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Reproductive Strategies of the Big Skate (Beringraja Binoculata) with Evidence of Multiple Paternity

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REPRODUCTIVE STRATEGIES OF THE BIG SKATE (*BERINGRAJA BINOCULATA)* WITH EVIDENCE OF MULTIPLE PATERNITY

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Jessica Ja-Jei Jang

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REPRODUCTIVE STRATEGIES OF THE BIG SKATE (*BERINGRAJA BINOCULATA)*

WITH EVIDENCE OF MULTIPLE PATERNITY

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DEDICATION

Dedicated to my parents that kept giving me their undying love and support through hardships and encouraging me to keep a positive outlook on pursuing this master's degree. You will realize that there's still so much to learn. I am looking forward to what the future brings as my pursuit for knowledge continues.

“Knowledge is power. Information is liberating. Education is the premise of progress, in every society, in every family”

-Kofi Annan

ABSTRACT

REPRODUCTIVE STRATEGIES OF THE BIG SKATE (*BERINGRAJA BINOCULATA)*

WITH EVIDENCE OF MULTIPLE PATERNITY

by
Jessica Ja-Jei Jang
Master of Science in Marine Science
California State University Monterey Bay, 2019

Beringraja binoculata is a large skate species commonly caught, raised, and exhibited in public aquaria, especially along the Pacific coast of North America. It is one of only two species in the Rajidae family the other species being *B. pulchra* found in the western Pacific able to produce multiple embryos within an egg case. Although recent studies have suggested this species might be the most fecund elasmobranchs currently known, there has not been a detailed study on whether this species' novel reproductive strategy is influenced by location and different environments (e.g. captive vs. wild). Specifically, if the reproductive strategies differ in egg case sizes, embryo numbers, embryo sizes, and if offspring sex ratios (OSR) were present. Specimens collected from NOAA trawl surveys between 2008-2016 showed evidence that egg cases and embryo sizes were larger in the wild than in captivity; 3-4 embryos in an egg case were the most common in the wild, while 2 was the most common in captivity. OSR were not significantly different between in the two environments, but in this study, there were more female than male offspring in both captive and wild settings. At 42 º North latitude, egg case sizes and embryo numbers peaked, suggesting that the region is considered a suitable habitat to raise offspring due to strong upwelling conditions from the California current ecosystem. Captive *B. binoculata* egg cases were raised to hatching to improve the description of the developmental stage process done by Hitz (1964). A growth curve was calculated to determine the development of the embryos among four observed stages. Paternity tests were conducted using four microsatellites primer sets to find multiple paternity exists within this species and that females may store sperm for a minimum of three months in captivity, suggesting that *B. binoculata* possesses several reproductive strategies.

TABLE OF CONTENTS

LIST OF TABLES

PAGE

LIST OF FIGURES

PAGE

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INTRODUCTION

Skates are one of the most diverse groups of elasmobranchs, comprised of four families, 38 genera, and over 300+ species, with most species being regional endemics (Ebert & Sulikowski 2007; Ebert & Compagno 2007; Ebert *et al* 2008a; Ebert *et al*. 2013; Last *et al*. 2016a). Skates are morphologically conservative with their dorso-ventrally flattened morphology, and with most skate species being benthic dwellers, their association with benthic habitats makes them susceptible to fishing practices such as bottom trawling and long-lining. Because most species of this family look quite similar, current U.S. fisheries management often lumps all skate species caught into a single generic skate category (Robinson *et al*. 2007). This classification poses many issues, as numerous skate species have variable sexual maturity sizes/age, breeding seasons, and different habitats where they lay their egg cases (sandy bottoms vs rocky reliefs) (Ebert & Compagno 2007). Therefore, understanding how variable the reproductive cycles are in this speciose family will help with management practices.

