ESTIMATING THE FECUNDITY OF MONCHONG, *EUMEGISTUS ILLUSTRIS*, AT CROSS SEAMOUNT

A SENIOR THESIS SUBMITTED TO THE GLOBAL ENVIRONMENTAL SCIENCE UNDERGRADUATE DIVISION IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

BACHELOR OF SCIENCE

IN

GLOBAL ENVIRONMENTAL SCIENCE

JANUARY 2014

By

Chase R. R. Roberts

Thesis Advisors

Kevin Weng

Christopher Kelley

We certify that we have read this thesis and that, in our opinion, it is satisfactory in scope and quality as a thesis for the degree of Bachelor of Science Global Environmental Science.

THESIS ADVISORS

Kevin Weng Department of Oceanography

Christopher Kelley Department of Oceanography

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to both of my advisors, Dr. Kevin Weng and Dr. Christopher Kelley, for the continuous support of my senior thesis project. Their patience, motivation, enthusiasm and immense knowledge helped me throughout my study and writing of this thesis. Special thanks to Dr. Kevin Weng for allowing me the opportunity to work on this project.

I also would like to thank Andrew Gray for teaching me how to use all the equipment necessary for this study, as well as providing assistance in gathering samples for my project.

Additional thanks to the Honolulu Fish Auction and the various Cross Seamount fishermen who were able to provide these samples.

Thank you to Jeffrey Drazen for allowing me to use his lab and equipment.

I also would like to thank Bob Humphreys and Ed Demartini for their contributions to my paper.

I would like to express my appreciation to Mike Seki, NOAA PIFSC, and PFRP for helping fund this research.

Lastly, I would like to thank my family and friends who supported me throughout this project.

ABSTRACT

The lustrous pomfret or monchong (*Eumegistus illustris*), is an important resource for the fishing industry here in Hawaii. The main objective of this study was to understand oocyte maturation, seasonality of spawning, and estimate fecundity of this species, to improve our understanding of its biology and inform management of the fishery. Results of this study suggest that E. illustris exhibit asynchronous ovary development. Five ovaries were sampled from monchong specimens caught at Cross Seamount, of which three showed oocytes at advanced stages of maturation. These fish were obtained from three of the four seasons in a year: one from spring, one from summer and three from fall. Fecundity estimations from this study suggest that the lustrous pomfret population at Cross Seamount are probably spawning year round. Fecundity calculations estimate the number of eggs a female can release at one time during the spawning season. Oocyte size distributions were obtained by taking aliquots from each ovary, photographing groups of oocytes from each aliquot under a microscope, and using image analysis software to count and measure the oocyte diameters. Batch fecundity was estimated either by counting hydrated oocytes, or counting oocytes in the mode undergoing maturation. E. illustris were estimated to spawn an average of 167,613 +/- 38,405 oocytes in each batch and 28,791 +/- 8,617 oocytes per kilogram.

iv

TABLE OF CONTENTS

Acknowledgements	iii
Abstract	iv
List of Tables	vii
List of Figures	viii
Chapter 1: Introduction	1
Eumegistus illustris	1
Reproduction	3
Reproduction strategies of teleosts	5
Fecundity	7
Stages of oocyte growth	9
Key Questions	12
Chapter 2: Methods	13
Obtaining ovary samples	13
Oocyte samples	13
Image analysis	14
Fecundity estimations	15
Chapter 3: Results	17
Size-frequency distributions	17
Advanced staged oocytes	22
Estimated Fecundity	23
Chapter 4: Conclusion	24
Oocyte maturation	
Seasonality of spawning	24
Fecundity	25

Imp	plications for fisheries	26
Futi	ure studies	26
Bibliogra	aphy	28

LIST OF TABLES

	<u>Table</u>	Page	
1.	Fecundity estimations	23	

LIST OF FIGURES

	<u>Figure</u> <u>Page</u>
1.	Lustrous Pomfret (<i>Eumegistus illustris</i>)1
2.	Cross Seamount fishing boat and catch 3
3.	Typical pattern of spawning species 6
4.	Size-frequency diagrams of the three types of ovary development7
5.	Most developed mode in a species with asynchronous development 9
6.	Oocyte stages of the Gilthead seabream (Sparus aurata) 11
7.	Oocyte samples
8.	Size-frequency distribution of FISH 852 18
9.	Size-frequency distribution of FISH 841 19
10	. Size-frequency distribution of FISH 80819
11	. Size-frequency distribution of FISH 851 20
12	. Size-frequency distribution of FISH 813 21
13	.Healthy vs Degraded Oocytes22
14	Largest 10% VIT and FM oocytes

