To the SEP Faculty:

The leatherback (*Dermochelys coriacea*) has been listed as endangered since 1970 and critically endangered since 2006. Of all the sea turtles, leatherbacks are unique in they have the large migrations in both the Pacific and Atlantic oceans. In 1979, protection of surrounding waters off nesting beaches was established as critical habitat for leatherbacks in Sandy Point, St. Croix, US Virgin Islands. Majority of conservation and protection efforts are concentrated on nesting beaches, but adults who migrate outside of the critical habitat zones are unprotected. A portion of the Pacific leatherback population migrate across the Pacific Ocean from nesting grounds in Indonesia to foraging grounds in neritic waters off California, Oregon, and Washington. Animals forage on large scyphozoan jellies during late summer and fall before moving south into warmer water for the winter months. In 2009, a revision to the critical habitat designation was proposed to include the US West Coast pelagic zone as a foraging ground. The extension of critical habitat designation to pelagic foraging grounds will help to protect mature adults who migrate to foraging grounds between breading seasons.

For my project I worked in collaboration with the Scott Benson of South West NOAA Fisheries and the National Marine Fisheries Service aerial observation team. Scott asked me to develop a method to help him quantitatively count surface aggregations of large jellies. I was responsible for developing the sampling design, training the observer team, and obtain funding for equipment. I was able to secure a grant of $1,200 to pay for equipment from the Dr. Earl H. Myers and Ethel M. Myers Oceanographic and Marine Biology Trust and the National Science Foundation, and was only one of two undergraduates to receive this award. This project taught me how to lead a team while conducting a field experiment, and how to work independently on my results. I really want to thank Lisa Webb of Moss Landing Marine Laboratories for guiding me through the entire process.

The goal of my scientific inquiry capstone was to develop an inexpensive, efficient method to quantify surface aggregations of large medusa to detect significant annual changes in the two most common prey for leatherbacks, the brown sea nettle (*Chrysaora fuscescens*) and moon jellies (*Aurelia* spp.). A photo sampling method was used to quantify surface aggregations of jellies for the Central California coast. Jellies for both species were manually counted, but due to the amount of time spent on analyzing one image an automated detection algorithm was developed. Quantifiable estimates of abundance will help critical habitat designation efforts for Pacific leatherback foraging grounds in neritic waters off the US West Coast.

I expect my findings to be consistent with the qualitative pattern Benson et al. (2007) observed. However, developing this new method to quantify jellies will allow more complex ecological questions regarding jellies or leatherbacks and their jellies prey to be addressed in the future.

Sincerely,
Erin Frolli

Mapping the distribution of *Dermochelys coriacea* by proxy: a method to quantify surface aggregations of *Chrysoara fuscescens* and *Aurelia* spp.

A Capstone Project
Presented to the Faculty of Science and Environmental Policy
in the
College of Science, Media Arts, and Technology
at
California State University, Monterey Bay
in Partial Fulfillment of the Requirements for the Degree of
Bachelor of Science

by
Erin Frolli
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Abstract
The leatherback turtle (*Dermochelys coriacea*) has been listed as endangered since 1970 and critically endangered since 2006. Major decreases in the global population have elicited conservation efforts. Waters surrounding nesting beaches in the US Virgin Islands have been designated as critical habitat. In January 2012 the pelagic foraging grounds along the US West Coast were also deemed critical habitat for this species. This was the first time pelagic foraging grounds have been designated as critical habitat for a turtle species. Critical foraging habitat must have sufficient quantities of prey available. The goal of this study was to develop an inexpensive, efficient method to quantify the most common prey of the leatherback turtle, the brown sea nettle (*Chrysaora fuscescens*) and moon jellies (*Aurelia* spp.), so that significant inter-annual changes in prey availability can be detected. An aerial photo sampling method was used to quantify surface aggregations of jellies, which serve as a proxy for total available prey, for the Central California coast. Manually counting jellies in aerial photos is time consuming. An automated detection algorithm was developed to produce population estimates. Estimated counts were compared with manual counts of the same photos. There was no significant difference between the methods indicating that the automated detection algorithm is a sufficient replacement for manually quantifying individual jellies in aerial photos of surface aggregations. The availability of these two jelly species will be used to help define important pelagic foraging habitats for leatherback turtles along the US West Coast.

Introduction
Leatherback turtles (*Dermochelys coriacea*) are the largest living reptiles lives most of their lives in Open Ocean (NOAA Fisheries 2010). On average adults weight over 800kg and are 2m in length (NOAA Fisheries 2010). Of all the sea turtles, leatherbacks are unique because they have large migrations in both the Pacific and Atlantic oceans (Benson et al. 2007a, Benson et al. 2007b, NOAA Fisheries 2010). Leatherbacks were listed as endangered throughout their range by the US Endangered Species Act in 1970 (US Endangered Species Act 35 FR 8495, 2 June 1970, NOAA Fisheries 2010). Risks for Pacific leatherbacks include nesting beach degradation by commercial and residential development, egg harvesting, and fisheries bycatch (Spotila et al. 2000, Sarti Martinez 2010). Published estimates of the mature adult female population have been reduced by at least 70% of the global population (Sarti Martinez 2010). Due to the major reduction in mature reproductive individuals over a ten year period, the World Union Conservation Red List listed the leatherback turtle as critically endangered in 2006 (Sarti Martinez 2010).

With major decreases in the global population, conservation efforts have been made to secure the future of the Pacific leatherback. In 1979, the protection of surrounding waters off nesting beaches was established as critical habitat, under the auspices of the Federal Endangered Species Act, for leatherbacks in Sandy Point, St. Croix, US Virgin Islands (NOAA Fisheries 2009). The majority of conservation and protection efforts are concentrated on nesting beaches, but adults who migrate outside of the critical habitat zones are largely unprotected. A portion of the Pacific

leatherback population migrates across the Pacific Ocean from nesting grounds in Indonesia to foraging grounds in neritic waters off California, Oregon, and Washington. Animals forage on large scyphozoan jellies during late summer and fall before moving south into warmer water for the winter months (Hughes et al. 1998, Benson et al. 2007, NOAA Fisheries 2009, NOAA Fisheries 2010). In 2009, a revision to the critical habitat designation was proposed to include the US West Coast pelagic zone as a foraging ground (Benson et al. 2007a, NOAA Fisheries 2010). The extension of critical habitat designation to pelagic foraging grounds will help to protect mature adults who migrate to foraging grounds between breeding seasons.