All skates are oviparous (egg-laying), with the females depositing keratinous egg cases, commonly known as 'mermaid purses', from two functional uteri, on the seafloor (Ebert & Compagno 2007). These egg case shapes are morphologically unique to each species and may be used to identify specific species (Ebert 2005; Ebert & Davis 2007; Ishihara *et al.* 2012). Most skate species lay a single enclosed egg case that will develop into a single embryo prior to hatching (Ishihara *et al*. 2012). Embryos are known to take 3 to 15 months to develop, but longer development times, sometimes 2-4 years dependent on the ambient water temperature (Luer & Gilbert 1985; Compagno 1990; Hoff 2010; Hoff 2016; Salinas-de-León *et al*. 2018).

Studies have found that skate species exhibit one of three different reproductive temporal patterns. These patterns are defined as: (1) reproductively active year-round; (2) a partially defined annual or biennial with one or two peaks; (3) a well-defined annual or biennial (Musick & Ellis 2005; Ebert *et al*. 2008a, b; Conrath & Musick 2012). Species can vary among these three distinct reproductive patterns. Some species may exhibit late sexual maturity and slow growth like other elasmobranchs like the Roughtail Skate (*Bathyraja trachura*) reach sexual maturity between 21 to 24 years (Winton *et al.* 2013), to other species such as the Little Skate (*Leucoraja erinacea*) maturing very quickly within seven to nine years (Cicia *et al* 2009).

While there are species that have the capability of reproducing actively year-round many of these species only lay single egg cases, whereas only two species of skates lay multiple embryos within in egg case (Zeiner & Wolf 1993; Ebert *et al*. 2008b; Winton *et al*. 2013; James *et al*. 2014). One of these species that exhibit this reproductive strategy of being reproductively active year-round and multiple embryo characteristic is the Big Skate (*Beringraja binoculata*) (J. Bizzarro and D. Ebert, Moss Landing Marine Labs, unpubl. data).

The genus, *Beringraja,* was described by Ishihara *et al.* (2012) to differentiate the Big Skate *Beringraja binoculata* (Girard, 1855) and the Mottled Skate *Beringraja pulchra* (Liu, 1932) from the North Pacific Assemblage (NPA) skate species. These two species were placed into this genus. Both species are unique from other skate species in that they exhibit similarities in clasper morphology and egg case structure (Figure 1A), and most importantly these are the only two species whereby multiple embryos occur within each egg case (Figure 1B). This novel reproductive strategy is not found in any other skates or any other oviparous elasmobranch. Furthermore, genetic evidence supports that these two species are sister taxa and are a monophyletic group from the NPA (Naylor *et al*. 2012; Chiquillo *et al*. 2014). Recently, the four other species of the NPA were added, without explanation to this genus, increasing the number of this genus to a total of six (Last *et al*. 2016a, b). However, none of these four other species have multiple embryos within each egg case, and their egg case morphology is quite distinct from the Big and the Mottled Skates.

The Big Skate (Figure 1C) is a shallow water species found from the Eastern Bering Sea in the north to Baja California, Mexico in the south (Figure 2) occurring from bays to depths rarely over 200 meters (Ebert 2003: Ebert *et al*. 2008b; Haas 2009; Bizzarro *et al*. 2014). It typically inhabits sandy and muddy bottoms (Bizzarro *et al*. 2014). Over the past decade, more species-specific life history information has been collected and reported on the Big Skate; a relatively common, but previously little-known species. Studies have included dietary, age and growth, and reproductive biology from throughout most of its range from Alaska to Southern California (Zeiner & Wolf 1993; McFarlane & King 2006; Gburski *et al*. 2007; Ebert *et al*. 2008b; King & McFarlane 2010).

Variation in sexual maturity and longevity of this species has been reported from three geographically distinct coastal study areas: the Gulf of Alaska, British Columbia, and central California. Sizes at sexual maturity range from 90-148.6 cm Total length (TL) (age at maturity from 5-9 years), with estimated life spans ranging from 12-26 years (Zeiner & Wolf 1993; McFarlane & King 2006; Gburski *et al*. 2007; Table 1). These estimated age studies were validated using bomb radiocarbon analyses with a probability of 70% that the age estimates are accurate to within 2 years (King *et al*. 2017).

