

Building the Quadrat and the Support Structure

A custom-made 25 × 25 cm quadrat (standard quadrat size used in ecological monitoring programs) was constructed to enable rapid imaging of the same area (Figure 1). In order to find the best working distance to get the highest possible resolution for the 25 × 25 cm quadrat, we performed test shots in a 500 L water tank (80 W × 120 L × 60 H cm): we submerged all the setup and checked on the camera Viewfinder (VF) for a distance that showed all the corners of the quadrat. Once the right distance was found, we measured it from the quadrat plane to the tray handles plane, to later build the spacer rods at a determined length of 65 cm. The structure was built using 25 mm-diameter PVC pipes and its parts glued together to create a strong and solid structure that can hold the camera and strobes on land and underwater in a position that enables the diver to take multiple images at the same camera position, without damaging the reef and with minimal

TABLE 1 | Full list of the equipment needed for the speed-FluorIS.

Product	Quantity	Cost \$	Total \$	Seller
Converted D850 full spectrum	1	3,350	3,350	Lifepixel.com
Nikon 35 mm 1.8 Lens	1	530	530	Bhphotovideo.com
Memory card	1	30	30	Bhphotovideo.com
Nauticam NA-D850 housing	1	3970	3970	Nauticam.com
Nauticam macro port 60	1	480	480	Nauticam.com
M67 flip filter holder	1	270	270	Nauticam.com
Barrier filter	1	50	50	Backscatter.com
Excitation filter	3	190	570	Backscatter.com
Schott-BG39	3	80	240	Shop.schott.com
400 mm aluminum arm	2	60	120	Nauticam.com
Triple clamp	2	50	100	Nauticam.com
Nauticam clamp	4	45	180	Nauticam.com
200 mm aluminum arm	1	55	55	Nauticam.com
Standard 1" ball mount	1	40	40	Nauticam.com
YS-D2 strobe	5	690	3450	Seaaandsea.jp
Nauticam to Sea & Sea dual fiber optic cable	2	230	460	Housingcamera.com
Eneloop AA rechargeable	5	20	100	Bhphotovideo.com
Ni-MH batteries (pack of 4)				
Subtotal			13,995	

Cells in green correspond to pieces of equipment that need to be doubled in quantity to build the original two-camera FluorIS system.

addition to its weight (~1 pound) and drag. The quadrat frame was attached to the camera housing using zip-ties (Figure 2B), facing down at a distance of ~65 cm measured from the quadrat plane to the sensor plane.

Study Sites

Both the Gulf of Eilat and Bermuda are situated at ~30°N, however, environmental conditions differ significantly, as Eilat is within an enclosed oligotrophic bay, whereas Bermuda is an oceanic island situated in the oligotrophic Sargasso Sea.

In Bermuda, two study sites in the Bermuda platform that varied in environmental conditions were selected (Figure 4): a shallow reef (~5 m depth; 32.45733N, 64.83475W) and a mesophotic reef (~45 m depth; 32.49145N, 64.85449W), located at 4.3 km from each other. Temperatures at shallow reefs sites in Bermuda vary from 22.8 to 29.5°C, and from 22.2 to 27.8°C at the mesophotic reef (Goodbody-Gringley et al., 2015). The clarity of oceanic water surrounding Bermuda greatly extends the euphotic zone, where the 10% photosynthetically active radiation (PAR) depth varies seasonally from ~30 to ~60 m (Siegel et al., 1995). Macroalgal cover up to 45 m depth is low mainly because of the slope and storm wave action. Fish richness, abundance and biomass increase with depth from the shallow to the mesophotic zone, with community structure strongly changing across depths (Pinheiro et al., 2016).

In Eilat, the survey was conducted in the shallow (~5 m depth) and mesophotic (~45 m depth) reefs adjacent to the Interuniversity Institute (IUI) for Marine Sciences (29.50221N, 34.91660E) (Figure 4), located at a distance of around 100 m from each other. Annual monitoring at these reefs sites records temperatures that range from 20.7 to 29.8°C in the shallow reef

and 20.9–27.8°C at 40 m depth (National Monitoring Program, Eilat). Light attenuation varies significantly with the annual cycle, with the depths of 6% PAR varying between 30–60 m depth (Dishon et al., 2012). Fleshy algae flourish at mesophotic depths and are sometimes present on top of reef structures at a higher coverage than in shallower environments. Reef fish assemblages reveal a change along the depth gradient of the Red Sea due to the steep bathymetry of the region. The abundance, biomass, and density of the most common herbivorous fish decreased along the depth gradient in the Gulf, potentially leading to reduced grazing pressure on MCEs (Brokovich et al., 2010).