CHAPTER 1. INTRODUCTION

Eumegistus illustris

The lustrous pomfret (*Eumegistus illustris*) is one of two species locally known in Hawaii, as monchong. This species inhabits deep ocean environments and is known for its oval-shaped, black body with hard scales, as seen in Figure 1. Unlike the pelagic sickle pomfret (*Taractichthys steindachneri*), *E. illustris* appears to associate with bathymetric features such as deep slopes and seamounts (i.e. bentho-pelagic). Monchong are the target of an important fishery at Cross Seamount, located 300 kilometers south of Honolulu. Fishing pressures around the area have raised concern with fisheries management. The North Pacific Fishery Management Council (NPFMC) and NOAA are interested in sustainability of the fishery in order to protect a valuable resource.



Fig. 1: A lustrous pomfret (*E. illustris*) caught at Cross Seamount.

Cross Seamount has been a popular fishing spot for many years, with fisheries targeting bigeye tuna and more recently monchong. Figure 2 shows a

fishing boat at Cross Seamount along with some of their catch. There has not been a stock assessment conducted for the latter species. The two monchong species have not always been separated in catch reports, and so there is not reliable time series information for *E. illustris*. However, since the seamount population has persisted over time, the question of how recruitment occurs to the population is of interest. Because spawned eggs are usually swept away with the currents, one can assume several possibilities for the continuous recruitment. One possibility is that the Cross Seamount population receives recruits from distant populations, maybe from other seamounts or island slopes of the Hawaiian Archipelago. Alternatively, there may be some mechanism for a limited amount of local retention. Another possibility could be that the monchong are depositing the eggs onto some substrate in order to keep them from migrating away, though the biology of the species suggests that this is unlikely. Unfortunately, very little information is available on the life history of any pomfret species. The focus of this study is to gain a better understanding of the E. illustris reproductive cycle. Previous studies on the fecundity of commercial value fish species have provided valuable information for fisheries science. However, in order to estimate fecundity, reproductive biology of the species in question must be determined.



Fig. 2: On the left, a fishing boat at Cross Seamount. On the right, a variety of fish caught at Cross Seamount.

Reproduction

The majority of fish species are sexually reproducing organisms, involving a male and female. When males and females reach sexual maturity (i.e., become reproductively capable), their gonads, testes for males and ovaries for females, start producing sperm and eggs, respectively. When fish spawn, they release these "gametes", at which point the sperm fertilize the eggs resulting in the formation of a zygote. Further cell division and development leads to the formation of embryos, frys, juveniles and eventually adults of the parental species who will be capable of sexual reproduction themselves. Although the process of recombination of DNA is generally the same in all sexually reproducing organisms, there are many different reproductive strategies that can vary between species. For example, some fishes are born as one sex and remain the same throughout their life, which is called gonochorism. Other species are born as one sex, but can change to the other at some point during their lives, which is called successive hermaphroditism (Jalabert, 2005). Successive hermaphroditism can be sub-divided into two types: protogy and protandry. Protogynous species will grow up as female and may change to male, while protandrous species will develop as males and may change to females (Lubzens et al. 2010).

Another reproductive strategy that can vary among species is the means by which the eggs are fertilized. Two broad egg fertilization methods exist within marine vertebrates, internal and external fertilization. Internal fertilization is more commonly seen in the following marine vertebrates: reptiles, mammals, cartilaginous fishes. Within the category of internal fertilization there are three strategies for embryonic and fetal development: oviparity, ovoviviparity, and viviparity. An animal exhibits oviparity when the eggs are fertilized internally and later deposited outside of the body of the mother in order to complete development. In ovoviviparity, the fertilized eggs are kept alive, feeding off of the egg yolk, within the mother until they are completely developed and born. Viviparity also occurs when the young develop within the mother, but the eggs receive energy directly from the blood of the mother instead of relying on the egg yolk.

External fertilization is the most common method of egg fertilization in bony fishes. In this process females will produce and release their eggs into the water column or attach them to some type of substrate. Once the eggs are released, the females will simultaneously release their sperm in order to fertilize the eggs. Synchronization between individuals is very important in the coordination of these spawning events. Eggs are very susceptible to predation and have high mortality rates. Many organisms try to counter that effect by producing massive quantities of gametes so that a few will survive to adulthood.