The Big Skate has been assessed as a species of 'Least Concern' by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Farrugia *et al.* 2016a). However, in the Western Pacific, its sister species, *B. pulchra,* have been extensively overexploited in commercial fisheries (Kang *et al.* 2013; Im *et al.* 2017). While there is not a direct fishery for the Big Skate in California waters, they are commonly taken as bycatch and sold as food for human consumption in Asian countries (Ebert 2003; Haas 2009; McKnight 2011). Hence, additional information about the reproductive strategies for *B. binoculata* will provide fundamental data for better management plans if commercial demands should increase.

The Big Skate is known to be the largest skate in the eastern North Pacific Ocean, with a female reaching a record length of 214 cm total length (TL), while males may reach 184 cm (King & McFarlane 2010). Interestingly, specimens of these extreme sizes have been found only in the higher latitudes in the Gulf of Alaska (Ebert *et al*. 2008b). These observations have prompted questions about whether Bergmann's rule also holds true in this species. Bergmann's rule is an ecogeographical rule named after Carl Bergmann (1847) who observed that large-bodied animal species tend to live further north than their small-bodied counterparts. However, this rule is still controversial due to his observations pertaining to endotherms as many studies have argued that resource presence plays a more important role to a species' size rather than location (Ray 1960; Blackburn *et al.* 1995). Although, other studies have suggested that Bergmann's rule does apply to several taxonomic poikilotherms such as amphibians, nematodes and several species of teleosts (Ray 1960). While it is noted that in northern latitudes, *B. binoculata* is known to be larger than their southern latitude counterparts, it is unclear whether their reproductive strategies are also affected. One study found that larger body sizes are positively correlated with the fecundity in male *Syngathus leptorhynchus* (Bay Pipefish) a

polygynous (mating with multiple females) teleost fish (Wilson 2009). Specifically, males with larger body sizes along a latitudinal gradient were able to maintain their potential reproductive rate despite decreased thermal gradients (Wilson 2009). This study prompted the question of whether oviparous elasmobranchs, such as the Big Skate also exhibit similar fecundity patterns. If Bergmann's Rule applies to reproduction in Big Skates, we may expect that egg case dimensions and embryo numbers per egg case will increase at higher latitudes.

Current studies have suggested that Big Skates are arguably the most fecund of any known elasmobranch (Ebert & Davis 2007; Ebert *et al*. 2008b). Evidence supporting this observation come from three features found in this species: 1) an earlier sexual maturity (5-8 years) than most species of skates (Ebert *et al*. 2008b), 2) the unique characteristic of producing multiple embryos (range: 1-8) within a single egg case (Hitz 1964) and 3) being reproductively active year-round. It is estimated that a single female during her lifetime may produce around 48,000 embryos (Ebert & Davis 2007; Ebert *et al.* 2008b). These calculations have prompted a question as to whether multiple paternity (MP) is present due to the unique mode of oviparity of this species.

Some species have developed a unique reproductive strategy to increase their chances of producing more genetically mixed offspring, called polyandry. Polyandry is defined as the action of a female mating with more than one male. Polyandry usually results in multiple paternity (MP) where a single brood is often sired by multiple males (Daly-Engel *et al*. 2010). Evidence of polyandry and multiple paternity are well documented in different taxa such as amphibians, birds, mammals, and teleosts (Coleman & Jones 2011). This strategy contributes to maintaining a genetically diverse population; species that exhibit MP are expected to have increased effective population size (N_e) (Sugg & Chesser 1994). However, the benefits of such a strategy are still being explored in taxa that do not exhibit additional parental care such as elasmobranchs (DiBattista *et al.* 2008a; Coleman & Jones 2011).