Testing the Imaging Method in the Field

In order to find the right camera settings, test shots with the system were made on the reef adjacent to the Interuniversity Institute (IUI) of Eilat. Repetitive co-located images with and without filters of different stony corals and algae were captured during daytime at depths of approximately 6 m and 40 m. To capture two sequential shots with the shortest interval possible, the camera was set to shoot in continuous mode of 7 Frames Per Second (FPS), which is the highest frame rate possible for this camera model. During image post-processing, the Image Calculator function of the FIJI software (Schindelin et al., 2012) was used for subtracting the fluorescence effect in daylight, in order to assess the quality of the shots.

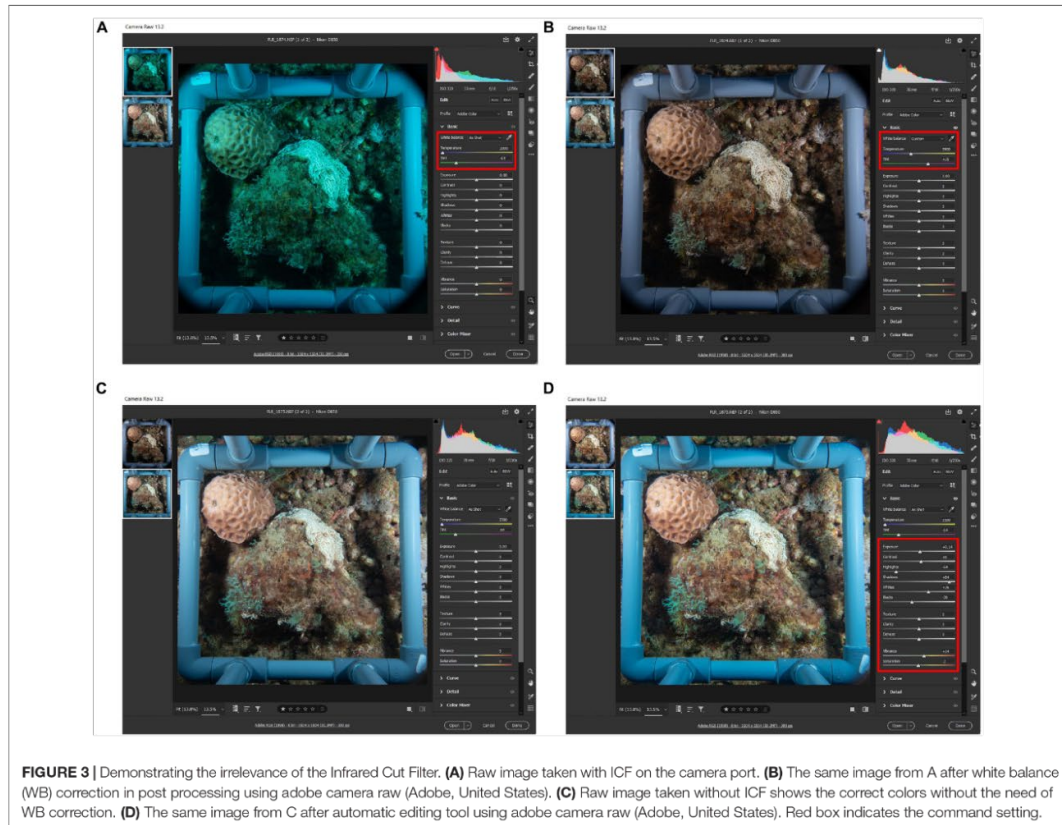
Survey of Coral Recruits Using the Speed-FluorIS

Once found the optimal camera settings with the test shots, co-located reflectance and fluorescence image-pairs were captured during daytime to survey coral recruits among the shallow and mesophotic reefs of Eilat (April 2019) and Bermuda (July 2019). Image locations were chosen randomly at each depth (5 and 45 m) in both sites. In Eilat, 46 random locations were selected for the shots at 5 m and 36 were selected at 45 m. In Bermuda, 17 random locations were selected at 5 m and 21 were selected at 45 m.

