

FINAL TECHNICAL REPORT

BELIZE BARRIER REEF CROSS SHELF CONNECTIVITY PROJECT

STATUS OF RESEARCH

The Cross-Shelf Connectivity Project of the Marine Management Area Science (MMAS) program was a one-year investigation of the hypothesis that back-reef habitats in the Belize barrier reef and atoll lagoons are essential as nursery areas for the production of commercially important members of the snapper (Lutjanidae), grunt (Haemulidae), and grouper (Serranidae) families. Lagoon mangrove and seagrass habitats are under increasing threat from real estate development. Real estate values are partly contingent upon quality of life and the environment, setting up a trade-off between habitat liquidation for guest and resident housing versus habitat preservation to safeguard the generation of food, recreational, and other living resources. This trade-off ultimately determines the net wealth that Belize can generate from its natural assets.

The primary objective of the Belize Cross Shelf Connectivity project was: to sample coastal habitats to identify larval settlement and juvenile nursery areas for select fishery species, and use stable isotope analysis to determine primary migration corridors across the shelf for these early life stages, to inform actions to preserve the habitats most critical to population survival and sustained economic production. Secondary objectives of the project were more specific and included: identifying critical habitats for larval settlement and juvenile production of key fishery species; mapping settlement and migration corridors of key species, from near shore to offshore; developing actions to conserve the most threatened critical habitats; and building capacity of Belizean scientists in the fields of marine ecology and stable isotope analysis. Two kinds of data were collected: visual estimates of juvenile abundance along fish transects, and stable isotopic ratios of muscle tissue to identify dietary signals that might later be used to interpret life history movements.

All field research and analysis have been completed. From the original objectives set at the beginning of the project, all have been attained except work on the otoliths. After otolith analysis is completed, approximately the end of the current year, the data should hopefully re-enforce the current results obtained from the size and abundance distribution and stable isotopes. Our data already shows how important nursery habitats are to commercially important reef fishes and this should be an integral part in how these habitats are managed. The final step after all the data is analyzed is to ensure that policy makers make use of the available data in these critical marine natural resources and make decision based on them. It is imperative that all stakeholders are involved in dialogue to ensure that resources are properly distributed and that decisions involve the input of all parties.

Publications are being drafted for submission to suitable ecology or fisheries related journals. The cross shelf poster is still being finalized but should be completed and printed by the end of October. There have been major delays in getting the final product from the designer. However, all the payments have been committed for this poster and accounted for in the final financial report.

METHODOLOGY

The CSC (Belize) project sampled the cross shelf of Belize from Placencia lagoon to Glover’s Reef atoll; and included four sampling locations: an estuary - Placencia Lagoon; a lagoonal reef - Laughing Bird Caye; a barrier reef - Gladden Spit; and an oceanic reef system - Glover’s Reef Atoll (Figure 1). Three different habitats were sampled mangrove, sea grass and back reef areas. At each location multiple sites were surveyed and varied in number among the locations. Placencia had a total of 4 sites, Laughing Bird Caye – 6 sites, Gladden Spit – 8 and Glover’s Reef Atoll – 10 sites. Sampling was conducted on a quarterly basis during the months of March, May, August and October of 2009. Stratified random sites were chosen in order for the project to be representative of the locations sampled and attain the goals and objectives of the project.

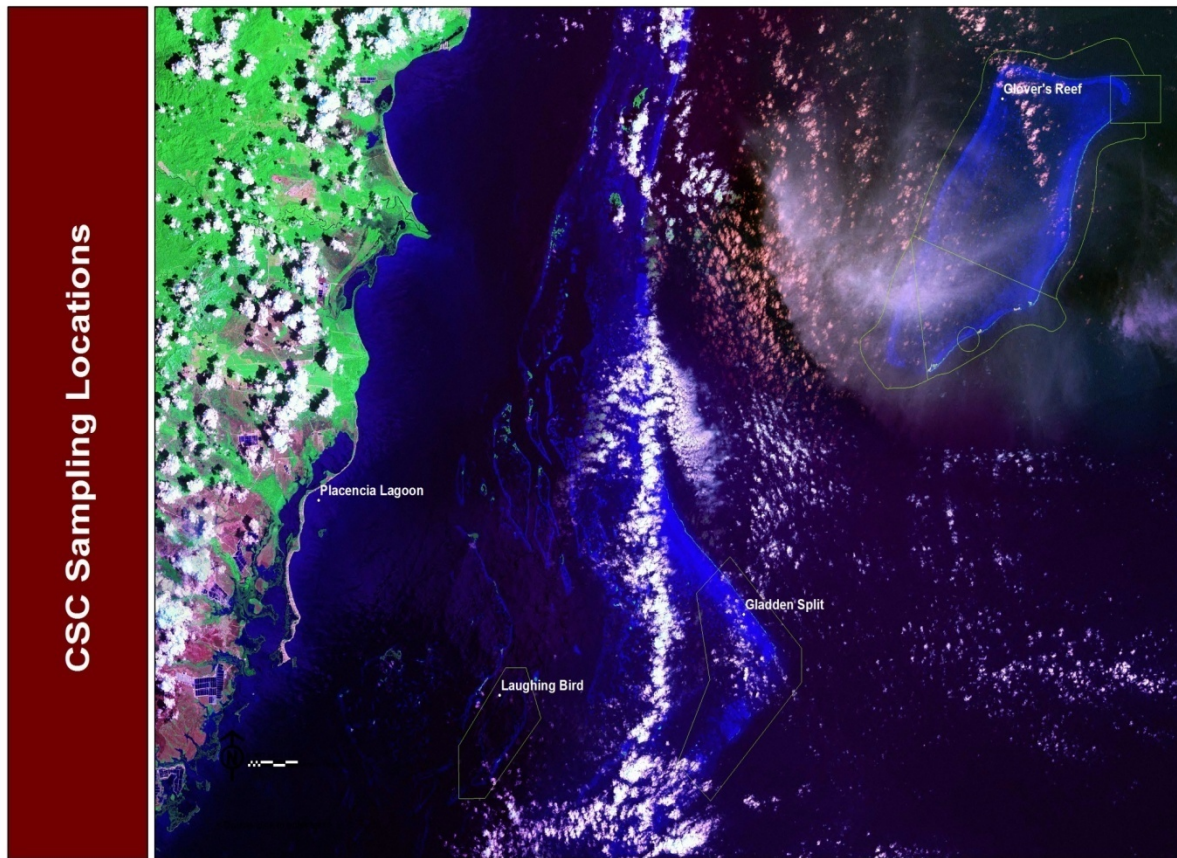


Figure 1 Sampling locations across southern Belize for the Cross-shelf Connectivity project.