In viviparous (live-bearing) elasmobranch reproduction studies, the advantages of MP are evident in males. Having more mates increases a male's potential to father more of the offspring (Griffiths *et al*. 2011). Yet, the indirect benefits for females are debatable (Feldheim *et al*. 2004; DiBattista *et al*. 2008 a, b; Daly-Engel *et al.* 2010; Coleman & Jones 2011; Griffiths *et*

al. 2011; Conrath & Musick 2012; Fitzpatrick *et al*. 2012). While MP will also increase the female's fitness, during copulation, they incur a greater risk of injury. Copulation results in large investment of energy, increasing the female's vulnerability to infections and disease (DiBattista *et al*. 2008a, b; Daly-Engel *et al*. 2010; Boomer *et al*. 2013). However, if the number of males is greater than females, it may be more advantageous for the females to be receptive than resistant. Therefore, MP might be a result of a behavior to avoid sexual conflict (Portnoy *et al*. 2007). Other hypotheses suggest that MP contributes to a genetically diverse and robust population that can withstand natural disturbances such as outbreaks of disease or abrupt changes in the environment (Feldheim *et al*. 2004; Daly-Engel *et al*. 2010). Polyandry and MP have also been suggested by commercial fisheries as strategies in species that are size and sex-selective (Gosselin *et al*. 2005; Chevolot *et al.* 2007a, b). However, there is little research into whether MP occurs and if this method is advantageous in oviparous (egg-laying) elasmobranchs since many lay multiple egg cases within a breeding season(s).

Although fecundity is higher in oviparous species than viviparous species, studies of the presence of MP in oviparous species have been sparse. There are two oviparous species in the Atlantic Ocean that are known to exhibit MP: The Small-spotted catshark (*Scyliorhinus canicula*) (Griffiths *et al*. 2011) and the Thornback ray (*Raja clavata*) (Chevolot *et al.* 2007a), yet these two species only produce one embryo per egg case (estimated: ~29-69 egg cases/yr in *S. canicula*; ~180 egg cases/yr in *R. clavata*) (Holden 1975; Griffiths *et al.* 2011). The Big Skate unlike other oviparous elasmobranchs has multiple embryos within a single egg case. However, whether MP occurs in this species is currently unknown. Therefore, this study will also investigate whether MP exists in the Big Skate with its novel and unique adaptation of producing multiple embryos within a single egg case.

The objectives of this study were: 1) Observe if Bergmann's rule is applicable in the reproductive strategy of *B. binoculata*, e.g. if egg case dimensions and embryo numbers from northern populations are greater than their southern counterparts. 2) Examine egg cases and the size of embryos to see if they differ among captive between wild *B. binoculata* species. 3) Modify and revise the developmental stages proposed by Hitz 1964 *B. binoculata* by adding embryonic morphometrics for stages 1-5. These additional data will include total length, disc

width, yolk diameter and yolk volume. 4) Model the growth curve observed during embryo development. 5) Investigate whether sex ratios differ from 50:50 and whether sex ratios differ in offspring produced in captive and wild settings. Lastly, 5) Determine if multiple paternity is present in specimens raised from Monterey Bay Aquarium (MBA) and whether if one parent influence the sex ratio of the offspring.

METHODS

Egg case collection

Egg cases were collected and raised under San Jose State University (SJSU) Institutional Animal Care and Use Committee (IACUC) protocols: 2014-D and Ebert/Jang 1017. Big Skate egg cases were collected opportunistically from NOAA's Fishery Resource Analysis and Monitoring Division (FRAM) surveys in 2008, 2014, 2015, and 2016. A typical skate egg case has several components including horns, tendrils, a main portion, which contains the fertilized yolk, and finally anterior and posterior aprons (Figure 3; Ishihara *et al*. 2012). Anterior and posterior sides of the egg case are determined by the direction of where the egg case is positioned in the oviduct of the mother (Ishihara *et al*. 2012). Egg cases were measured from the anterior to the posterior apron for their length (ECL) and width of the main portion, and then dissected to determine fertility status. If embryos were present, measurements such as total length (TL), disc width (DW), yolk diameter, and yolk weight were recorded. Yolk volume was calculated from the assumption that the yolk is shaped as a sphere:

$$V = 4/3 * \pi * r^3 \qquad [1]$$

Where r is the radius or half the diameter of the egg yolk, and π is a constant value.

The developmental stages of each embryo were also determined based on descriptions in Hitz (1964), ranging from stage 1 to 5, with 1 as least developed to 5 being well-developed (Table 2). If embryos were developed enough, sex was recorded depending on the presence and absence of claspers. Embryonic tissues were also collected for DNA extractions.