Reproduction strategies of teleosts

Teleostei is the most diverse group of ray-finned bony fishes, which include most of the living fish species seen today (Arratia, 1999). External fertilization is the most common method of egg fertilization in teleosts. The common pattern of an external fertilizing fish, or spawning fish, is presented in Figure 3. There are two general types of spawners, total spawners and batch spawners (Brown-Peterson et al. 2011). Total spawners are species that develop a single group of immature egg cells, known as oocytes, during the reproductive season. Batch spawners develop multiple groups of oocytes during the reproductive season and may release them continuously or in several batches spaced out though the season (Brown-Peterson et al. 2011). These batches of oocytes develop until they reach a certain stage where they are hormonally triggered to be released into the environment (Jalabert, 2005).



Fig. 3: The typical pattern of a spawning species. Going from the top to the bottom, a group or batch of oocytes develops over time and eventually reaches a point where it is hormonally triggered and then released.

There are three types of ovary development in fishes: synchronous, group-synchronous and asynchronous, as seen in Figure 4 (Wallace and Selman 1981). Species with synchronous ovaries form and develop all of their oocytes at the same time. Group-synchronous ovaries have at least two clutches of oocytes. The group of larger oocytes will be released during the current spawning event while the smaller oocytes will be released during the next spawning event. Asynchronous ovaries appear to have a mixture of oocytes in several stages.



Fig. 4: Size frequency diagrams of the three types of ovary development. On the left, the size frequency pattern of a species with synchronous ovary development. In the middle, the size frequency pattern of a species with group-synchronous ovary development. On the right, the size frequency pattern of a species with asynchronous ovary development.

Fecundity

Based on the type of ovary development a species exhibits, the fecundity may be estimated. Fecundity is the measurement of how many eggs a female produces each year. There are two types of fecundity: determinate and indeterminate fecundity (Brown-Peterson et al. 2011; Hunter et al. 1992). Determinate fecundity is when it is possible to calculate the total number of eggs that will be spawned in one year (Hunter et al. 1992). This can be done if the species has synchronous or group-synchronous ovary development. If the will be developed in one year must be known. A key characteristic of determinate fecundity is a gap between primary growth oocytes and secondary growth oocytes (Brown-Peterson et al. 2011). Indeterminate fecundity is when it is not possible to calculate the amount of eggs that will be produced in one year (Hunter et al. 1992). This type of fecundity is characteristic of asynchronous and group-synchronous species if the number of batches spawned per year is difficult to estimate, due to the continuous development of oocytes.

Fecundity studies commonly use hydrated oocytes to represent the next batch to be released (Hunter et al. 1985). Once oocytes become hydrated they will be spawned with the next 24 hours. Therefore, hydrated oocytes provide the best estimate of the number of eggs that will be released in the next spawning event.

Without hydrated oocytes, it is more difficult to assess the next spawning batch of oocytes. Figure 5 shows the difference between two samples with asynchronous development, one having an identifiable mode in the advanced stages and the other without. If no hydrated oocytes are present in the ovary, the most advanced staged oocytes will likely be the next to spawn. In this situation, finding the point at which the VIT oocytes transfer into the FM stage is key to determining the next spawning batch of oocytes.



Fig. 5: On the left, the most developed mode in a species with asynchronous ovary development as it goes through maturation. On the right, there is no identifiable mode of advance staged oocytes present in the distribution of a species with asynchronous ovary development.

The timing and frequency of spawning events vary widely between fish species (Jalabert, 2005). Teleost species are capable of spawning: only once in their lives, once every few years, once a year, or multiple times in a year. Fecundity estimations from the different seasons of a year allow researchers to determine annual spawning patterns. The timing and frequency of spawning events can be influenced by many things including: photoperiod, food availability, temperature and social factors (Jalabert, 2005).