Sampling produced two types of data: target species abundance and size, and specimens for stable isotope and laser ablation of otoliths. Belt transects (visual surveys); traps (Chevron traps), Spear, Hand line, Seine nets, and Fyke nets were the main means of data collection across all habitats. The belt transects were conducted in both mangrove and sea grass habitats and were used to get counts on fish abundance. The transects were 30m by 1m belt transects for sea grass, parallel to shore and nothing deeper than 2m. The mangrove transects were all 30m but in line with the prop-roots and varied in depth. The area visually surveyed included from the transect line to as far as the surveyor could see within the prop-roots; which in most case did not go beyond 1m. All target species were identified and their size estimated. For all transects, mangrove and sea grass, the depth and GPS point at the beginning and end of each transect were recorded, type of habitat and disturbed vs. undisturbed sites were also noted. All snappers observed in all transects were recorded by name and sized.

Fyke net and seine nets were only used at habitats whose topography and depth allowed. Chevron traps were used at all locations specifically targeting juvenile to adult target species (Figures 2b&c). Traps were baited and left overnight to capture specimens for tissue samples and otolith. All traps were properly anchored and re-sampled during every quarter. Spears were used in shallow areas where free diving was possible. No spearing was done on SCUBA and only the fish species of interest were captured in areas where visual transects were conducted. Hand lines were used in the areas sampled, as an additional means of capturing larger fish species of interest. Seine nets were used in shallow sea grass beds and back reef habitats. Seine nets targeted juvenile and late juvenile target species as well as silver sides (bait fish). Seine nets were used parallel to shore and all specimens were used for tissue samples (Figure 2d). Fyke nets were only used at mangrove habitats due to the lack of available anchoring structures at the other sampling locations. Samples from fishermen were a major supplemental source of specimens for stable isotope and otolith. This involved samples from all sampling sites where fishermen are known to fish the target species not collected during sampling of a location and for which samples were needed. All samples caught were used for tissue samples for stable isotope testing.

Tissue samples for Stable Isotope

The samples needed to conduct the stable isotope test were from the species of interest (snappers, grunts, groupers and parrotfish), their main food source, and the primary producers (mangrove leaves, and sea grass; both green and dry) from the habitat being sampled. For fish samples, a $\frac{1}{2} \times \frac{1}{2}$ cm sample of the muscle tissue was dissected and placed on foil paper, labeled with the fish's ID name and number. For silversides that were too small to obtain tissue sample the entire fish was placed on foil paper. For all fish, the standard length was measured and recorded. Crustaceans and primary producers' leaves were placed whole on foil paper. After all samples were properly labeled and placed on a metal tray, the tray was placed on an oven with a controlled temperature for the specimens to dry completely. Once dry all samples were placed in a container and taken to the lab for further processing. Once in the BU lab, the samples were ground to a fine powder and placed in labeled vials. The samples were then weighed, any

animal 1g and any plant 2g, and then placed in specially design small foil containers and folded into a cube. The samples were then loaded into the Mass Spectrometer for the stable isotope test.



(a)



(c)



(b)



(d)

Figure 2 Data collection during CSC (Belize) sampling: (a) Eli and Reyland getting ready for a dive at Glover's Reef Atoll; (b) retrieving chevron trap from Placencia lagoon; (c) custom made chevron trap for trapping target species; and (d) Eli and Tim Smith (collaborator on the sampling of Placencia lagoon) seining on shallow seagrass beds of mangrove islands in the Placencia lagoon.

RESULTS TO DATE

Most of the results show that there is indeed a difference in standard length across habitats for most of the target species. Some species are well adapted to make use of some of the habitats over a longer period of time and across different life stages, like *L. synagris*. But one of the most important areas of knowledge reinforced by the study is that mangrove habitats are highly important as nursery habitats for most of the target species. The data not only suggests that there are differences in their abundance but also in size and stable isotope ratio (δC and δN). By simply comparing the records from Placencia lagoon to the ones from any of the other sampling locations that had significantly more visual sampling sites, it is evident how much mangrove habitats are being used by reef fishes (Table 1). This is due to the condition of the Placencia lagoon which has suffered a variety of anthropogenic impacts. The study shows how management of the lagoon and its surrounding habitats can help to improve environmental conditions thereby increasing habitat use by fish in the lagoon. Several studies have shown not only how important mangroves are to reef fishes, but also how small disturbances can have significant adverse effects (Mumby *et al.* 2004, Taylor *et al.* 2007).

Placencia Lagoon was occupied by very small individuals for all the species present. It may be a critical nursery for commercially important reef fishes. Gladden Spit and Glover's Reef had a slight difference in the size of target species (GR and GS, $p=0.042$) while Laughing Bird Caye did not differ significantly from Glover's Reef and Gladden Spit in the size of fish present (LB and GR, $p=0.9745$; and LB and GS, $p=0.1638$). These three sites had mainly juveniles and adults present. The size distribution of commercially important species indicate just how much some of the target species depend on mangrove and seagrass habitats during early development. Although size increases from mainland to offshore, abundance decreases for most species (Figure 3a-d). Sampling showed not only how important mangrove habitats are to commercially important fish species but also richness and size distribution (Table 1).