The availability of the leatherback's primary prey (large scyphozoan jellies) is used to determine the necessity of critical habitat protection for pelagic foraging grounds in neritic waters off the US West Coast (Benson et al. 2007a, NOAA Fisheries 2010). It is necessary to document the state of local jelly populations; an abundant, sustainable population is needed to provide evidence that the US West Coast is a viable foraging ground for migrating leatherbacks (Pers. Comm. Scott Benson).

In this study we will be developing a method to quantify two local species the brown sea nettle *(Chrysaora fuscescens)* and moon jellies *(Aurelia* spp.*),* both are known to be part of the leatherback diet (Benson et al. 2007a, Graham 2009, NOAA Fisheries 2009, NOAA Fisheries 2010). Aerial surveys off the central coast of California indicate leatherbacks and large jellies are found in retention zones (Benson et al. 2007a). Retention zones, or "upwelling shadows" (Graham et al. 1992), are hydrographic features where nutrient rich water is restrained nearshore in periods of wind relaxation during upwelling events (Graham et al. 1997, Graham et al 2001, Benson et al. 2007a).

In the past, aerial observers have used a qualitative scoring index to record surface aggregations of jellies during leatherback surveys (Benson et al. 2007a). Other existing methods are available to obtain jelly abundance include camera sleds, net trawls, side-scan sonar and bottom facing acoustic echosounders (Purcell et al. 2000, Graham et al. 2003, Houghton 2006, Graham 2009). However, these methods are not reliable in the upper few meters of the water due to orientation of the equipment (Purcell et al. 2000, Graham et al. 2003). Thus, there is minimal data for surface aggregations of jellies, where leatherbacks concentrate their dive time (Benson et al. 2007).

In this study we developed an inexpensive, efficient method to quantify surface aggregations of large scyphozoan jellies. Distribution data can then be used to calibrate the aerial observer's qualitative data from past flights to develop density estimates. This method can then be used during existing aerial transects, enabling quantifiable detection of inter-annual changes in jelly abundance. Quantitative estimates of jelly abundance will aid designation of critical foraging habitat for Pacific leatherback turtles in neritic waters off the US West Coast.

Methods

Field Methods

Aerial line-transect surveys were conducted for leatherback turtles in fall of 2010 off central California (N36:31; Fig. 1). All fine scale transects were flown using a twin engine fixed-wing aircraft (The Partenavia, P 68-OBS "Observer"; Fig 2). The aircraft was flown at a height of 198m above the surface of the water with a speed of 90-100 knots following methods described by Forney et al. 1991 and Benson et al. 2007a.

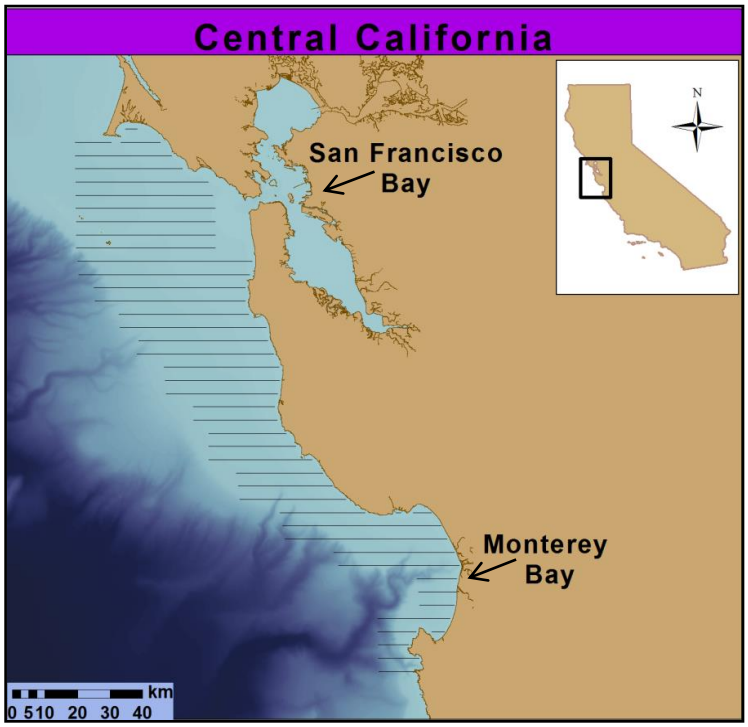

Figure 1: Map of the central California study site with aerial transect lines (<30km in length) ranged from Half Moon Bay (north) to Carmel Bay (south)

A five man crew conducted all surveys. An experienced pilot and data recorder sat in the nose of the plane. Two observers made animal sightings through the bubble windows directly below the wings on ether side of the aircraft. The third observer lying in the belly of the plane, and made animal sightings through the floor window positioned to see everything directly below the aircraft. All animal sightings were called out to the data recorder, through radio headsets (Fig. 3a), who took a GPS position of the siting and recorded aspect, angle, and species information.

Figure 2: The Partenavia, P 68-OBS "Observer"

During all transacts photographs of jelly aggregations were taken with a 10.1 megapixel digital still camera (Canon Rebel XTi, DS126151). The camera was set to aperture priority mode and a ISO of 1600 to ensure an appropriate shutter speed for all photographs. To increase color contrast of moon jellies compared with their surrounding water, white balance was set as a customized value. A photograph of a white piece of paper taken in full sunlight was used as the white value in the white balance setting. Resolution was set to the highest setting and focal length was set to 51mm to replicate the image seen by the observer's eye.

The camera was secured in a custom designed mount just forward of the belly observer (Fig. 2b). This allowed the aerial observer to take photos of jellies during transect lines while still observing the water for leatherback turtles and other species of interest. The camera lens extended through a rectangular hole in the top portion of the plexiglas, observing window to prevent image distortion.