Stages of oocyte growth

Oocytes go through four general stages of growth during their development (described in Wallace and Selman 1981). Figure 6 shows the comparison of known oocyte stages in seabream compared to the estimated oocyte maturation stages of *E. illustris*. The first stage is called Primary Growth (PG). This stage begins when the fish are still juveniles and continues throughout their life. Immature female reproductive cells called oogonia undergo partial meiosis to form cells called oocytes. PG oocytes are made up of a central nucleus (germinal vesicle) with some cytoplasm enclosed by few follicle cells (Wallace and Selman, 1981). The following three stages occur only once a fish has reached maturity. The second stage of oocyte growth is called Cortical Alveoli (CV). A distinguishing feature appears for the first time during this stage, yolk vesicles (Wallace and Selman, 1981), which prepare the oocyte to accept yolk during the next stage.

During the third stage, vitellogenesis (VIT), a large amount of fat and protein is taken up by the oocyte, which will be later used by the growing embryo. The vitellogenic stage is long and as a result, has been separated into three different stages in previous papers (Brown-Peterson et al. 2011; Lowerre-Barbieri et al. 2011), but for the purposes of this study the whole process was considered one stage. Vitellogenin is a type of protein that is a precursor of the yolk in an egg. It is currently believed that vitellogenin is created in the liver in response to a form of estrogen (estradiol - 17β) and other hormones (Jalabert, 2005; Lubzens) et al. 2010). Once synthesized, vitellogenin is transported in the blood to the oocyte where it is taken in and used to create yolk proteins (Jalabert, 2005). Yolk granules, the accumulation of yolk proteins, first appear during this stage (Wallace and Selman, 1981). Another event that takes place during this stage is the displacement of the ooplasm from the center to the periphery of the oocyte surrounding the yolk mass (Wallace and Selman, 1981). Some species may have the oil droplets in the oocytes that among other things provide buoyancy once the egg is released (Brown-Peterson et al. 2011). Initial oil droplet formation can be seen at this point in the oocyte maturation.

The last stage that oocytes go through is called Final Maturation (FM). At this point, the oil droplets coalesce forming fewer and larger drops until there is one left. At this stage, most species that have external fertilization of pelagically spawned eggs will undergo oocyte hydration (Brown-Peterson et al. 2011). This phenomenon, which is hormonally triggered, results in a rapid size increase (Wallace and Selman, 1981). Once hydrated, the oocyte breaks out of the surrounding follicle and migrates into the ovarian lumen (cavity of the ovary) where it waits to be released during spawning.



Fig. 6: Comparison of the known oocyte stages of maturation in the Gilthead seabream (*Sparus aurata*) to the lustrous pomfret (*E. illustris*) (Lubzens et al. 2010).

At the end of the reproductive cycle, atresia may occur. Atresia is the process of oocyte and follicle resorption from oocytes that fail to complete the maturation process (Lubzens et al. 2010; Murua et al. 2003). Evidence of atresia

can be observed beginning at the VIT stage and continuing into the regenerating stage, which is the process of new oocyte growth to get ready for the next spawning season (Brown-Peterson et al. 2011). Characteristics of atresia include: the disintegration of the nucleus, other organelles (mitochondria, cortical alveoli) and the follicle (Miranda et al. 1999).

Key questions

In this study, I aimed to answer the following questions:

- 1. Does *E. illustris* show synchronous, group-synchronous or asynchronous development?
- 2. Does it spawn all year or during a certain season?
- 3. What is the batch fecundity of *E. illustris*?

CHAPTER 2. METHODS

Obtaining ovary samples

All of the *E. illustris* ovaries used in this study were caught at Cross Seamount by commercial fishermen. Once acquired, the fish weight, date caught and date at which the samples were obtained were recorded. Upon extraction, both lobes of the ovary from each fish had two incisions made, approximately dividing each lobe into thirds. Next, the ovaries were placed into a glass jar and filled with 10% buffered formalin. The incisions allowed the 10% buffered formalin solution to quickly penetrate the ovarian lamellae and fix the oocytes. Ovaries were later transferred into glass jars with 70% ETOH for long term storage and sampling.

Oocyte samples

At the time of the study the ovaries were removed from their containers and measured to the nearest 0.001g wet weight. Each ovarian lobe, now divided roughly into thirds (made by previous incisions), had one sample taken from each section, as seen in Figure 7. Every ovary sample taken was weighed to the nearest 0.001g and added to a 1.5ml test tube. The next step was to add fresh water and shake the tubes in order to rinse the ethanol off the samples, as well as help separate the oocytes.



Fig. 7: Oocyte samples were taken from each section (ovary divided into thirds) of the ovarian lobes.