To determine whether multiple paternity is present in this species, fertile Big Skate egg cases (n=10) were acquired from the MBA and raised in the Moss Landing Marine Laboratories (MLML) aquarium room from May 2015 to January 2016. The average incubation period for

this species is between five to six months in captivity (Chiquillo *et al*. 2014). The aquarium room runs on a sand-filtered-open-water-system pumping fresh saltwater from the Pacific Ocean. These ambient water temperatures provided suitable raising conditions for the egg cases to grow and develop. Additional aeration devices were placed behind the egg cases to ensure optimal hatching conditions. An incision was made in each egg case and a plastic film cover was glued to observe the embryos as they developed (Figure 4A). Each egg case was numbered and put in glass tanks (Figure 4B). Embryos that died prematurely were measured for TL, DW, stage of development, and yolk diameter, and sex was determined if possible before preserving them in 90% ethanol for DNA extraction. For offspring that successfully emerged, morphometrics and sexes were also recorded. These individuals were named from the corresponding egg case where they emerged from followed by either A, B, C, or D for sequential purposes. Then each neonate was separated to keep track of when the remaining littermates would emerge. From these 10 egg cases, 29 embryos were raised, and only 25 embryonic tissue samples (10-20 mg) were used (Table 3); DNA extraction was not successful on four individuals. Five adult Big Skates tissue samples were also acquired (three females, F_0007, F_2918, F_6528, and two males, M_2920, M_1479) from the aquarium. These adults were present in the exhibit before and after the egg cases were laid and collected by the MBA aquarists.

Microsatellite Genotyping

By observation of egg case laying, mothers for each case were known. Microsatellite markers were used to determine paternity between two captive males. They are tandem repeats of short sequence motifs of 1-6 nucleotides found in the nuclear genomes of most taxa (Hancock 1999; Selkoe & Toonen 2006). The pattern is arranged without interruption by any other additional base or motif. Microsatellites are also thought to be selectively neutral, usually polymorphic, and codominant (Hancock 1999; Chevolot 2006a). These characteristics are useful tools for paternal and kinship analyses as well as for studying population genetic structure, evolutionary relationships, and gene mapping in species (Kashi & Soller 1999; Webster & Reichart 2005; Selkoe & Toonen 2006). Due to their high levels of polymorphism

they are widely used in population genetics and used to determine family relatedness especially in polyandrous species (Portnoy & Heist 2012; Chabot 2015).

Genetics protocol

Tissue samples (muscle, embryonic tissue, and egg case samples) (10-20 mg) were collected, preserved in 90% ethanol, and stored in room temperature in 1.5 mL microcentrifuge tubes until processed (Table 3). DNA was extracted using Wizard® Genomic DNA Purification kit from Promega (Promega, Madison, WI). DNA were extracted from these tissues using Wizard® Genomic DNA Purification kit from Promega (Promega, Madison, WI). Samples were tested for quality and concentration using the nanodrop and diluted to 10 ng/µL in preparation for Polymerase Chain Reactions (PCR) using *Taq* PCR kit from New England Biolabs (NEB, Ipswich, MA). Five microsatellite primer sets (RP 30, RP 35, RP 39, RP 43, RP 44) were used to test for compatibility for this species (Table 4; Kang *et al*. 2012). Since the primers were created for a sister species (*B. pulchra*) closely related to the study species, these were the best published primers set to conduct the paternity tests for the study species, *B. binoculata*. M13(-21) universal primer tails were added only to the forward primers (5'-TGT AAA ACG ACG GCC AGT 3'), this was needed to label PCR products with appropriate dyes (NED, VIC) in a secondary PCR using the M13 primers (Schuelke 2000).