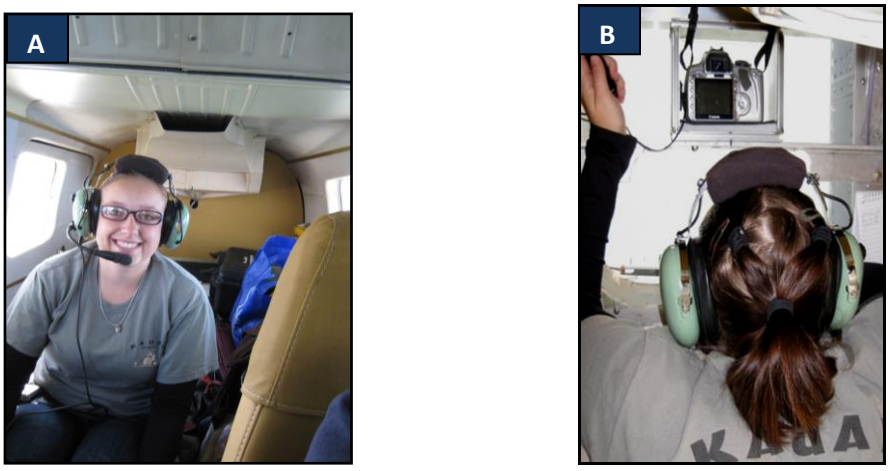

Figure 3: A. Belly observer siting up during takeoff. **B.** Belly observer in lying position with camera mount through the floor window of aircraft

For each transect, the data recorder recorded GPS locations when changes in jelly densities were seen by the three person observer team. As described in Benson et al. 2007a, observer density categories change on order of magnitude and individual jellies can be seen from the surface of the water to about 1m in depth. Figure 4 is three aerial images that depict the jelly categories that aerial observers could see during any given transect.

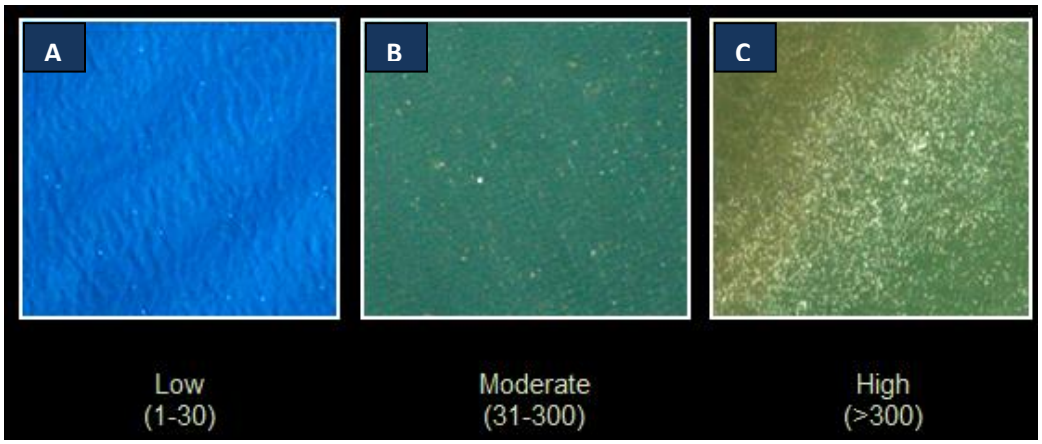

Figure 4: Examples of the three moon jellies observer density categories. **A**. Image of low: 1-30 individuals **B.** Image of moderate: 31-300 individuals **C.** Image of high: $\geq$ 301 individuals

Both species of interest brown sea nettle (*Chrysaora fuscescens*) and moon jellies (*Aurelia* spp.) are large enough to be identifiable from the aircraft (Benson et al 2007a, Graham 2009; Fig. 5). Individuals can be distinguished between based on color and shape. The brown sea nettle has a red-brown color with relatively long oral arms and the moon jellies aperies white with relatively short oral arms (Fig. 6).

Figure 5: Aerial image of two jelly aggregations

Photographs were taken randomly upon sightings of jelly aggregations. No images were taken when jellies were not present, to save space on the cameras memory card. The GPS location and elevation, from the radar altimeter for the aircraft, were recorded for photographs whenever possible by the data recorder. The camera's clock was synced with the GPS's clock in order to insure the correct altitude for all images.

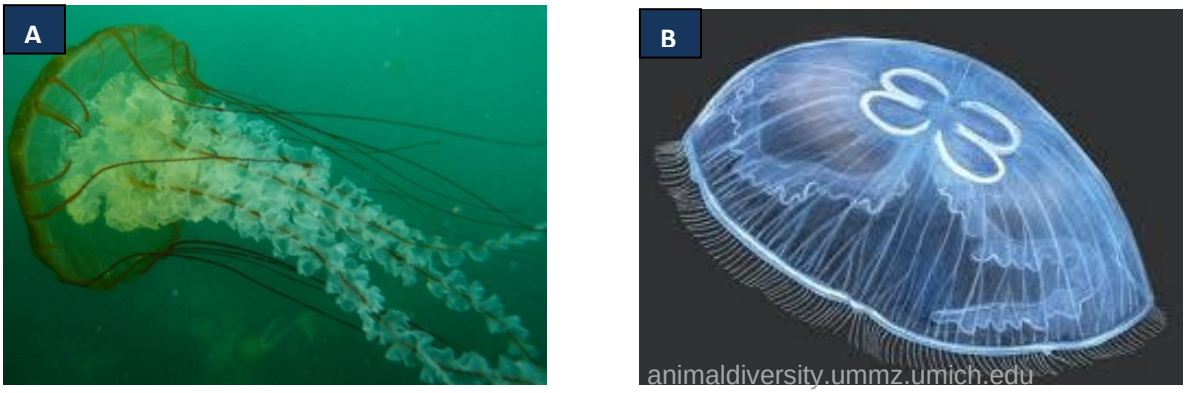

Figure 6: **A.** Image of brown sea nettle (*Chrysaora fuscescens*) **B.** Image of a moon jelly (*Aurelia* spp.).

Analytical methods

A total of 406 images were taken during aerial transects. All images were categorized by species to match the observer qualitative jelly density categories of low (1-30 individuals), moderate (31-

300 individuals), or high (≥ 301 individuals) using the image time stamp. To insure that images could be used to count jellies, photographs were eliminated from the data set by factors such as glare, no known jellies, and fog. Two hundred photographs remained and 31 images were analyzed using two programs, ArcGIS and MATLAB. ArcGIS was used for the manual count, because a point feature could be used to count and mark individual jellies. MATLAB was used to create an automated detection algorithm, because of its capability to upload and manipulate high resolution images. Each image took at minimum 5 hours to process, so only sixteen random images were used to analyze the brown sea nettles and fifteen images were used for moon jellies.