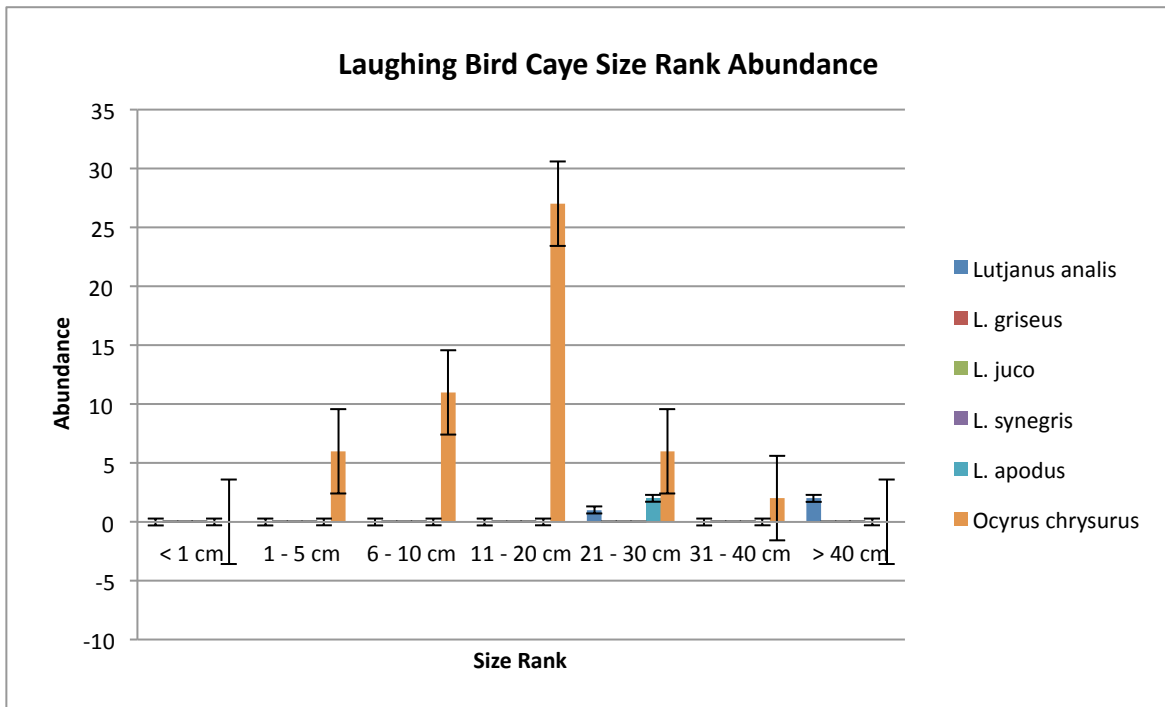
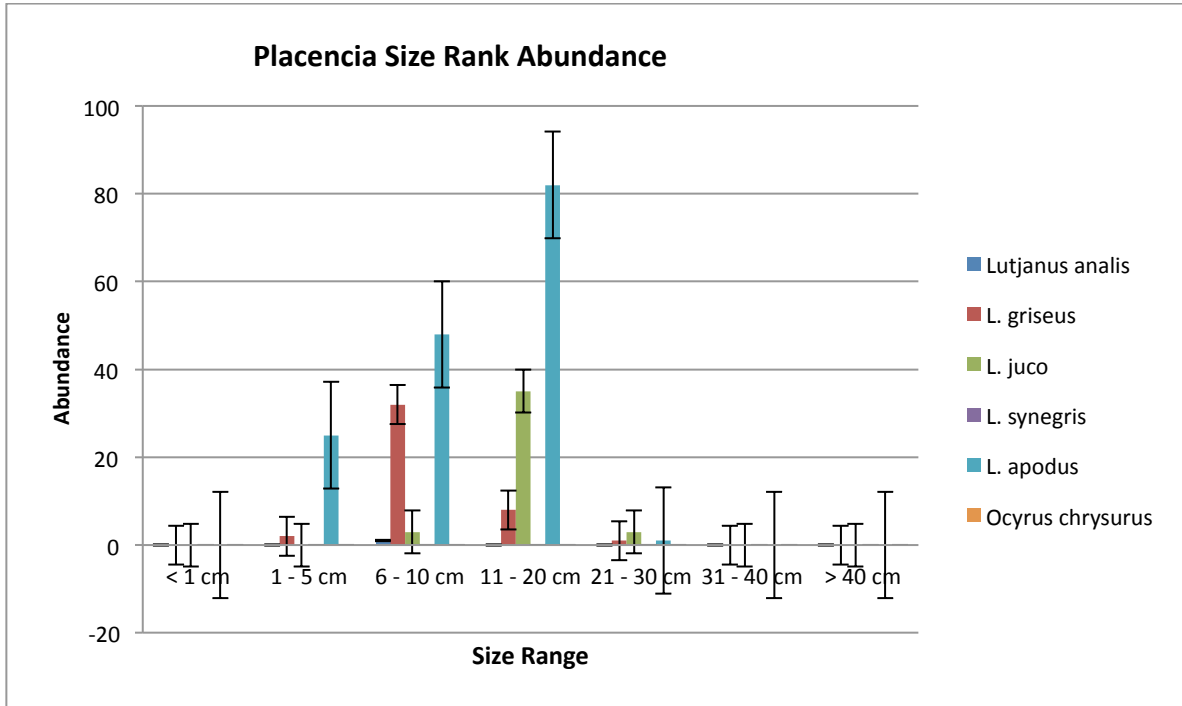


Figure 3 a-b Average size distributions between habitats of lutjanids: (a) Placencia lagoon and (b) Laughing Bird.