Once the oocytes were washed with fresh water, a pipette was used to extract the oocytes and place them onto a glass slide. Using a microscope, the oocytes were sorted into four stages, the primary growth stage (PG), the cortical vesicle stage (CV), vitellogenesis stage (VIT), and final maturation stage (FM). The distinguishing features of each class were determined by the appearance of yolk granules, oil droplets and size (Wallace and Selman, 1981).

Image analysis

The PG and CV oocytes were not used in this study and as a result were removed from the pictures as best as possible. Photographs of each oocyte were taken in order to determine their size and stage. Pictures of the oocytes were taken using a Nikon T2i Rebel camera attached to an Olympus SZX9 microscope under 25X, 32X, 40X, 50X and 57X magnifications. The pictures were later imported into an image organizing program, Picassa. Using XnView, each picture had its date at which it was taken, zoom, species, fish identification number, picture number, and the amount of copies of the same image written on the top-left hand corner of the image.

An image processing program, ImageJ, was used to measure the diameter of each oocyte. If the oocyte was a circular shape, the diameter was measured from the top to the bottom. Oocytes with any other shape had the greatest distance between the two farthest points, while going through the nucleus, measured. Any oocyte showing evidence of a broken outer layer or atresia was not included in the measurements.

Resulting data were entered into a computer program, JMP Pro 10, for statistical analysis. Histograms were made with the number of observations as the dependent variable and the diameter of the oocyte (in microns) as the independent variable.

Fecundity estimations

Fecundity estimates were conducted using two different methods, the Hydrated Oocyte Method and the Oocyte Size-Frequency Method, both described by Hunter et al. (1985). The Hydrated Oocyte Method takes the number of hydrated oocytes to estimate the amount of eggs that will be spawned in the next batch. Our initial observations confirmed the existence of hydrated oocytes, allowing us to use this method. Due to the lack of information on the monchong, the size at which the VIT stage oocytes transition into FM stage oocytes in unclear. In the two samples that had hydrated oocytes (fish 841 and 808), a cutoff size was determined to separate the VIT and FM stages. The

cutoff size was calculated by taking the average diameter of the largest VIT oocyte found in each sample with hydrated oocytes. Any oocyte with a diameter above that cutoff size was presumed to be hydrated and released in the next spawning event.

The Oocyte Size-Frequency Method uses size-frequency graphs and takes the number of oocytes in the developing mode to estimate the amount of eggs that will spawned in the next batch (Hunter et al. 1985). This method was used for calculating the batch fecundity of fish 813, which did not have any hydrated oocytes. The developing mode of VIT oocytes was determined by visual interpretation of the size-frequency distribution.

Three samples were taken from the lobe of each ovary to determine if there was any variance in the distribution of oocyte stages. The number of oocytes in the batch (N) was counted by either the hydrated oocyte or size frequency method. The total weight of the ovary (W_o) was divided by the total weight of the oocyte samples (w). The result was multiplied by N to get the absolute batch fecundity (A).

(1) $A = (W_o / w) * N$

Dividing the absolute batch fecundity (A) by the weight of the whole fish (W_f) produced the relative batch fecundity (B).

(2) $B = A / W_f$

CHAPTER 3. RESULTS

Size-frequency distributions

The size frequency distributions of *E. illustris* oocytes from five different fish caught at different times of the year are presented in Figures 8 – 12. Each histogram shows a similar pattern of a skewed distribution with the largest proportion found in the lower ranges of oocyte sizes (between 200 and 400 microns) and a smaller number in the upper ranges. Modes were difficult to distinguish because the oocyte size distribution was closely spaced together.

A cutoff size to distinguish between the VIT and FM stages was calculated to be approximately 885 +/- 5 microns based on the largest non-hydrated oocytes in fish 841 and 808. The most developed mode of oocytes above 885 microns was used for the fecundity estimations in these two individuals (see Fig. 9 and 10). The smallest measured hydrated oocytes were 920 and 950 microns in diameter (Fig. 9 and 10, respectively) and the largest corresponding VIT oocytes taken from the same fish were 885 and 890 microns in diameter (Fig. 9 and 10, respectively).

For ovaries without hydrated oocytes, the developing batch of oocytes was used for the fecundity calculations, based on the presence of a mode in the size frequency distribution. Fish number 813 showed an identifiable mode in the upper size classes of the distribution, above 700 microns, which was used for to estimate fecundity (Fig.12). Fish number 852 and 851 did not have any

identifiable modes in the upper size classes of their distributions, therefore no fecundity estimates were made using these fish (Fig. 8 and 11, respectively).