PCRs were conducted in 10 µL reaction mixture containing 0.25 U *taq* DNA polymerase (New England Biolabs, Ipswich, MA), 2 x PCR buffer, 100 µM dNTP mix, 10 µM of forward primer, 10 µM of the universal primer (M13) with the fluorescent tag (NED or VIC), and 10 µM of reverse primer, and 2-5 µL of the template DNA (10 ng/µL). The forward primer without the M13(-21) tail was only ¼ the amount of the reverse primer, this was to make sure the forward primer with the M13(-21) tail was able to amplify and the fluorescent tag would attach to the finalized PCR product (Schuelke 2000). PCR conditions are as follows: 4 min at 94° C for initial denature process; followed by 35 cycles of denaturation at 94° C at 30 s; 1 min at the annealing temperature specialized for each of the 5 primers (61-64° C); initial extension at 1 min at 68° C, followed by 8 cycles at 94° C for 30 s, annealing temperature at 53° C, initial extension at 1 minute for 68° C, and a final extension for 68° C for 10 min.

Genotyping protocols

PCR samples were visualized with an ABI PRISM 3130xl Genetic Analyzer at San Francisco State University's (SFSU) Genomics Transcriptomics Analysis Core (GTAC). 1 µL of PCR product was added to 10 µL of Hi-Di™ Formamide and 0.5 µL of GeneScan™ 600 LIZ™ dye Size Standard in each well. The 600 LIZ™ dye Size Standard was used as a reference to determine the size and fluorescent intensity of PCR products. Samples were heated to 95^{o} C for 3-5 min before loading onto the sequencer which generated FSA files. For adults, PCR and electrophoresis was repeated three times. FSA files are fragment analysis data files created by DNA genetic analyzers that record the sizes and fluorescent intensities of PCR products by size which then can be read by genotyping software.

Genetic analyses and statistical analyses

FSA files were viewed using the Microsatellite plugin in Geneious (Biomatters, New Zealand) and the strongest two PCR products were scored as alleles, assuming this species is diploid. For adults, allele sizes were determined by averaging the product sizes, as estimated by the Geneious-plug-in. For embryos, PCR was repeated only for some samples and loci when initial results did not produce scorable PCR products. Overall, the microsatellite primers were expected to produce products of about 150-230 bp based on Kang *et al.* (2012), though a larger size range of 100-250 was considered as this species had not been previously characterized for these loci. For each locus, alleles were expected to differ by multiples of 2 bp.

Multiple paternity was investigated first by examination by eye of Table 10 A& B. The mothers were known from observing them laying egg cases. Knowledge of the maternal genotype allowed the paternal allele in each embryo to be identified. Because only two males were potential sires in this captive group, it was possible to determine whether alleles unique to each male were present among case-mate embryos (Figure 5). This would be evidence for multiple paternity in this species and within cases

For comparison to manual analysis, several programs were used to determine population genetics, relatedness, sibship and parentage. Multiple paternity analysis was conducted using

the program COLONY by Jones and Wang (2010) (http://www.zsl.org/science/research-projects/software/colony,1154,AR.html). CO-ANCESTRY was used to confirm the validity of the genotyping and potential sibship.

COLONY **(**2.0.6.4**)** was used because this program considered species that are polyploid, whereas most programs only accommodate diploid species, and cannot perform analyses on both sibship and parentage (Jones & Wang 2010). Currently, polyploidy in elasmobranchs is unknown as there is variability in genome sizes for different species (Range: 1 GB to 11 GB) (G. Naylor, pers. Communication, College of Charleston), but most elasmobranch paternity studies assume diploidy (2N). Furthermore, COLONY uses group maximum likelihood ratios to assign individuals into sibling groups and considers multiple mating systems and skewed reproductive success (Jones & Wang 2010; Larson *et al*. 2011).

Finally, CO-ANCESTRY (V. 1.01.9) was used as this program implements seven different relatedness methods (TrioML, Wang, LynchLi, LynchRd, Ritland, QuellerGt, and DyadML) that determine pairwise relatedness using control references between individuals, including or excluding inbreeding accounts and genotype errors in the data (Wang 2011). Average relatedness was calculated among all the programs.