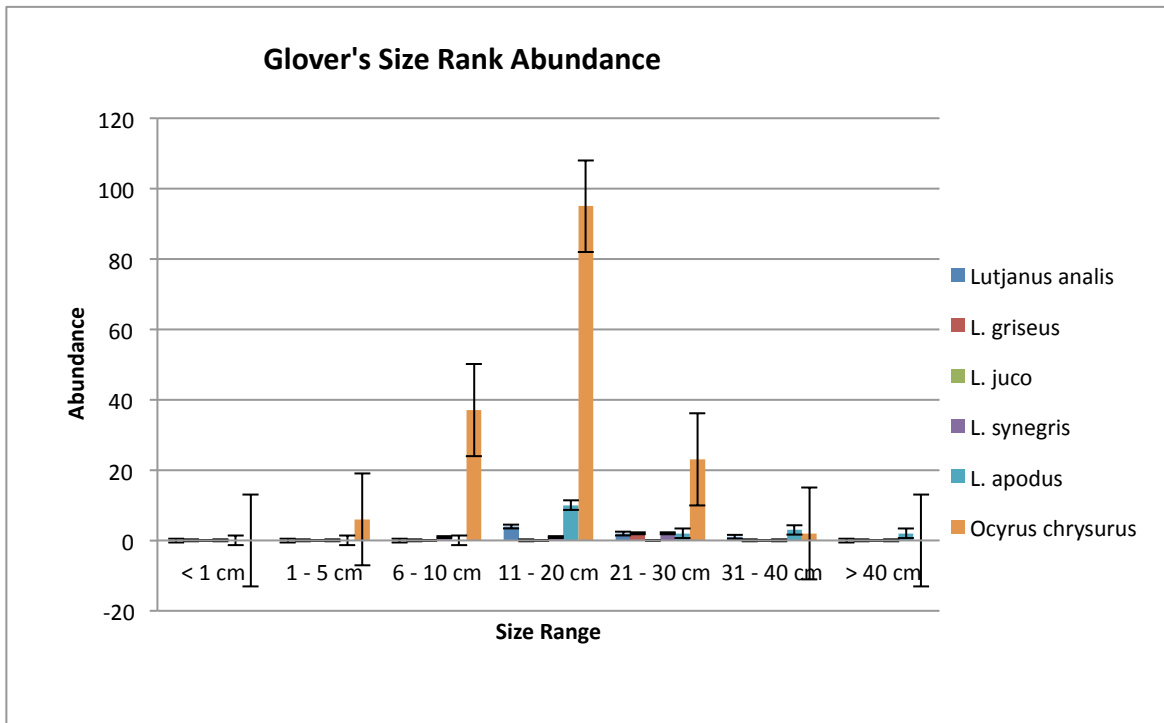
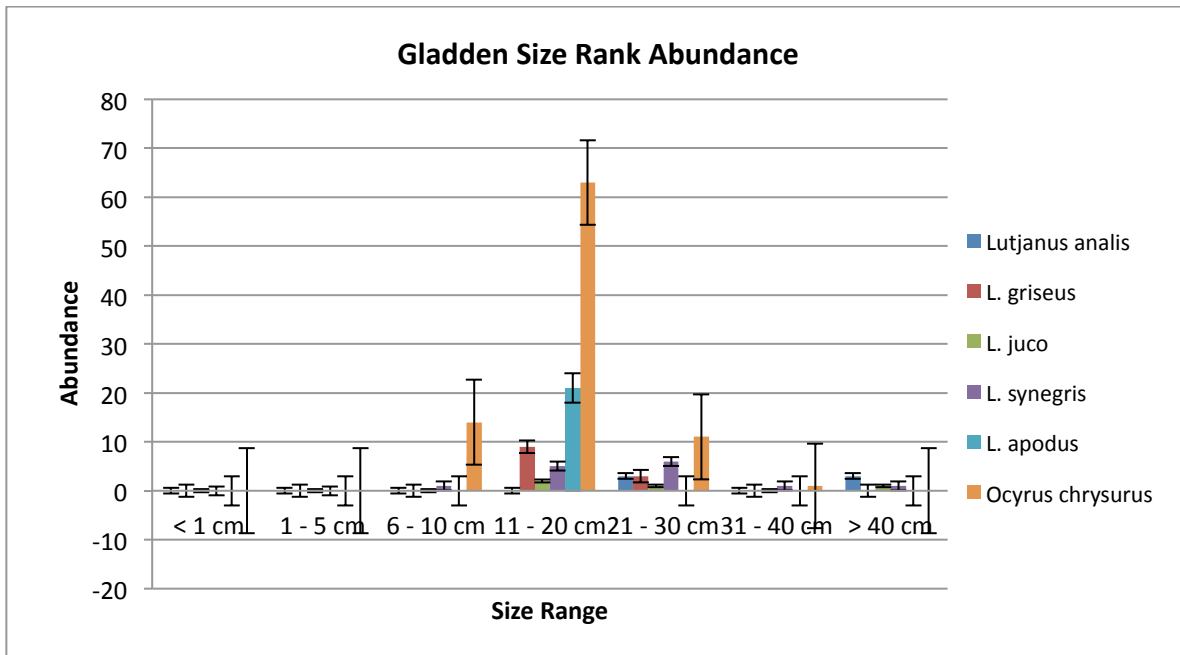


Figure 3 c-d Average size distributions between habitats of lutjanids: (c) Gladden Spit and (d) Glover's Reef atoll.