FISH ID 852

Fig. 8: The distribution of vitellogenic (VIT) stage oocytes from an ovary of an *E. illustris* caught during the fall season (October). On the far left, an early VIT stage oocyte has limited yolk granule accumulation with some follicle development. As the oocytes develop through the VIT stage, increased densities of vitellogenin and yolk granules along with follicle development occurs. This fish was not used in the fecundity estimation due to the lack of hydrated oocytes and mode of advanced VIT stage oocytes.



Fig. 9: The distribution of vitellogenic (VIT) stage and hydrated oocytes from an ovary of an *E. illustris* caught during the fall season (October). From left to right there is an increasing density of yolk granules until 880 microns. The largest VIT oocyte is 880 microns and the smallest hydrated oocyte is 920 microns. The increased size, coalescence of oil droplets and transparency of the oocyte indicate hydration. All oocytes above 885 microns were considered to be released during the next spawning event. Degradation of the VIT oocytes is obvious by the gap between the nucleus and outer zona radiata. Size classes marked by * were counted for fecundity estimations.



Fig. 10: The distribution of vitellogenic (VIT) stage and hydrated oocytes from an ovary of an *E. illustris* caught during the spring season (May).). From left to right there is an increasing density of yolk granules until 880 microns. The largest VIT oocyte is 890 microns and the smallest hydrated oocyte is 950 microns.

The increased size, coalescence of oil droplets and transparency of the oocyte indicate hydration. All oocytes above 885 microns were considered to be released during the next spawning event. Healthy VIT oocytes is obvious by the lack of a gap between the nucleus and outer zona radiata. Size classes marked by * were counted for fecundity estimations.



FISH ID 851

Fig. 11: The distribution of vitellogenic (VIT) stage oocytes from an ovary of an E. illustris caught during the fall season (October). On the far left, an early VIT stage oocyte has limited yolk granule accumulation with some follicle development. As the oocytes develop through the VIT stage, increased densities of vitellogenin and yolk granules along with follicle development occurs. This fish was not used in the fecundity estimation due to the unidentifiable cutoff size inferred by the smallest hydrated oocyte.

FISH ID 813



Fig. 12: The distribution of vitellogenic (VIT) stage oocytes from an ovary of an *E. illustris* caught during the summer season (July). On the far left, an early VIT stage oocyte has limited yolk granule accumulation with some follicle development. As the oocytes develop through the VIT stage, increased densities of vitellogenin and yolk granules along with follicle development occurs. This fish was used in the fecundity estimation due to the identifiable mode of advanced VIT oocytes between 700 and 950 microns. Size classes marked by * were counted for fecundity estimations.

Oocytes were found in various conditions when looking through the microscope. Figure 13 shows the difference between healthy and degrading oocytes. Healthy vitellogenic oocytes have minimal distances between the nucleus and zona radiata, an outer cell layer (Fig. 13). Once a fish dies, the degradation of oocytes begins. If proper preservation is not performed quickly, the oocytes will show signs of degrading, by a gap between the zona radiata and nucleus (Fig. 13).



Fig. 13: A healthy oocyte on the left, shows minimal distance between the zona radiata and the nucleus. The oocyte on the right shows evidence degradation by the gap relatively large gap between the zona radiata and the nucleus.

Advanced staged oocytes

Hydrated oocytes were found in two out of the five monchong samples used in this study. It is believed that hydration provides a water reservoir for the new embryos as well as assisting with the buoyancy of the eggs when it is released into the water column (Lubzens et al. 2010). The presence of hydrated oocytes also indicates that the species uses external fertilization.

The average size of the top ten percent of the oocytes found in each fish is provided in Figure 14. These averages can be used to indicate the maturation stage of the fish. Fish samples that lacked large oocytes indicate fish that are not in or near spawning condition. Any fish with their largest oocytes above the cutoff size for the VIT-FM transition can be assumed to be mature. Standard deviations also help to reflect the maturation stage of the fish. Large standard deviations reflect that there is a greater variation of oocyte sizes, inferring a mature fish.



Fig. 14: The average size of the largest 10% oocytes and the standard deviations from each fish sample. The red line represents the cutoff size (885 microns) between the VIT and FM stage.