During image post-processing, the FIJI software was employed to subtract the strobes-off images from the strobes-on images, using the Image Calculator function. Coral recruits between 1 mm and 2 cm of diameter expressing FP fluorescence were counted using the Cell Counter plugin in FIJI. Reflectance FS images under white light illumination were used to verify that the fluorescent organisms detected in the fluorescence images corresponded to coral recruits by shape.

Statistical Analysis

Recruit count data were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Brown-Forsythe test). As the assumption of normality was violated, a non-parametric Mann-Whitney test was used to compare recruit numbers between depths at the same location. Significant groups have a value of $P \leq 0.05$. The GraphPad Prism software version 8.0.2 (GraphPad Inc.) was used to perform the statistical tests. Results are presented as mean \pm standard error.



All raw data used in this study are accessible through the electronic notebook¹.

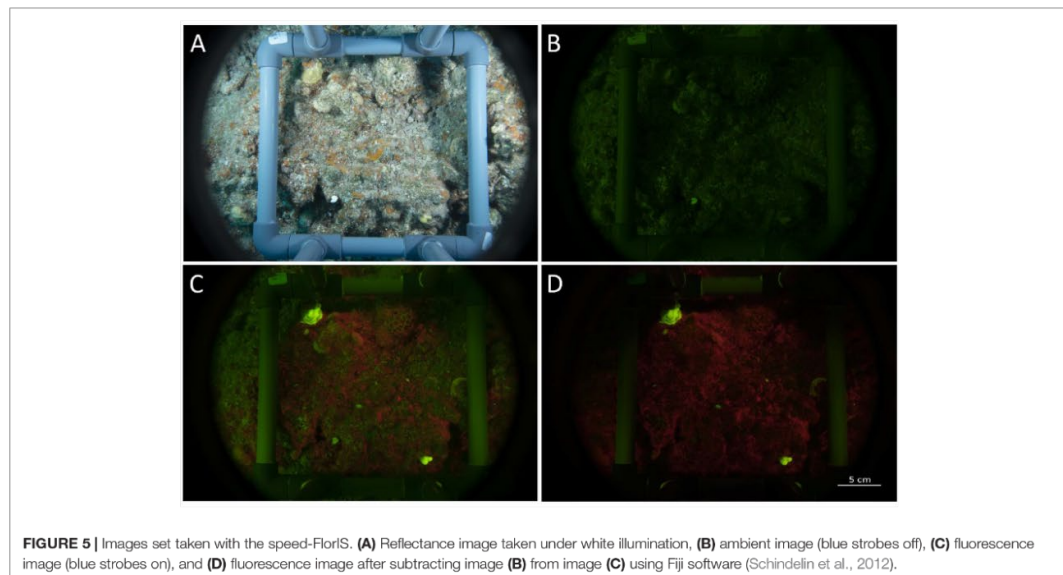
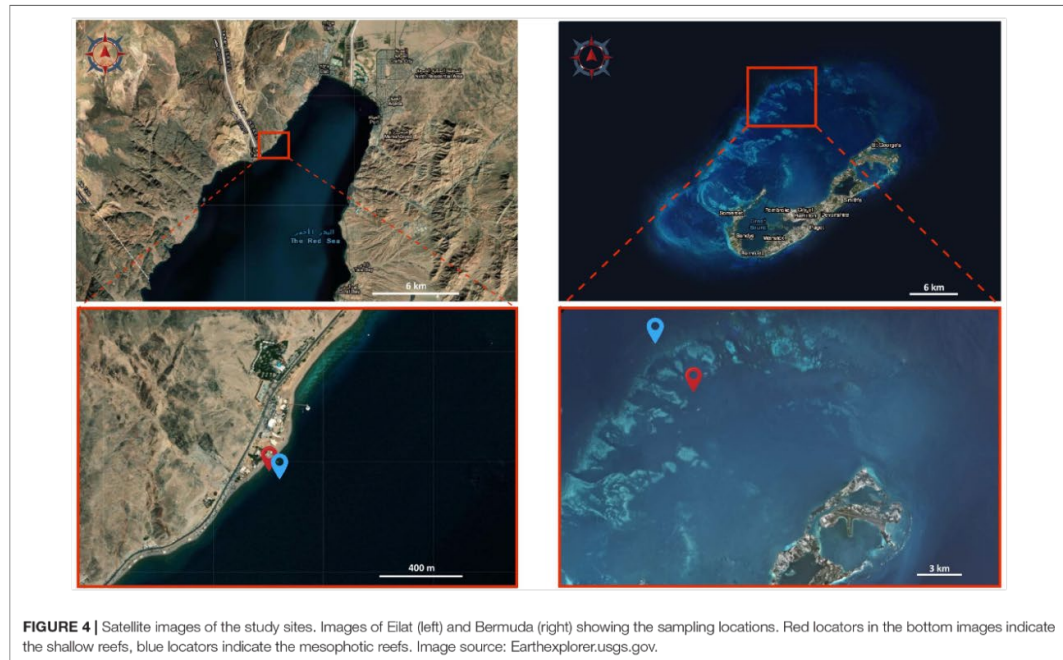
RESULTS