Table 1. Adult =A, J= Juvenile; E.J = Early Juvenile; N.S= Newly Settled. * = Occurred in the area but not recorded. Estuary =Placencia Lagoon; Lagoonal reef=Laughing Bird; Barrier Reef=Gladden Split; Oceanic=Glover’s Reef Atoll

GRUNTS (by rank abund)	Estuary	Lagoonal Reef	Barrier Reef	Oceanic	Primary NS Habitat
<i>Haemulon flavolinatum</i>	J, E.J, N.S	A, J	A, J, E.J, N.S	A, J, E.J, N.S	Shallow sea grass bed
<i>H.sciurus</i>	<i>J,E.J, N.S</i> *	A	A, J	A, J, E.J, N.S	Shallow sea grass bed
<i>H.plumieri</i>		A	A, J	A, J, E.J	Shallow sea grass bed
<i>H.carbonarium</i>	<i>J,E.J, E.S</i>	J, A *	A,J *	A, J	
<i>H. parra</i>	<i>J, E.J, N.S</i>	A, J *	A, J *	A, J	Shallow mangrove roots
<i>H.chrysargyreum</i>	<i>J, E.J</i>	A, J, E.J *	A, J *	A, J *	Shallow mangrove roots
<i>H. aurolineatum</i>		A, J, E.J *	A, J *	A, J *	
SNAPPERS (by ranked abundance)					
<i>Ocyurus chrysurus</i>	<i>J, E.J</i> *	A, J	A, J	A, J	Shallow patch reefs and back reefs
<i>Lutjanus. apodus</i>	<i>A, J, E.J</i>	A, J	A, J	A, J	Shallow mangrove roots
<i>L. griseus</i>	<i>A, J, E.J</i>	A, J *	A	A	Shallow mangrove roots
<i>L. jocu</i>	<i>J, E.J</i>		A		Shallow mangrove roots
<i>L. analis</i>	<i>J, E.J</i>	A	A	A	Shallow sea grass and around mangrove islands
<i>L. synagris</i>	<i>A, J, E.J</i> *	A	A	A	Shallow patch reefs and back reefs
<i>L. mahogoni</i>		A *	A	A *	Shallow patch reefs and back reefs
<i>L. cianopterus</i>	<i>J, E.J</i> *	A, J *	A, J *	A, J *	

Standard length of lutjanids by habitat had no specific patterns other than varied sizes by species and location (Figure 4). *Lutjanus juco* had the highest size range across locations and *L.synegris* and *L.mahogoni* had the smallest size range across habitats. For the other species like *L.griseus*, *L.analis*, *L.apodus* and *O.chrysurus* the size range distribution across habitat was slightly larger but not as large as that of *L.juco*. Although there was no conclusive size distribution pattern for lutjanids, when all species were compared, there was a significant difference between species at all locations. There also appears to be an increase in mean size as we move across the shelf, at least up to Gladden Split, with that pattern discontinuing at Glover's Reef atoll (Figure 4).

Abundance of lutjanids was not evenly distributed, but clearly showing that coastal mangroves are an important nursery habitat to many reef fishes. Placencia lagoon only had 3 visual survey study sites but still had the largest abundance of lutjanids; Laughing Bird on the other hand had the lowest abundance followed by Gladden Split and then Glover's. Most target species were recorded on all sampling sites through visual sampling, except *Haemulon aurlineatum*, *H.sciurus*, *O.chrysurus*, *L.synagris*, *L.cianopterus* and *L.mahogoni*. Species like *H.sciurus*, *O.chrysurus*, *L.synagris* and *L.cianopterus* were not recorded visually but are known to spend some part of their life history at mangrove estuaries and/or coastline from samples collected at this habitat or historical data.

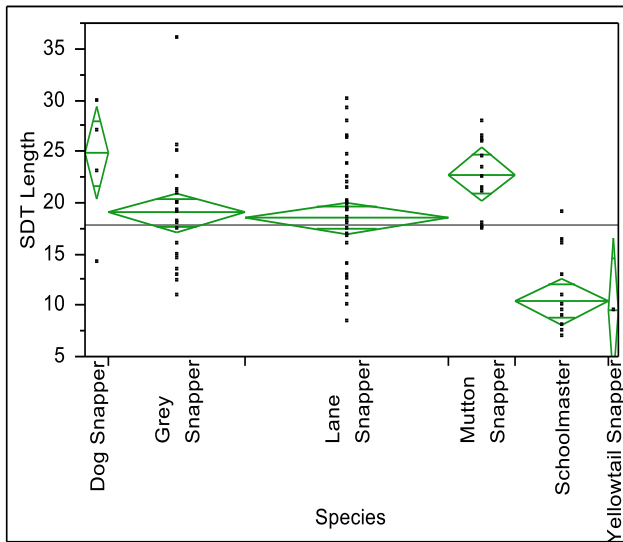
δ C stable isotope ratios

The δC isotopic ratio varied only for some species across habitats. *Lutjanus griseus* had the largest variation in its δC isotopic ratio, with the lightest carbon at Laughing Bird and the heaviest at Placencia lagoon. The other species that showed varied δC were *L.analis* and *O.chrysurus*, but with lower degree of variation between locations. The δC of *L.analis* was in two main groups, Laughing Bird and Gladden Split both had the lighter δC and Placencia and Glover's both had the heavier δC . *Ocyurus chrysurus* had varied δC , but more evenly separated by location. The δC were distributed from Laughing Bird to Gladden Split to Glover's to Placencia in the order of lightest to heaviest.

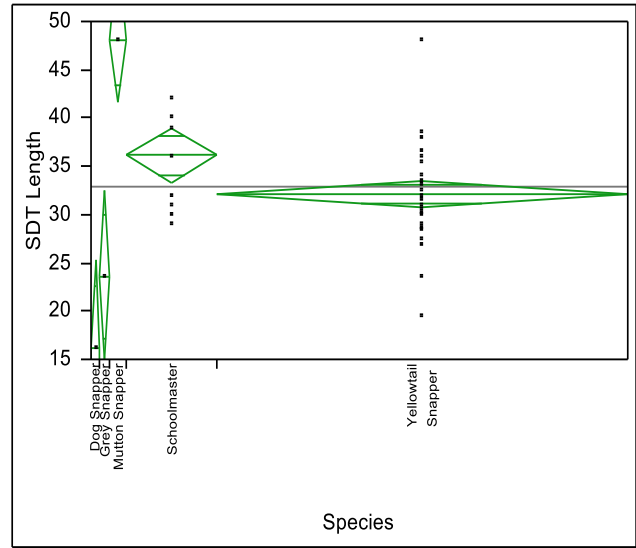
All other sampled lutjanids showed little variation in their δC across habitats, with *L.apodus*, *L.juco* and *L.mahogoni* having the smallest deviation and *L.synagris* varying slightly. Comparing all lutjanids to each other, there is a significant difference between species at all locations with an ANOVA, Placencia, Laughing Bird and Glover were 0.0001* and Gladden Split was 0.0019*.