Estimated fecundity

Table 1 shows the absolute fecundity and relative batch fecundity estimations made for each fish we sampled. Absolute batch fecundity is the measurement of the number of total oocytes to be spawned in the next batch. Relative batch fecundity is the estimation of how many oocytes are spawned per kilogram of weight of the fish sampled and was derived from the absolute batch fecundity.

FISH ID	Fish weight (kg)	Absolute batch fecundity (oocytes)	Relative batch fecundity (oocytes/kg)
841	5.9	114,906	19,487
808	7.7	205,320	26,627
813	4.5	182,614	40,259

Table 1: Estimated absolute and relative batch fecundity for each fish sample.

CHAPTER 4. CONCLUSION

Oocyte maturation

Although the timing between batches has yet to be determined for *E*. *illustris*, they do seem to exhibit batch spawning behavior. Batch spawners are characterized by multiple lines of evidence including: group-synchronous development, asynchronous development and continuous recruitment of oocytes (Brown-Peterson et al. 2011). Asynchronous development was evident by the observation of oocytes in every stage when looking at the ovary samples under a microscope. Similar patterns of a large mode at the lower end of the size distribution with multiple modes of larger oocytes can be seen in the spring, summer and fall seasons. What the large, lower mode may represent is continuous oocyte recruitment into vitellogenesis throughout the year. No evidence of gaps between modes further suggest that *E. illustris* exhibit an indeterminate fecundity pattern, also a characteristic of batch spawners.

Seasonality of spawning

The continuous supply of oocytes into the maturation stages suggests frequent spawning, and the existence of this condition in three seasons during which fish were caught suggests that spawning is likely throughout the year. Species known to exhibit constant spawning behavior year-round include: dolphinfish (*Coryphaena hippurus*, mahimahi) which spawn every 2 days (Alejo-Plata et al. 2011), sailfish (*Istiophorus platypterus*) which spawn every 1.89 days

(Chiang et al. 2006) and skipjack tuna (*Katsuwonus pelamis*) which spawn every 1.18 days (Hunter et al. 1986).

Fecundity

The fecundity estimates calculated in this study were the first known estimates for the monchong, *E. illutsris*. Some reasonable boundaries on absolute batch fecundity for the *E. illustris* population at Cross Seamount are between 114,906 and 205,320 oocytes with a mean of 167,613 +/- 38,405 oocytes. Relative batch fecundity estimates ranged from 19,487 to 40,259 oocytes per kilogram with a mean of 28,791 +/- 8,617 oocytes per kilogram. These fecundity estimates provide a small insight to the reproductive biology of the lustrous pomfret, *E. illustris*. However, further work with a much higher number of samples is required before a better understanding is possible.

The most reliable fecundity estimates were obtained for two fish that had hydrated oocytes using the Hydrated Oocyte Method (Hunter et al. 1985). A less reliable estimate was produced for a fish without hydrated oocytes, by looking at for a mode in the size-frequency distribution. While this method is less reliable due to the shape of the monchong oocyte distribution, the batch fecundity value obtained was similar to those obtained using hydrated oocyte counts. Therefore it appears that the size frequency method can be used where there are visible modes.

Implications for fisheries

Fecundity estimates provide fisheries with a measurement of the reproductive capability of monchong. Highly fecund species are more likely to be able to withstand high levels of fishing pressure. Species with lower fecundity are more susceptible to overfishing and need to be managed conservatively in order to maintain a healthy population. Our estimated batch fecundity of 167,613 eggs seems to fall in the lower range of fecundity when compared to the dolphinfish (*Coryphaena hippurus*, mahimahi), a known species of high fecundity, which has between 45,022 – 1,903,245 eggs every 2 days.

E. illustris seem to be spawning continuously for at least three out of the four seasons in a year. This suggests that there is a continuous recruitment of individuals back into the Cross Seamount population, and although there is no evidence for the winter season, it seems likely that there is constant spawning year round.

Future studies

This study provided a small insight into the reproductive biology of the lustrous pomfret, *E. illustris* and highlighted areas for future research. Our study estimated batch fecundity for *E. illustris*, but to know the annual fecundity we must know how many batches are spawned per year. Estimating batches per year, or spawning frequency, has been conducted using a number of approaches, such as the postovulatory follicle method (which requires histology), or the hydrated oocyte method (a cruder method not requiring histology) (Hunter

et al. 1985; Hunter and Macewicz, 1985; Hunter et al. 1992). Alternately, if a species can be brought into captivity, accurate estimates of spawning frequency can be made. If this study were to be repeated, a larger sample size should be obtained, preferably with hydrating females from each season. Multiple samples from each month of the year will strengthen the statistical evidence for seasonal trends.