Camera Settings Optimization

Setting the camera to shoot at the highest frame rate (i.e., 7 FPS) does not provide enough recharging time to the excitation strobes, that with the highest output (GN32) can fire at less than 1.5 s between shots. As a result, the first image of each set is full power fluorescent shoot, and the subsequent shoot is reflectance (ambient) light (Figure 5). If ambient light is absent or weak, for example at depth or at sunrise and sunset, we found that there is no need to make a reflectance image for subtraction, since the fluorescence is strong enough (Figure 1D). However, at shallow depth and with strong light during the day, there is an advantage in using the software developed with the FluorIS (Zweifler et al., 2017) or with FIJI software for subtracting and

emphasizing the fluorescence effect in daylight (Figure 5). The result of the subtraction is a fluorescence image excited solely by the blue strobes (Figure 5D). Alternatively, the system can be used without the need for subtracting by eliminating as much ambient light as possible with few basic adjustments and considerations before and during the dive. First, the exposure value of the camera should be set to a value that minimizes ambient light, allowing at the same time as much strobe light as the sensor can record. To accomplish that, we found that the shutter needs to be set to the highest synchronization speed allowed by the camera. With the Nikon D850 this corresponds to 1/250th of a second (note that each camera model can present a different value for synchronization speed). The aperture of the lens has to be set to a value that allows a good depth of field and sharpness but gives enough strobe light to penetrate to the sensor. We found that such aperture value corresponds to f10. The sensor sensitivity was set to a final value of ISO320, so to summarize, the ultimate exposure parameters were set to 1/250th, f10, ISO320. Using these settings, we reduced the acquisition time to an average of 10 s for a set of 3 images produced on each quadrat.

¹https://github.com/Mass-Lab/Fluorescence_imaging_of_coral_recruits



Another two points that are recommended when removing the ICF and using a barrier filter is to set the camera White Balance (WB) to 2300K or less and to

set the tint to green. This setting can be done in the camera WB menu, or by adjusting a preset WB, or in post processing if the images file format is set to RAW.

We found that these two points allow complete control on the final picture.