ArcGIS Method

Each photograph was given the projected coordinate system NAD 1983 UTM Zone 10N. A personal geodatabase was created for each photograph and projected to the same coordinate system. Distinct individuals were identified based on shape and color, and were marked with a single point feature to obtain a proxy of the total number of jellies seen in the photograph (Fig. 7). All photos were analyzed by the same person to minimize human error. After each photograph was thoroughly checked for jellies the total number of jellies was recorded. Average time to analyze an image was 4hours.

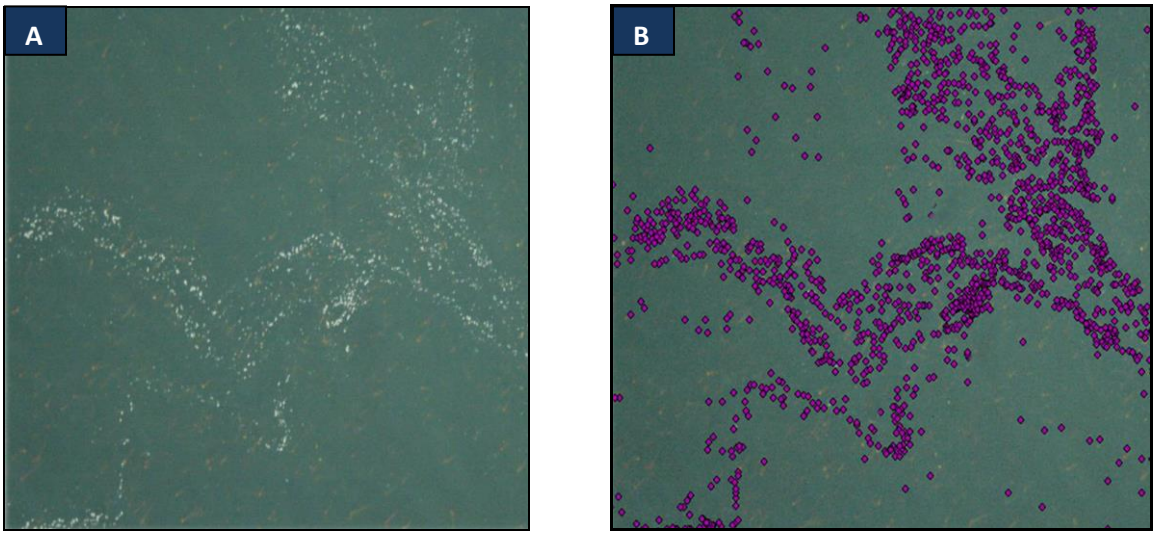

Figure 7: **A.** Original aerial image with a large moon jelly aggregation **B.** same image after individual moon jellies were marked with a single point feature (purple).

MATLAB Methods

After the manual count was completed in ArcGIS, each photograph was analyzed using an automated detection algorithm in MATLAB (Appendix). For this method a custom code was developed using pixel's red, green and blue values (RGB values) to obtain a total surface area of the image that contains jellies. RGB values were collected for each species in different areas of the image to account for water color, glare and other oceanographic conditions. The maximum and minimum RGB values were used to define the algorithms selecting requirements for pixels

that contain jelly color. An “If Than” loop was used to examine all individual pixels of the image systematically; starting with the first pixel in the top left corner of the image to the bottom right pixel. Once the program came upon a pixel containing the appropriate requirements, it was counted and the blue value was changed to the highest reflection value (225). Once the loop assesses all pixels within the image, the image is displayed with the new tagged pixels for ocular verification that the program is selecting appropriate pixels that contain jellies (Fig. 8).

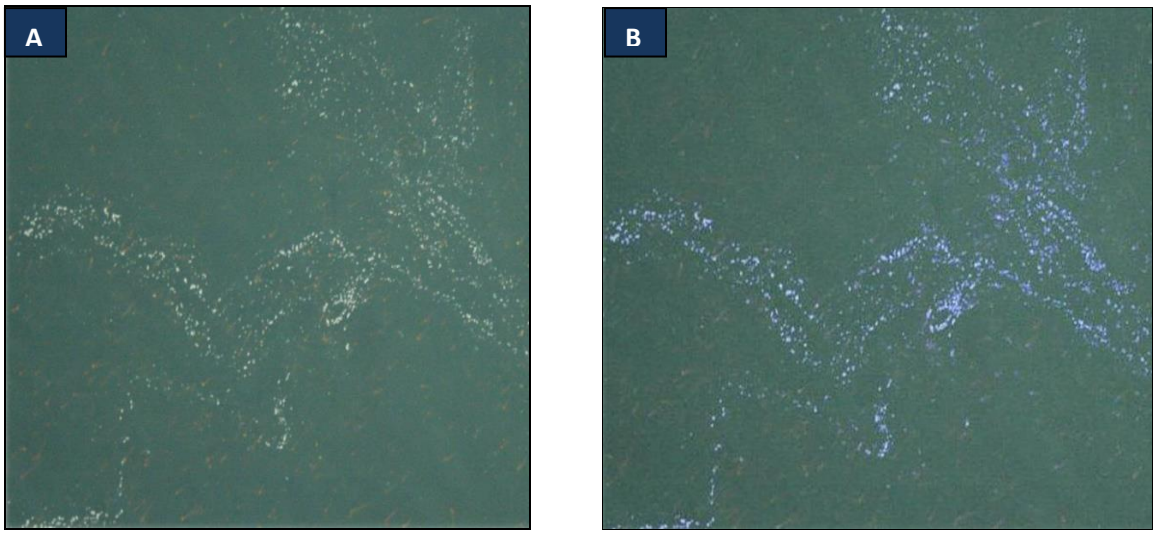

Figure 8: **A.** Original aerial image with a large moon jelly aggregation **B.** same image after pixels containing the appropriate RGB values were counted and the blue values were changed to the highest reflection value (light blue).

The total number of jellies registered by the program is

$$T_{Jellies} = \frac{N_{Pixels}}{\bar{x}_{SA}}$$

where N_{Pixels} is the total number of jelly colored pixels counted by the program and $\bar{x}_{SA}$ is the average surface area in pixels per species of jelly. Average time to analyze an image was 15 minutes.

To find the average surface area of jellies for each image the original photograph in ArcGIS was used. A vector was made for each photograph to include all the values from 1 to n, were n is the number of jellies counted in the photograph. Using the program R (R Development Core Teams. 2006) the vector was randomly sampled from using a sampling function to obtain 100 randomly selected jellies.