Pomfrets in general, are currently not well understood. Many opportunities are available to learn more about their behavior, diet, population dynamics, and reproduction. Monchong populations exist throughout the Hawaiian Archipelago. It is unknown if they are moving between these different locations. Current research is being done to understand the movement patterns and behavior of *E. illustris* at Cross Seamount (Gray et al. unpublished).

BIBLIOGRAPHY

- Alejo-Plata, C., Díaz-Jaimes, P., and Salgado-Ugarte, I.H. 2011. Sex ratios, size at sexual maturity, and spawning seasonality of dolphinfish (*Coryphaena hippurus*) captured in the gulf of Tehuantepec, Mexico. *Fisheries Research* 110(1): 207-216.
- Arratia, G. 1999. The monophyly of Teleostei and stem-group teleosts Consensus and disagreements. *Mesozoic Fishes 2 – Systematics and Fossil Record* 265-334.
- Brown-Peterson, N.J., Wyanski, D.M., Saborido-Rey, F., Macewicz, B.J., and Lowerre-Barbieri, S.K. 2011. A Standardized Terminology for Describing Reproductive Development in Fishes. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science* 3(1): 52-70.
- Chiang, W.C., Sun, C.L., Yeh, S.Z., Su, W.C., and Liu, D.C. 2006. Spawning Frequency and Batch Fecundity of the Sailfish (*Istiophorus platypterus*) (Istiophoridae) in Waters off Eastern Taiwan. *Zoological Studies* 45(4): 483-490.
- Jalabert, B. 2005. Particularities of reproduction and oogenesis in teleost fish compared to mammals. *Reprod. Nutr. Dev.* 45: 261-279.
- Ganias, K. 2013. Determining the indeterminate: Evolving concepts and methods on the assessment of the fecundity pattern of fishes. *Fisheries Research* 138: 23-30.
- Hunter, J.R., Loe, N.C.H., and Leong, R.J.H. 1985. Batch Fecundity in Multiple Spawning Fishes. *In* R. Lasker (editor), An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy (*Engraulis mordax*), p.67-77. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 36.
- Hunter, J.R., Macewicz, B.J., Lo, N.C.H., and Kimbrell, C.A. 1992. Fecundity, spawning, and maturity of female Dover sole *Microstomus pacificus*, with an evaluation of assumptions and precision. *Fishery Bulletin* 90(1): 101-128.
- Hunter, J.R., Macewicz, B.J., and Sibert, J.R. 1986. The spawning frequency of skipjack tuna, *Katsuwonus pelamis*, from the South Pacific. *Fishery Bulletin* 84(4): 895-903.
- Kjesbu, O.S., Hunter, J.R., and Witthames, P.R. 2003. Report of the working group on modern methods to assess maturity and fecundity in warm-and cold-water fish and squids. *Bergen, Havforskningsinstittuttet, Institute of*

Marine Research 1-140.

- Lowerre-Barbieri, S.K., Ganias, K., Saborido-Rey, F., Murua, H., and Hunter, J.R. 2011. Reproductive Timing in Marine Fishes: Variability, Temporal Scales, and Methods. *Marine and Coastal Fisheries* 3: 71-91.
- Lubzens, E., Young, G., Bobe, J., and Cerdà, J. 2010. Oogenesis in teleosts: How fish eggs are formed. *General and Comparative Biology* 165: 367-389.
- Miranda, A.C., Bazzoli, N., Rizzo, E., and Sato, Y. 1999. Ovarian follicular atresia in two teleost species: a histological and ultrastructural study. *Tissue Cell* 31, 480-488.
- Murua, H., Kraus, G., Saborido-Rey, F., Witthames, P.R., Thorsen, A., and Junquera, S. 2003. Procedures to Estimate Fecundity of Marine Fish Species in Relation to their Reproductive Strategy. *J. Northw. Atl. Fish. Sci.* 33: 33-54.
- Wallace, R.A. and Selman, K. 1981. Cellular and Dynamic Aspects of Oocyte Growth in Teleosts. *American Zoologist* 21(2): 325-343.