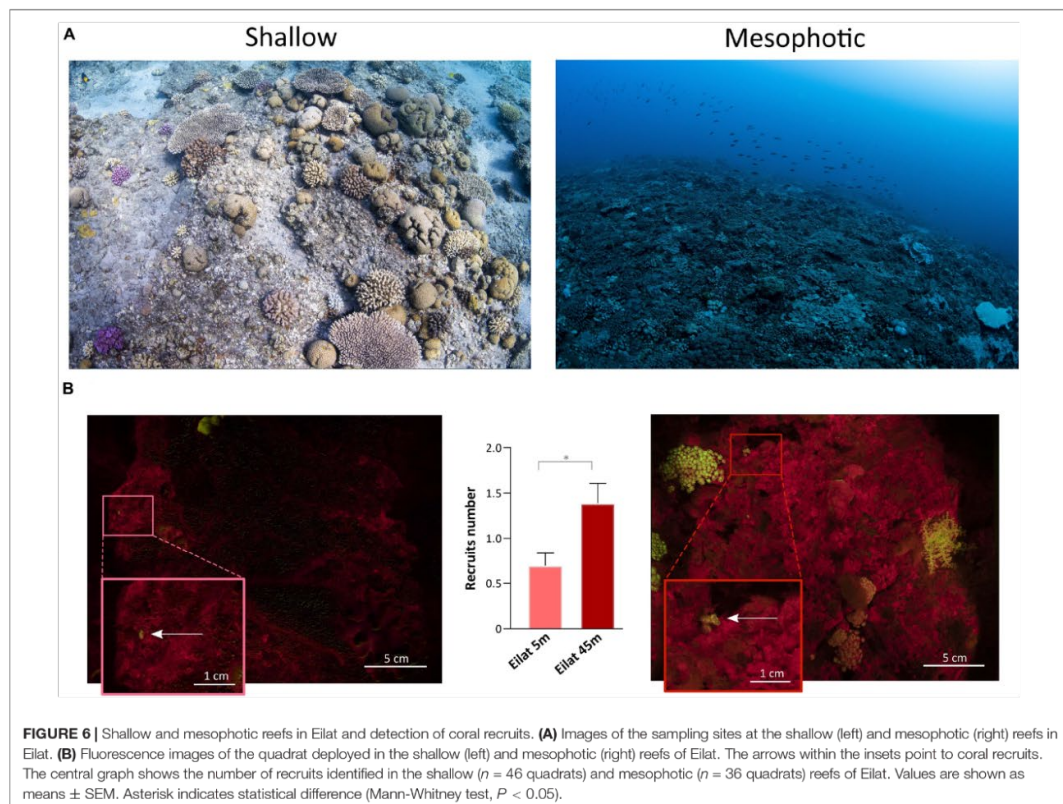
Because light underwater fades rapidly with increasing distance from the source, it is crucial to position the strobes as close as possible to the subject without the strobe body appearing in the image. To achieve this, we used 40 cm Nauticam light mounting arms that can hold the strobes to the housing handles and give the flexibility to move and lock the strobes in the desired position (Figure 2D). However, we observed that the use of the excitation filters significantly reduces the strobes power, therefore it is crucial to set them on full output and to place the strobe approximately 20 cm from the target. The two FS strobes can instead be placed in a larger distance or set on lower output, as their power is not affected by filters. Finally, the surveyor's body can be used to block the ambient light on the subject. Even with the exposure value mentioned above and maximal excitation strobe output, the intensity of direct sunlight at noon in tropic areas is strong enough to overcome the fluorescence emission and to be undetected by the camera. To avoid this, the surveyor should plan the dives to early morning or afternoon, but if diving at noon time, the surveyor can hover right above the quadrat and shade it with the diving gear and body, so that the intensity of

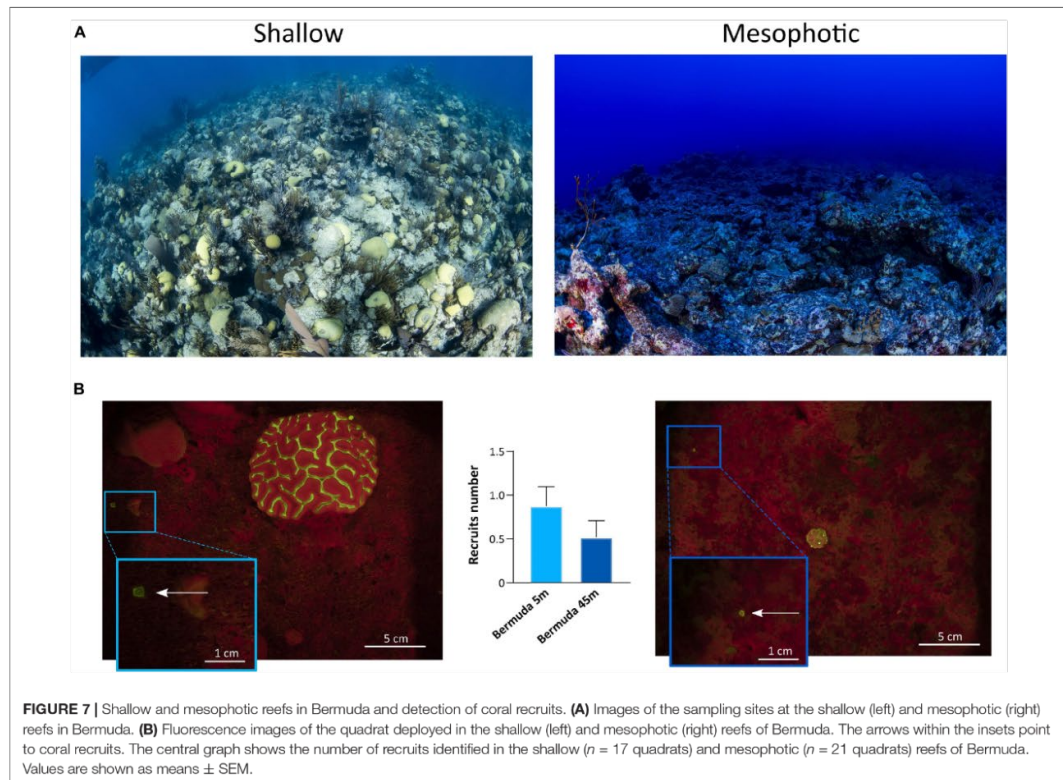
the ambient light in the image drops dramatically and a good reflectance image can be easily captured.