Various statistical tests were run on R 3.4.4 (R Core Team 2018) such as T-tests and ANOVAs (Analysis of Variance) to test whether there was difference between egg case dimensions and embryo numbers per egg cases among the two environments. T-tests were conducted to see if average number of embryos differed between captive and wild egg cases. Generalized liner models (GLMS) were used to see if there were differences between embryo sizes among the two environments. GAMs (Generalized Additive Models) were chosen to determine if there was a latitudinal gradient among egg case dimensions and embryo numbers because initial plots with the egg case TL and embryo numbers as response variables did not show a linear regression relationship with increasing latitude. Also, biologically, the egg case length is dependent on the size of the female producing the egg case, and that would also restrict the number of embryos produced within an egg case. Therefore, a linear model would not biologically explain these relationships. GAM analyses were conducted using the mgcv package v. 1.8-2.4 and following certain parameters listed in Wood (2006).

A relationship was determined between disc width and embryo TL, using GLM. It is well known that an allometric growth curve is observed in many taxa during crucial

developmental stages, therefore the equation was log-transformed to determine the values. Using R, the null deviance and a residual deviance were calculated to calculate the pseudo R^2 value proposed by Faraway (2016):

$$1 - \frac{\text{ResidualDeviance}}{\text{NullDeviance}} \quad (2)$$

Finally, to determine if there were Offspring Sex Ratio (OSR) in both captive and wild specimens Chi-squared tests were conducted with a Yate's correction factor for continuity, with α value of 0.05.

RESULTS

Egg case dimensions and Big Skate productivity

Wild egg case length ranged from 20.00-33.33 cm; wild egg case width ranged from 9.30-17.00 cm. Captive egg case length ranged from 19.30-22.50 cm and 8.50-9.30 cm width. T-tests analysis was conducted to determine if the egg case dimensions differ between the captive and wild populations (Figure 6). I found that both dimensions (egg case length [**ECL**] and width [**ECW**]) from captive (n=10) and wild egg cases (n=103) were significantly different, with the wild egg cases being much larger [ECL: T= -12.71, df= 28.90, $p<0.0001$]; [ECW: T=-18.13, df= 63.17, $p<0.0001$]. On average, the wild egg cases were 6.71 cm larger in length than the captive eggs. A linear relationship was determined (Figure 7) between egg case length (ECL) and width and both were significantly correlated ($F=11.57$, df= 129, $p<0.0001$, $R^2=0.53$). Since ECW and ECL were highly correlated with each other, ECL was selected as the response variable for future analyses. 103 egg cases were collected from the FRAM surveys with, of which 59 were fertile and 44 infertile (Table 5). Of the 59 fertile egg cases, embryo counts ranged from 1 to 6 (Figure 8), with an average embryo number of 3.62 per egg case. In egg cases collected from MBA (n=10), the embryo counts ranged from 2 to 4 (Figure 8) with an average of 2.90. Excluding the empty egg cases, three embryos were the most common per egg case in the wild (Figure 8). Egg cases with only two embryos were the most common in captive

egg cases (N=2). Embryo numbers were significantly different among captive and wild egg cases [T=6.02, df=67, p=0.02].

Latitudinal patterns in egg case lengths and embryo numbers

Big Skate egg cases were largest in length at 42° N around 32 cm TL (dev. Expl: 28.02%, p-value: $3.71e^{-03}$) (Table 6, Figure 9). Although not significant (dev. Expl: 12%, p-value: 0.06), embryo numbers per egg case also peaked at 42° N (Table 7; Figure 10). In this region of the coast, egg cases were larger and contained more embryos than other locations.

Allometric growth curve

The final equation to explain the relationship between disc width and embryo TL, (Emb_TL in the model) was log(disc_width) =-3.90 + 2.16*log (Emb_TL) (Figure 11). A pseudo R^2 value was calculated with a of 0.87, indicating that this allometric model was able to explain around 87% of the variables in the relationship. Embryos raised in captivity tend to have a larger disc width and a shorter total length ratio.