δ N stable isotope ratios

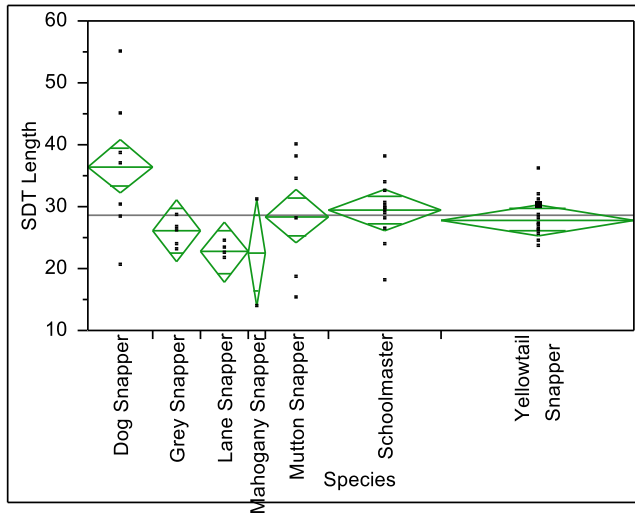
There was a lot more variation for the δN of all target species, except *L.mahogoni*, which only samples for Gladden were collected. The biggest variation on the δN isotope ratios were expressed by *L.juco*, *L.griseus* and *O.chrysurus*, although the pattern on which they varied across habitats was different for each species. For *L.juco* its lightest δN was Placencia followed by Gladden then Laughing Bird and then Glover's with the heaviest. *L.griseus* on the other hand had its lightest δN at Laughing Bird, getting heavier at Gladden then Placencia and heaviest at Glover's. *O.chrysurus* was lightest at Gladden and heavier at Glover's but even heavier at Placencia and Laughing Bird, which grouped together. The variation was still present on *L.analis*, *L.apodus* and *L.synagris* but much less



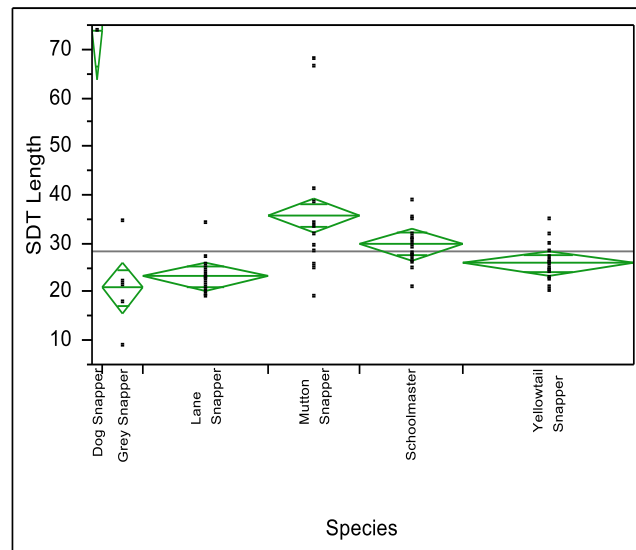
(a)



(b)



(a)



(d)

Figure 4 The average size distribution between habitats of lutjanids across habitats, (a) Placencia lagoon ($p < .001$) (b) Gladden Split ($p < .0029$), (c) Laughing Bird ($p < .001$) and (d) Glover's Reef atoll ($p < .001$).

compared to the latter three species. The pattern was also different between these species by locations. Apart from all the differences within species there were significant differences between species by location, this was true for all locations except Glover's (ANOVA: PL-0.007*; LB-0.0001*; GS-0.0003* & GR-0.0673).

To get a better understanding of how the stable isotope ratios differed between some species, we compared schoolmaster (*L.apodus*) and yellowtail snapper (*O.chrysurus*) and found that both species differed in how they used different habitats for feeding (Figure 5). The carbon SI ratios showed that target species change their carbon source, shifting from light sources in mangrove based estuaries to heavier seagrass and plankton sources (Figure 5a-b, 6). The nitrogen SI ratio showed how most of the target species are on higher trophic levels with increasing size (Figure 5c-d, 7). N can also be affected by N from inland sources and can also vary between species.

Even the relationship between size and stable isotope of these two species will need verification when samples are calibrated. These results are yet not conclusive until we properly calibrate all samples based on the primary production isotope ratios obtained from a common sea grass species on all four locations, turtle grass (*Thalassia testudinum*). This is the area where collaboration with other scientists in the area becomes important, mostly with Tim Smith, a independent consultant working in Belize and Will Heyman, a professor at Texam A&M University.