For images containing moon jellies, the *Select Attribute* function was used to locate each of the 100 randomly selected individuals. Moon jellies appear round in an aerial view, so bell diameters were measured in pixel width (assuming horizontal orientation) and recorded in the attribute table (Fig. 9). The bell diameters were used to calculate the average surface area of moon jellies in pixels.

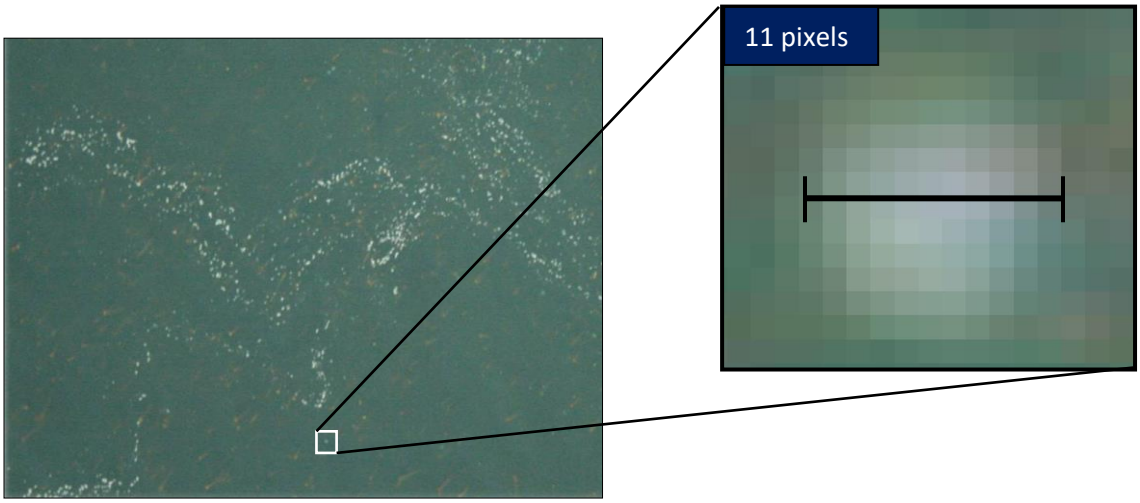

Figure 9: A magnified single moon jelly with bell diameter

Brown sea nettles appear to have a rectangular shape, because they are most often seen horizontal in the water column with both bell and oral arms extended (Fig. 10). Surface area was treated as a rectangle with length and width measurements in pixels and recorded in the attribute table. Average time to measure jellies was 1hour.

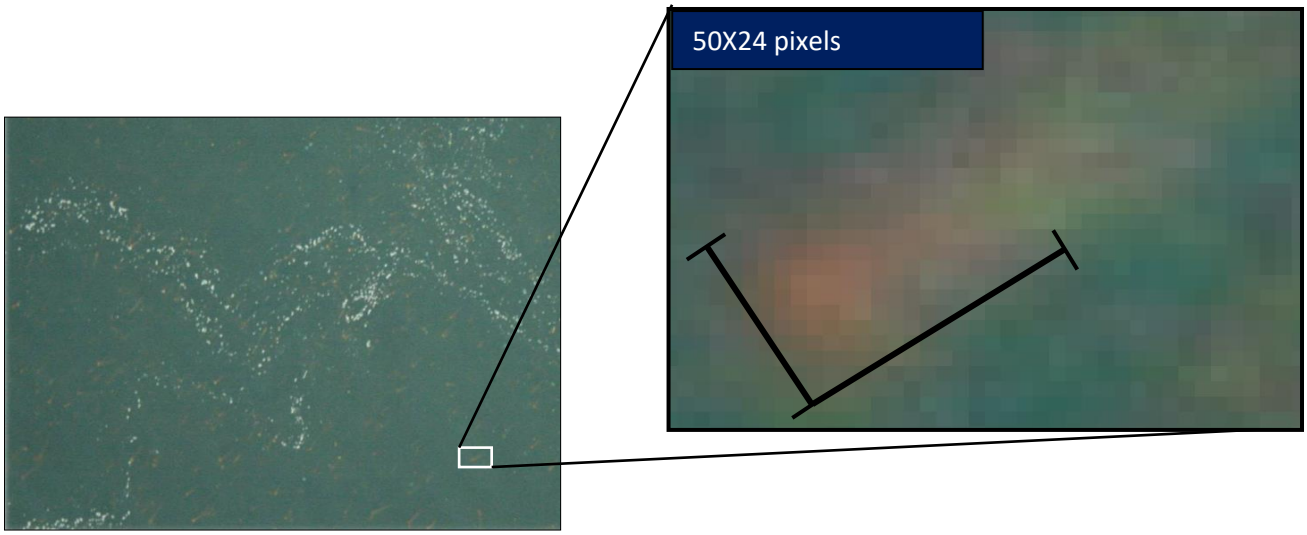

Figure 10: A magnified single brown sea nettle with length and width

Analyzing the Aerial Observer Counts:

Habitat maps were made for each flight day and contained three important factors 1) the starting and ending points of each transect 2) the changes in jelly abundance categories along all transects, and 3) turtle locations were included in all maps. The transect end points were used to measure the total length of each transect and to indicate when observers were "on effort" or when observers were recording animal sightings. Points were also recorded every time the overall abundance in jelly aggregations changed. These points were used to measure the length of each density category segment. Turtle sittings were used to show turtle locations in relation to jelly aggregations.

To determine the jelly density seen during any given transect, strip transect areas were used. Since the camera focal length was set to that of the human eye (51mm), the image width can be used as a proxy for strip-transect width. The *Law of Sines* was used to determine the image width. The camera's angle of view was determined by taking photographs of known measurements at a known height and perimeter. Since all photographs were taken at the same focal length the angle of view was the same for each image. The time stamp was used to locate the altitude of the camera when the photograph was taken. If we assume that the height of the plane is perpendicular to the transect line, then the actual width distance of the area covered by the photograph can be determined (Fig. 11). If θ_w is the angle of view for the photograph

$$W_{Photo} = Tan\ (\theta_w) * a$$

where W_{Photo} is the width of the area covered by the photograph and a is the altitude of the camera.