Changes in Reef Structure With Depth and Coral Recruits Survey

The topography of the sampling sites differed significantly across depths in each geographic location (Figures 6, 7). In Eilat, scattered stony coral colonies with mainly branching morphology in the shallow reef are replaced by encrusting or plate-like colonies in the mesophotic reef, which leads to a flattening of the reef structure with increasing depth (Figure 6A). Differently in Bermuda, the high density of stony and soft corals substantially declines at mesophotic depths, where a non-coral dominated reef with a high topographic complexity is predominant (Figure 7A).

In Eilat and Bermuda, the total distance that we sampled corresponded to around 100 m in both shallow and mesophotic reefs. Considering the total time needed to complete each dive, we estimated that we employed a maximum of 1 min to sample each single quadrat. Using the speed-FluorIS system we recorded wide FOV fluorescence images of coral recruits from 1 mm to 2 cm in diameter (Figures 6B, 7B). In Eilat, we found





significantly higher numbers of recruits in the randomly placed 25×25 cm quadrat at 45 m compared to 5 m (Mann-Whitney, $P = 0.009$) (Figure 6B). In Bermuda instead, we found no significant difference between the shallow and mesophotic reefs (Mann-Whitney, $P > 0.05$) (Figure 7B).

DISCUSSION

The development of the FluorIS system has demonstrated the biases of *in situ* visual surveys of coral recruits, by providing a higher precision and standardized method to accurately assess coral recruitment (Zweifler et al., 2017). Such a system eases and speeds up the detection of small and highly cryptic coral recruits (Figures 6B, 7B), overcoming the limitations of non-standardized and labor-intensive visual recruit counts. Here we show that the speed-FluorIS system greatly facilitates high sensitivity *in situ* fluorescence imaging of coral ecosystems, especially by eliminating the excess weight and encumbrance of a double-camera system and by reducing the time needed to acquire all sets of images. In fact, the use of speed-FluorIS significantly reduces the acquisition time to an average of 10 s for a set of 3 images, reducing the overall time employed to

sample a single quadrat to 1 min. This time frame is significantly shorter compared to the sampling time of the original FluorIS system, which corresponds to ~ 6 min per quadrat (Zweifler et al., 2017; data not shown). As survey depth increases, the dive complexity increases as well. For example, technical dives are needed to go beyond recreational dive limits, which require heavier and bulkier equipment and are strictly limited in time. Therefore, conducting underwater surveys with speed-FluorIS brings several advantages for the diver, including safety, ease of navigation, reducing fatigue, and simplifying the logistics of conducting fluorescent surveys in the field. Moreover, the speed-FluorIS system is much more economical compared to the original dual-camera system, reducing the cost of the full set-up of about 40% (Table 1).

In this study, we demonstrate that speed-FluorIS enables a fast imaging of fluorescence to study coral recruitment patterns over a broader range of depths and reef topographies than previous fluorescence methods (Figures 6, 7; Schmidt-Roach et al., 2008; Roth and Knowlton, 2009; Hsu et al., 2014; Zweifler et al., 2017; Ramesh et al., 2019), providing a standardized survey methodology that can be applied in different sites across the world. Solely based on the results of our survey (Figures 6B, 7B), it is difficult to estimate recruitment

dynamics in the shallow and mesophotic reefs of Eilat and Bermuda, considering that coral recruitment greatly varies between seasons and years (Jouval et al., 2019, and references therein). Further long-term surveys would significantly improve the resolution and accuracy of temporal and spatial data, revealing the full picture of coral recruitment patterns and assessing the true status of the reef. Moreover, environmental data such as light, temperature, nutrients, algae and fish abundance of the sampling sites also need to be assessed, as they have a strong influence on coral recruitment patterns (Brokovich et al., 2010; Kramer et al., 2019; Loya et al., 2019; Stefanoudis et al., 2019).