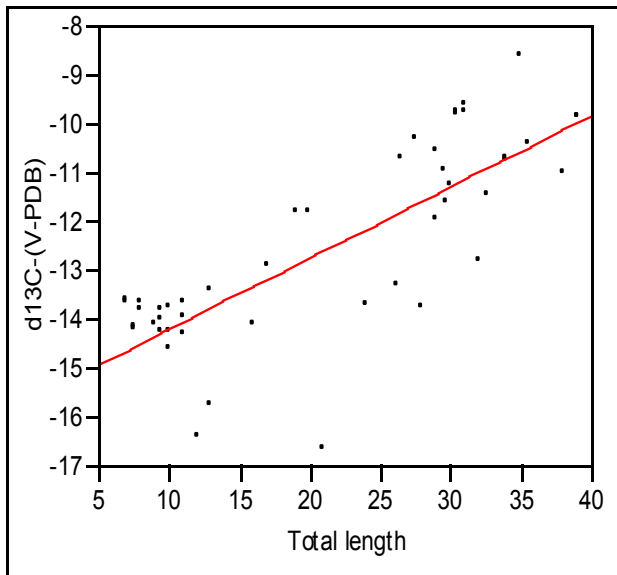


Figure 5a. Bivariate fit of d13C (V-PDB) by total length for *L. apodus*

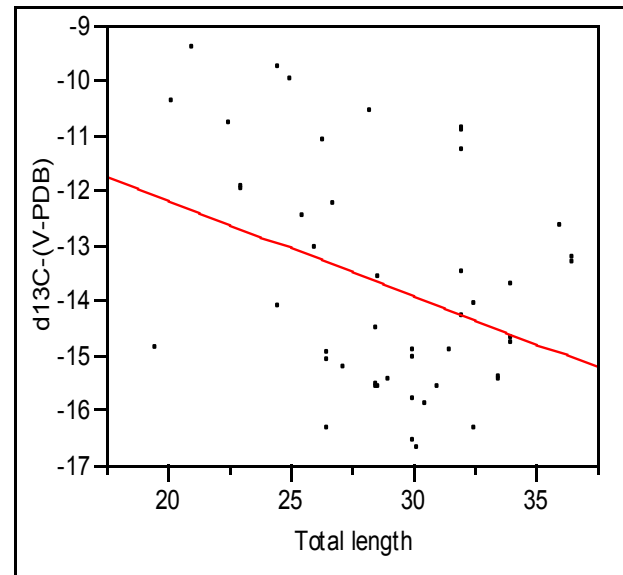


Figure 5b. Bivariate Fit of d13C-(V-PDB) By Total length for *O. chrysurus*

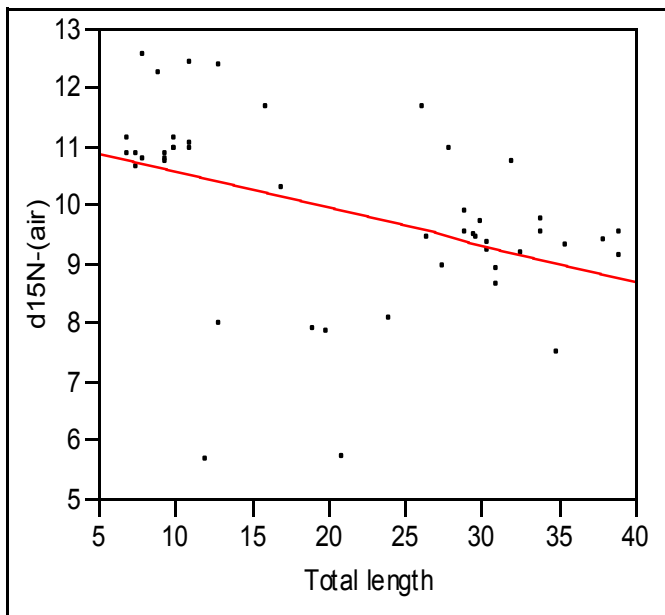


Figure 5c. Bivariate Fit of d15N-(air) By Total length for *L. apodus*

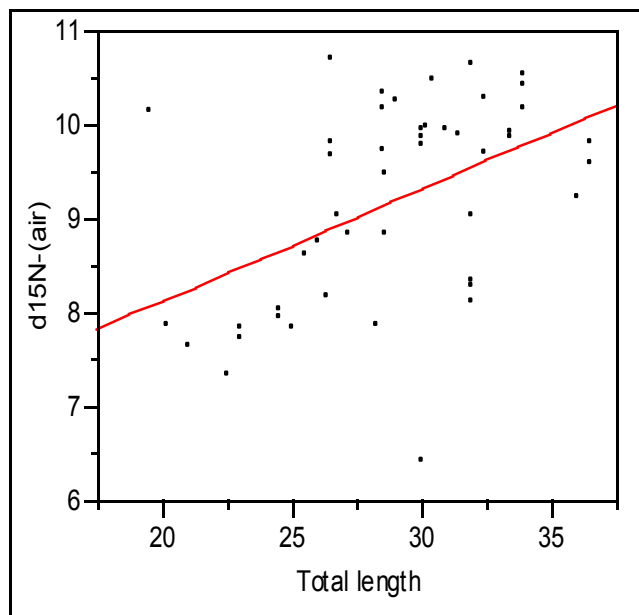


Figure 5d. Bivariate Fit of d15N-(air) By Total length for *O. chrysurus*

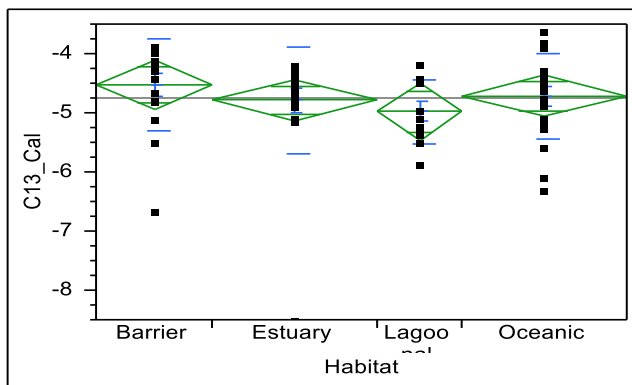


Figure 6 One-way Analysis of C13_ based on of habitat *L. apodus*

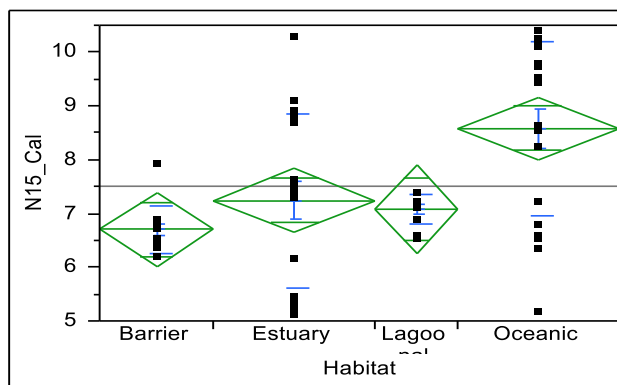


Figure. 7 One-way Analysis of N15 based on habitat of *L. apodus*

Grouper, particularly juvenile grouper, were too rare to draw inferences regarding juvenile habitat use. This in itself is of some practical concern. Juveniles of all of the commercially important snapper and grunt species observed during this study were concentrated, as expected, in mangrove and seagrass habitats in shallow back-reef and lagoonal environments. Gray, schoolmaster, dog, mutton, lane and yellowtail snapper all exhibited a clear pattern of the smallest juveniles occurring in greatest abundance within lagoons and close to shore, with the density maxima successively closer to barrier and fore-reefs with increasing size. However, lutjanid species exhibited a distinctive pattern of habitat use across the shelf. It was noted that schoolmaster snapper depend heavily upon mangroves, especially those near the coast and on offshore reefs as juveniles and the adults are found farther offshore. This species therefore relies strongly on inshore habitats to recruit, then shift across the shelf to coral reefs as they mature. Yellowtail snapper show a more variable pattern and are mostly an offshore species with all sizes occurring in vicinity of the mid-shelf, barrier, and atoll reefs. However they are still strongly reef associated, acting as zooplanktivores by day on coral reef and benthic carnivores by night in seagrass beds. They seem to benefit from the close proximity of seagrass and coral reef and don't rely heavily upon mainland nurseries.

The study determined that the life cycles of commercially important fishes are intricately linked to varied habitats across the Belize Barrier Reef shelf, from estuaries to inner shelf mangrove lagoons to the barrier reef and oceanic reefs. When looking at habitat connectivity along the continental shelf of Belize, mangrove habitats are a critical nursery ground for several commercially important fish species, and their removal can have adverse effects on populations of commercial species. The inner lagoon seagrass beds support juvenile life history stages while the barrier reef and oceanic reefs support a mix of sub-adults and adults. This was demonstrated through the abundance and size distribution of fishes with size increasing from mainland to offshore. Juveniles of all of the commercially important snapper and grunt species observed during this study were concentrated, as expected, in mangrove and seagrass habitats in shallow back-reef and lagoonal environments. Lutjanid species exhibited a distinctive pattern of habitat use across the shelf. Species such as the schoolmaster snapper relies strongly on inshore habitats to recruit then shift across the shelf to coral reefs as they mature. In general, commercially important reef fishes utilize the entire barrier reef shelf throughout their life histories, depending on mangroves and seagrass beds during early life history stages and moving to coral reefs in the larger stages as adults. Stable isotope analyses indicate that mangroves are used primarily as shelter, with most of the feeding that supports early growth of snapper and grunts occurring in the nearby seagrass beds and water column, principally at night. In order to ensure a future for its reef fisheries and tourism industries, it is essential that Belize maintain its fabric of mangrove, seagrass, reef, and inter-reefal habitats as a continuous belt throughout its national waters.