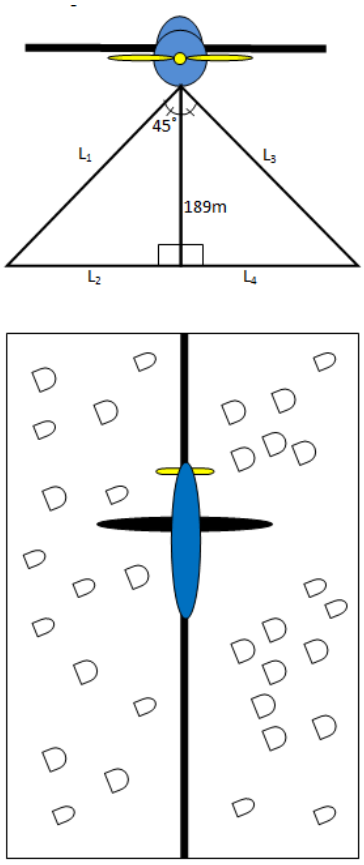

Figure 11: A schematic of the aircraft flying along the transect line with camera angle depicted at the top of figure

The area for each jelly density category (low, moderate, and high) was calculated individually along all transects (Fig. 12). The length between each density change point was measured using spatial analysis tools in ArcGIS and multiplied by the strip width (photograph width) to determine the total area.

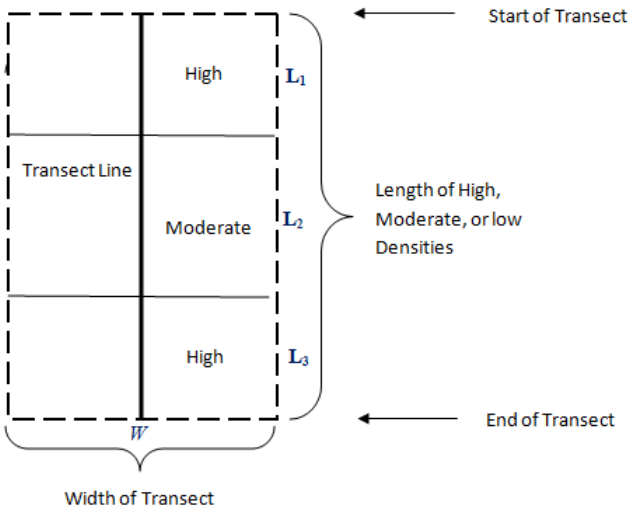

Figure 12: Example transect with changes in jelly densities. The solid lines are the extension of the GPS points marked for the change in jelly densities. *W* is the width of the strip-transect and L1, L2 & L3 are the lengths of each density category.

The mean jelly density was calculated for the whole transect using the average number of jellies for a given image of each category. An ArcGIS interpolation method was used to estimate the total abundance of jellies between each transect line for the Monterey Bay survey area. New maximum and minimum jelly densities were calculated for each abundance category.

Statistical Methods

We tested the hypothesis that there was no difference in the means of the two counting methods. With the alternative being that there was a difference between the two methods. We tested our hypothesis for each species, because they aggregate differently. A Student's t-test was used for data that met the assumptions. A Wilcoxon rank sum test was used when count data were not normally distributed.

Observer density category guidelines were adjusted to calibrate the observer counts with the actual counts obtained with the automated detection algorithm. Thirty additional randomly selected images were analyzed by the automated detection algorithm only. Five images for each category type and species were analyzed in addition to the 31 original images to obtain the maximum and minimum number of jellies for each category.

Results

Fifteen images were analyzed using both counting methods. There was no significant difference in brown sea nettle counts derived by ArcGIS and Matlab counting methods (Fig. 13, W = 119.50, p-value = 0.763).

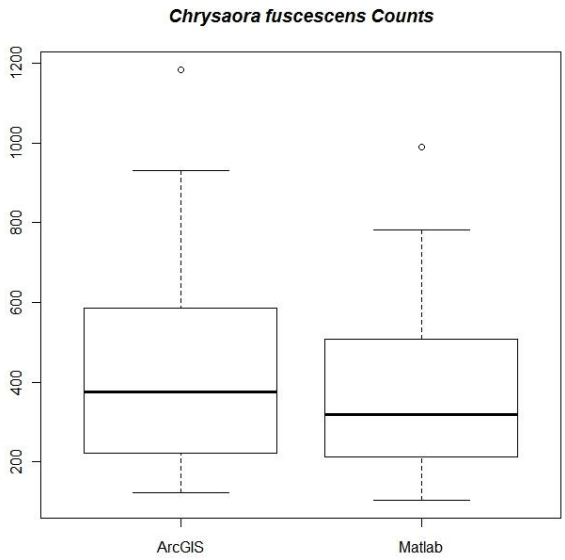

Figure 13: Box plots of total brown sea nettle (*Chrysaora fuscescens*) counts using ArcGIS (manual) and Matlab (automated) counting methods

There was no significant difference in moon jelly counts derived by ArcGIS and Matlab counting methods (Fig. 14, df = 28, p-value = 0.21).

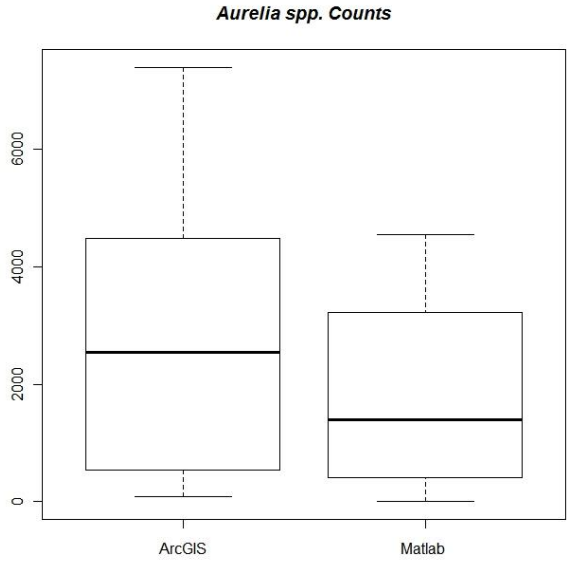

Figure 14: Box plots of total moon jelly (*Aureila* spp.) using ArcGIS (manual) and MatLab (automated) counting methods

Fifteen original images and the additional 15 images using only the detection algorithm were used to create new observer guidelines for brown sea nettles (Table 1).

Table 1: Observer Guidelines for *Chrysaora fucescens*

	Low	**Moderate**	**High**
Historic Guidelines	1-30	31-300	301+
Revised Guidelines	1-200	201-500	501+

Sixteen original images and the additional 15 images containing moon jellies were analyzed using only the detection algorithm were used to create new observer guidelines for moon jellies (Table 2).