In mesophotic reefs, *in situ* surveys of recruitment patterns have mostly been limited to the northern Red Sea and Western Australia, where artificial settlement tiles have been employed to count new coral recruits (Turner et al., 2018; Kramer et al., 2019; Shlesinger and Loya, 2021). Several studies have evaluated *in situ* coral recruitment patterns in the shallow reefs of Eilat and Bermuda through visual underwater identification of coral recruits on settlement tiles or on the natural substrate (Smith, 1992; Glassom et al., 2004; Abelson et al., 2005; Glassom and Chadwick, 2006; Martinez and Abelson, 2013; Shlesinger and Loya, 2016; Guerrini et al., 2020). Although being performed on the same site, these surveys revealed high variation in coral recruit numbers. Inconsistent findings in recruitment studies using different methods have been attributed to the differences in substrate, method of attachment, and duration and depth of tile deployment (Harriott and Fisk, 1988; Mumby, 1999; Glassom et al., 2004; Abelson et al., 2005). In addition, the use of settlement tiles of different sizes may also affect abundances of coral recruits, which in turn will affect the number of individuals per unit area (Birkeland et al., 1981). Thus, studies on coral recruitment based on this methodology can be difficult to compare, especially since the type and preparation of recruitment tiles greatly affect which organisms will settle on them (Brandt et al., 2019). Such lack of a standardized methodology hinders the comparison of recruitment patterns between reef sites worldwide (Glassom et al., 2004; Zweifel et al., 2017). Moreover, the labor-intensive demands of executing visual surveys and direct counts of recruits in the field make it difficult to generalize findings to broader geographic extents and across wide depth distributions (Shlesinger and Loya, 2016).

Fluorescent proteins fluorescence has been widely used for better detection of coral recruits by divers and in images (Piniak et al., 2005; Baird et al., 2006; Schmidt-Roach et al., 2008; Roth and Knowlton, 2009). However, FPs fluorescence alone might not be enough to identify young corals, since FPs fluorescence intensity greatly varies among species (Eyal et al., 2015), with some corals showing very weak or no FPs signal (Baird et al., 2006; Roth et al., 2010). Moreover, there are other organisms besides corals that contain fluorescent pigments, such as algae, sponges, and worms. Therefore, accurate identification is only possible by comparing each coral recruit identified in the fluorescence image to a high-resolution FS image, as we did in this study, which can also facilitate taxonomic identification (Baird et al., 2006).

The speed-FluorIS that we propose here represents an easy-to-use and non-invasive method that will help standardize surveys and long-term monitoring of coral recruits. In the future, speed-FluorIS can be applied via underwater vehicles for rapid and automated surveys. The recorded data could be uploaded to create universal and easily accessible databases that contribute to our understanding of the vital and delicate early life stages of corals. Importantly, if MCEs are in fact important lifeboats for coral survival as increasingly advocated (Bongaerts et al., 2010; Lesser et al., 2018), it is imperative to accurately investigate coral recruitment dynamics of these deeper reefs. Coral recruitment has been long identified as a key process in the ability of reefs to recover from disturbances (Hughes et al., 2007, 2010; Ritson-Williams et al., 2009). Therefore, understanding the recruitment process is essential for developing suitable reef conservation and management strategies to protect these vital ecosystems.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

HN, GG-G, and TM designed the research. HN, GG-G, SM, SE, AC, and TM performed the underwater surveys. FS and HN carried out the image analysis and recruit counts. HN, FS, GG-G, and TM wrote the manuscript. All authors contributed to improving, revision and approval of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2021.709175/full#supplementary-material>

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General Discussion

In this study, we optimized the FluorIS system by utilizing a single infrared-converted camera instead of the bulkier regular dual-camera system (Treibitz et al., 2015). We thus significantly reduced the system complexity and the time consumed for completing the underwater survey, while also broadening the types of terrain shapes and slopes that can be surveyed. With this faster, more flexible and easier to handle system we surveyed coral recruits under 2 cm in diameter across shallow and mesophotic reefs of the northern Red Sea and Bermuda. Our improved technique represents a valuable, non-invasive and rapid underwater tool to investigate coral reproductive ecology, *in situ* across wide depth ranges, that significantly increases the speed and accuracy of coral recruit counts.