DATA

All raw data, maps and pictures collected during the study have been uploaded to an ftp site for access and downloading by CI.

PUBLICATIONS & DELIVERABLES

There is currently one paper in the process of being prepared for a publication in either Ecology or Conservation Biology.

An illustrated cross shelf poster is being finalized and printed to highlight the importance of protecting cross shelf habitats to ensure healthy fish stocks and sustained fisheries.

Recommendations on protecting coastal mangroves as fish nursery habitats have been presented to the Ministry of Natural Resources and should be incorporated into the revised Mangrove Regulations.

SCIENCE TO ACTION

The preliminary findings from the cross-shelf project were presented at the Science to Action workshop held on February 5th 2010. Findings were also shared with NGOs, government agencies, students and researchers at the 4th NRM Research Symposium on August 18th 2010.

The cross shelf connectivity of habitats critical to support healthy populations of commercial fish species has been actively highlighted and promoted in the mangrove regulation amendments. These amendments are still being finalized but should take into consideration the protection of mangroves as cross shelf habitats.

The Science to Action component of MMAS has incorporated the key message that it is necessary to protect the nursery areas (seagrass and mangrove), into discussions with the Fisheries Department, NGO protected area managers and Fishermen (cooperatives) and the recommendation to increase the area under protection has been actively pushed focusing on the need to include these nursery areas in no-take zones. It assisted with the designation of the pelican cayes no-take zone within the SWCMR in 2009.

The study reinforced previous studies that should fish biomass is very low across all habitats in Belize. This message was incorporated into the "Scrap the Traps" campaign trying to eliminate destructive fish traps and prevent an influx of Jamaican traps into Belize which would further stress and impact the already overfished environment.

Finally the poster which will highlight these linkages in an attractive life cycle drawing is being developed and when completed will be disseminated to fisherman cooperatives, NGO protected area managers, Government agencies that review development application such as the Department of the Environment, Lands Department, Fisheries, etc. The poster will serve to bring awareness of the critical link of mangroves to the life history of species such as snappers and groupers.

CAPACITY BUILDING

The project has contributed to capacity building for fisheries management within Belize through the Field Coordinator/MMAS Fellow, Eli Romero who is currently completing his M.Sc. thesis at Boston University to transfer to the PhD program. In addition the field assistant, Reylando Castro, gained training in surveys and visual ID of juvenile fish species. He has now been hired as a biologist with the Southern Environmental Association working with the Laughing Bird Caye National Park and the Gladden Spit Marine Reserve. A small group of UB students also benefited from SCUBA and survey training, who provided assistance during the second sampling period. Most of those students have put that knowledge and training to use as student research volunteers with the Environmental Research Institute at the University of Belize.