Table 2: Observer Guidelines for *Aurelia* Spp.

	Low	**Moderate**	**High**
Historic Guidelines.	1-30	31-300	301+

Revised Guidelines	1-300	301-2500	2501+

Discussion

We found no significant difference between jelly counts obtained with the ArcGIS manual counting method and the Matlab automated detection algorithm indicating that the automated method is a viable replacement method for assessing the abundance of jellies. The amount of time spent processing photographs was reduced from three to four hours per photo with the manual method to 12 to 15 minutes per photo when using the automated method.

Potential limitations to this algorithm include misidentification and underestimation. Miss identification can occur when other objects in the water have RGB values that meet the algorithms requirements for identifying jellies. For example, the program may not be able to distinguish a patch of foam from several moon jellies based on surface area and color alone. In cases of moderate to high aggregations the ability of the algorithm to detect individuals may also be limited because individuals tend to stack on top of each other with overlapping bells and oral arms, thereby underestimating the number of jellies.

Despite the possibility of incorrectly counting some pixels as containing jellies when they do not, the advantage of using the detection algorithm is the dramatic decrease in time spent analyzing photos. The majority of the pixels containing jellies will have been identified and the highlighted image that results after running the program can be visually examined to count missed pixels. The time saved by using the automated detection algorithm allows larger data sets to be analyzed in a shorter period of time.

The ability to process numerous photographs in a short amount of time will enable count estimates to be produced more efficiently. While the count estimates only represent the surface aggregations and therefore are the minimum jelly densities, the automated counts provide a conservative, qualitative number of jellies observed. NOAA funding has been abated every year since the line-transect surveys were established for leatherbacks (Benson et al. 2007a, NOAA Fisheries 2009). The methods described here can produce additional data such as jelly size and distribution information that can be housed in a database. This will be a valuable addition to data collected through the existing surveys, but with minimal expense.

We found that the observer guidelines should not the same for both species, so when calibrating the past data we have to take into account that the spices aggregate differently. Though both species have the classic bell shape, their oral arms are different. Moon jellies don't have large oral arms, and seem to cluster closer together thereby allowing for observing more individuals at the surface of the water. Brown sea nettles seem to cluster further apart from each other with the extension of their larger oral arms (Graham et al. 2003). These aggregation patterns have been

seen over the years by the leatherback aerial observers, and were captured by the photographs taken during fall 2010 (Benson et al. 2007a).

The new estimates for observer guidelines can also be used to calibrate observer qualitative counts from flights conducted after 2010. The actual number of observed jellies was on the order of magnitudes larger than the guidelines that observers use to estimate density for both species. With the better foundation of the minimum surface density of each category, estimates of density from past data can be established for years prior to 2010. Since the observer flights have been established for leatherbacks by NOAA every year funding is available (Benson et al. 2007a, NOAA Fisheries 2009), this method can be reproduced as an addition to build a database for jelly size and distribution.

Development of habitat maps can be used to demonstrate were leatherbacks are consistently found in relation to jelly aggregations. Habitat maps will help conservation efforts for the designation critical foraging habitat, because they will visually demonstrate where surface aggregation hot spots (or dense aggregations) are and if they are consistent over the years. We can also monitor if these aggregation areas move spatially from year to year to predict were leatherbacks will be sighted in future years.

To improve this method, better technology is needed. The budget for this project was small. The camera was donated for the duration of the project and had a relatively low resolution. A camera with greater resolution may help the detection algorithm to distinguish individuals in greater density aggregations because of the improved ability to detect the edge of a jelly, but overlapping individuals may still pose a problem. Other improvements to the camera system should include attaching and altimeter and GPS (photogrammetry systems) so that the photograph data includes the exact GPS and measurements of plane height (Geodetic Services, Inc. 2006, Gilpatrick 1996). For the purposes of this project the ability of the algorithm to detect individuals was efficient and repeatable for future years at a relatively low cost.

A major limitation to this method is that we were unable to assess whole jelly aggregations including jellies at depth. The aerial observer method is limited to observing the surface aggregations to roughly 1m in depth depending on water clarity, light, and sea state (Benson et al. 2007a, Forney et al. 1991). Currently there is not a single technology able to capture the full extent of jelly aggregations (Purcell et al. 2000; Graham et al.2003; Houghton 2006; Graham 2009).

Our future goal is to apply the revised density category guidelines to data from past years to create revised and more accurate jelly density estimates. Assuming that jelly populations have aggregated at the surface in similar ways in any given year we can infer that the revised density category guidelines can be used to calculate qualitative estimates for jelly abundance for flights conducted for years prior to 2010. A database can then be established and monitored for changes in the abundance of jellies among years. These data can also be used to predict areas of high

density surface aggregations or “hot spots” that can be extremely important for leatherback foraging habitat (Benson et al. 2007a, NOAA Fisheries 2009).

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Appendix:

Jelly Counting Code (Used in MATLAB)

```
%% Jelly_Counting_code.m
% Written by Erin Frolli
%For copy of the m file please contact Erin Frolli at efrolli@csumb.edu

% This code was a product of Erin Frolli's 2010-2012 Capstone project @ CSU
% Monterey Bay.

% The code was designed to automate counting of individual
% jellies from Aerial photographs taken from the belly window of a
% twin-engine fix wing aircraft. All transects were conducted using three
% person observer team in a twin engine fix wing following methods
% described in Forney et al. 1991 and Benson et al. 2007. The height of
% the plane during transects was 198m with a speed of 90-100 knots as
% previously described in Forney et al. 1991 and Benson et al. 2007.

% For this code to work the photograph must be in a format that allows
% Matlab to surch through the digital RGB values of each pixel. Before this
% program is run you must also have the average surface area in pixels of
% each species for the last section of the code.

% NOTE: Replaces the Average_Jelly with the correct average surface area
% (in pixels) of the species of interest. Do so below
% for Average_Jelly_Surface_Area

Average_Jelly_Surface_Area = 380.92;

% Please note that this code has to be run one photograph at a time.

%% Uploading the Photo

% NOTE: make sure that the photos that you would like to use are in a
% folder in the Matlab directory. Make sure that the folder is opened so
% that Matlab is pulling the photos directly from this folder when the
% imread function is activated.

% The imread function is used to load your image into Matlab as a large
% matrix of both position and RGB values for each individual pixel of the
% photograph. F

% NOTE: For this function you must have the whole name of the photograph
% with file extension in (' ').

I=imread('IMG_0007.JPG'); %Loads the photo for use as a matrix. Note that
% named the photo I for future used and for simplicity.

% The imshow function will open a figure window that shows you the photo
```

```
% matrix as the actual photo. We bring up the photo first to make sure
% that we have the correct photo and second to collect X,Y coordinates of 5
% jellies around the photo in order to build our pixel database.

imshow(I) %brings the original photo up in a separate window

% Once the photo is up in the second window look at the top tabs.
% Tools > Data Courser
% This tool will turn the courser into a t-shape. This function will now
% allow you to find X,Y coordinates of 5 areas of interest. It would be best
% to zoom completely in on individual jellies so that you can select the
% appropriate pixel.  For the imcrop function that you will be using in the
% next section of this code you will want the top-left most corner of the
% jelly.  Only need this coordinate for the code.

% Its best if you see the photograph having four quadrates.  Then choosing
% a jelly that best represents the color that is seen in that quadrate
% (i.e. so that we can capture all attributes of this photo graph including
% glare, depth, watercolor, and fog).  For the fifth jelly pick a one that
% is in the center of the photo.

% Once this is done you can run the remaining code.  AFTER you imput the
% 5 coordinates and average jelly size.

%% Collecting Pixel Color Data

% This section crops the photo around the five individual jellies that you
% have chosen as your representatives of the photo.

% The imcrop function useses the original photo (in this case I) with a
% small matrix.  The first two values of the matrix are your X,Y coordinates
% of the jelly.  The second two numbers is the size of the area that you
% would like to crop.  For example, (I,[120 825 6 6]) says that I would like
% to crop from photo I into a 6X6 matrix starting at the top left corner
% at (120,825).  If you want to make sure that the program has cropped the
% correct area of interest please use the imshow code that has been made
% into a note by putting a % in front of the code.

C1=imcrop(I,[120 825 6 6]); % Crops first area of interest
C2=imcrop(I,[1039 444 6 6]); % Crops second area of interest
C3=imcrop(I,[3024 415 6 6]); % Crops third area of interest
C4=imcrop(I,[716 2469 6 6]);% Crops fourth area of interest
C5=imcrop(I,[1958 1296 6 6]); % Crops fifth area of interest

% Use imshow to shows your cropped areas of interest
%figure
%imshow(C1)
%figure
%imshow(C2)
```

```
%figure
%imshow(C3)
%figure
%imshow(C4)
%figure
%imshow(C5)

%% Export RGB Values

% If you would like to import the pixel data into excel you would use the
% impixel function to collect the RGB values for you to export.  The FULL
% range of coordinates has to be put into the two vectors in order to
% obtain the full all of the RGB values.

% Note this sections is noted out because we do not need to export the data
% in order to run the code.
% Xs=[120:126,1989:1996,673:679,3421:3428,1988:1995];% x coordinates range
% Ys=[825:831,683:690,2229:2235,2398:2405,682:689];% y coordinates range
% RGB_Values=impixel(I,Xs,Ys); % Stores all of the RGB values from all three
% sets into a single matrix
%% Finding the Max and Min RGB Values

% In this section of the code we are finding the maximum and minimum RGB
% values in order to define the strict range of color we would like MatLab
% to find.  This will use the cropped data from before.

% First we must combined all of the cropped values together in once matrix.
% Since we would like to stack the values we write the vector as such.

RGB_Values = [C1 % Vector of RGB values
   C2
   C3
   C4
   C5];

M_=max(RGB_Values);
MAX = max(M_);% Finds the OVERALL Max values of the RGB Values
M__=min(RGB_Values);
MIN = min(M__);% Finds the OVERALL Min values of the RGB Values
MAXr=MAX(1,1,1); % names the Max red value
MAXg=MAX(1,1,2); % names the Max green value
MAXb=MAX(1,1,3); % names the Max blue value
MINr=MIN(1,1,1); % names the Min red value
MINg=MIN(1,1,2); % names the Min green value
MINb=MIN(1,1,3); % names the Min blue value

%% Pixel Finder Algorithm

% In this section we will be finding the overall number of pixels contained
```

```
% in the photo that are in our specific maximum and minimum RGB values.

% We first need to find the size of the photo.  Which we have named [Y X
% color].
[Y X color]=size(I);

% We then need to define an empty vector to hold our overall number of
% pixels found with our algorithm.
count=[0]; % Starts the pixel count at 0

% We are going to use a double For-End lope that has an IF statement for
% each RGB color. This tells MatLab to start at the top-left corner of the
% photo matrix and move from pixel to pixel looking for the defined RGB
% requirements.

% NOTE: That the last column of the photograph is data storage of the
% photographs properties not color data.

% We are using the maximum and minimum values to define the IF statements
% for each RGB values
% 1 = r = red
% 2 = g = green
% 3 = b = blue

% Once a pixel is found it is tagged using the brightest red value
% (225 = Bright Red). This is done so that the technician can double check
% that the code was finding the correct values when searching through the
% photograph.

% Once the pixel is tagged red it is added to the count. This number
% always replaces the number before it in the count vector.

for y = 1:Y-1; % tells the code to start at the first pixel in the top-left
%  and go through the whole photo until the last row

for x = 1:X-1; % tells the code to start at the first pixel and go
% through the whole photo

if(I(y,x,1)>= MINr)&&(I(y,x,1)<= MAXr)...
&&(I(y,x,2)>= MINg)&&(I(y,x,2)<= MAXg)...
&&(I(y,x,3)>=MINb)&&(I(y,x,3)<= MAXb);

I(y,x,1)=225; % highlight all pixels that match the correct criteria

count=count+1; % includes this pixel to the count
end% ends first loop
end% ends second loop
end% ends third loop
```

```
% Once the lope is finished MatLab brings the original photo up in a
% separate window with new highlighted pixels shown.
imshow(I)
%% Species Counter

% Final Number of jellies estimated by the algorithm comes from the number
% of pixels tagged in the photograph (count vector number) divided by the
% average surface area (in pixels) of the species of interest.

% The format long function is used to expand your number of interest out to
% 15 decimal places for a more accurate number.

format long
No_Jellies = count./Average_Jelly_Surface_Area

% FINAL NOTE: Make sure that you Clear all and Close all when you start a
% new image so that you do not overload MatLab with several different
% large matrixes.
```