The speed-FluorIS that we developed (Nativ et al. 2021) represents an economical, non-destructive, and easy-to-use method that will help standardize surveys and long-term monitoring of coral recruits. Moreover, the speed-FluorIS is in order of magnitude faster to acquire images than the former two-camera systems on the market. With those improvements, it is possible to acquire images and take surveys in any practical depth and time of the day. In the future, it can be applied via underwater vehicles for rapid and automated surveys. The recorded data could be uploaded to create universal and easily accessible databases that contribute to our understanding of the vital and delicate early life stages of corals. Importantly, as coral recruitment has been long identified as a key process in the ability of reefs to recover from disturbances (Hughes et al., 2007; Hughes et al., 2010; Ritson-Williams et al., 2009), understanding the recruitment process is essential for developing suitable reef conservation and management strategies to protect these vital ecosystems.

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Supplementary:

Workflow demo. For In situ estimation of coral recruitment patterns from shallow to mesophotic reefs using an optimized fluorescence imaging system

<https://www.frontiersin.org/articles/10.3389/fmars.2021.709175/full#supplementary-material>

שיפור שיטת צילום פלורסנטית להערכת בריאות שוניות אלמוגים במגוון בתי גידול

חגי נתיב

תקציר

התיישבות אלמוגים מהווה מדד חשוב לבריאות ועמידות שוניות האלמוגים בעולם, החווים התמודדות מול שינויי אקלים והפרעות סביבתיות. לימוד והבנת תהליכים של התיישבות אלמוגים הם שלבים קריטיים בהערכת בריאותה של השונית וניהול נכון של אסטרטגיות השימור בתקופה של שינויים אקלימיים.

מערכת הצילום הפלורסנטית (FluorIS) פותחה על מנת להשיג תמונות ברורות ניתוח של התיישבות אלמוגים בדיגום שטח והוכיחה יעילות בשוניות רדודות. על כל פנים, עד עכשיו טרם הצליחו לבצע בעזרתה עבודה יעילה בשוניות עמוקות ובאזור המזופוטי בפרט, בשל העובדה שתפעול המערכת מסורבל והעבודה בעומק מאתגרת מאוד מבחינת יכולות צלילה ומגבלות זמן.

בעבודה זו שיפרנו את מערכת הצילום על ידי שימוש במצלמה אחת במקום בשתי מצלמות בהן בוצע שימוש במערכת המקורית, ועל ידי כך צמצמנו בסדר גודל את המורכבות בתפעולה והזמן שלוקח לבצע באמצעותה סקר מתחת למים עד לזמן ממוצע של 10 שניות לסט של 3 תמונות הנדרשות להפקת נתונים מריבוע דיגום אחד. בעזרת המערכת המשופרת, הפשוטה לתפעול והזולה יותר, ביצענו סקר עם ריבוע דיגום של 25X25 ס"מ בשוניות רדודות ועמוקות בברמודה ובאילת, מהשוניות הצפוניות ביותר בעולם, עם מאפיינים טופוגרפיים ומצע שונים זה מזה, והראנו שניתן להפיק מידע בר ניתוח ותוצאות של התיישבות אלמוגים על ידי שימוש במערכת הצילום שבנינו (Speed-FluorIS).

המערכת שפיתחנו מהווה יתרון משמעותי בביצוע סקרים ללא גרימת נזק לשוניות האלמוגים ותורמת ליצירת כלי אחיד וסטנדרטי המאפשר שימוש גם בתוכניות ניטור להערכת בריאות השונית מסביב לעולם וליצירת אסטרטגיות שימור בהתאם להבנת תהליכי ההתיישבות של האלמוגים.

שיפור שיטת צילום פלורסנטיית להערכת בריאות שוניות אלמוגים במגוון בתי גידול

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הפקולטה למדעי הטבע

ביה"ס למדעי הים על שם ליאון צ'רני

החוג לביולוגיה ימית

נובמבר 2021

שיפור שיטת צילום פלורסנטיית להערכת בריאות שוניות אלמוגים במגוון בתי גידול

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