Big Skate developmental stages

Wild embryos were 1.00 to 1.30 times larger than their captive counterparts in the same stages of development (Figure 12&13). Only four developmental stages (2-5) were observed throughout the study, no embryos were found in stage 1(Table 8). Hitz (1964) described stage 1 where the embryos are not physically formed yet, the yolks are fully separated, and blood vessels are present in each yolk sac. In this study, all embryos were physically developed and embryos measurements in both captive and wild settings were compiled to determine the ranges among the developmental stages. In stage 2, the embryos were white in color and had a tadpole-like body, had developed eyes, and had external branchial filaments covering their bodies. Embryos in stage 2 had a TL that ranged from 2.5-9.0 cm, with an average of 6.29 cm. The pectoral fins (wings) had started to develop, and disc width (DW) ranged from 0.4-4.5, with the average disc width of 1.06 cm. Yolk diameter ranged from 3.3-5.7 cm, with an average yolk

diameter of 4.48 cm. In stage 3, the pectoral fins became more developed and external brachial filaments were replaced with gill slits and spiracles had formed, but the embryos had a pale-cream coloration. Embryo TL and DW ranged from 5.0-13.4 cm with the average of 9.74 cm, and 1.0-5.0 cm with the average of 2.61 cm, respectively. Yolk diameter at this stage ranged from 2.4-5.4 cm with the average of 4.21 cm. In stage 4, embryos had pigmentation on the dorsal side consisting of a light brown and tan color, and the distinct eye spots on the wings had formed. TLs ranged from 10.50-20.1cm, with an average of 15.0 cm. Disc width ranged from 3.1 to 12.8 cm, with the average disc width of 7.21 cm. At stage 5, the embryos had dark brown dorsal coloration with faded splotches, distinct ocelli on the dorsal side of each wing were present, and dorsal spines on the head were formed for emergence. Embryo TLs ranged from 8.5 cm-21.00 cm, averaging 17.13 cm. Disc widths ranged from 7.0-14.0 cm, with an average of 11.03 cm. Yolk diameter ranged from 0.3-3.0 cm, averaging 1.11 cm. Embryo sizes among the two environments were also significantly different ($T=8.46$, $df=104$, $P<0.0001$)(Figure 12).

Offspring sex ratio (OSR) from FRAM surveys

A total of 219 embryos were collected in 2008 and from the years 2014-2016. Only embryos that were in the developmental stages of 4 and 5 (Table 9) were selected for sex ratio analysis (hypothesized at 1:1) because these were the stages where the sexes could be determined; of these embryos, there were 48 females and 37 males. There was no difference in OSR in the wild or ($\chi^2=0.38$, $df=1$, $p = 0.54$) or between the different environments (captive vs wild) ($\chi^2=0.20$, $df=1$, $p = 0.66$). Both tests were not statistically significant, therefore the null hypothesis was not rejected.

OSR from captive environment

Offspring sex ratio from the Monterey Bay Aquarium samples was 13 females, 7 males, and 5 unknowns. These five embryos had died prematurely before sex appendages were developed enough to determine their sex. There was no significant difference in OSR between these two sires ($\chi^2=0.41$, $df=1$, $p = 0.55$).

Genetic relatedness and multiple paternity presence

A total of 29 embryos were raised from the 10 captive-bred egg cases. Female F_0007 was observed to lay one egg case with three embryos. Female F_6528 laid nine egg cases with two to four embryos each. Female F_0007 was aquarium raised and known to never to have mated. Female F_6528 was wild captured and introduced to the mating group for approximately one year during which she laid three egg cases; the remaining six egg cases were laid over a three-month time period after she was removed from the mating group due to injury. Twenty-five embryos were successfully extracted for genomic DNA. Microsatellite locus RP 35 did not generate usable genotype data and was removed from the study.

The other loci displayed some puzzling characteristics. The difference in allele sizes were unexpectedly large (Table 10 A&B). For example, RP 30 had alleles of 158 to 250, much larger than the 14 bp difference in *B. pulchra* (Kang *et al.* 2012). A large difference was also seen in RP39 (130-174 bp). Dinucleotide repeats are expected to differ in allele sizes by multiples of 2 bp, yet both odd and even sizes were seen in RP43 (e.g., 125 bp and the next longer allele of 132 bp, a 7 bp difference). These unusual characteristics suggest that errors in allele calling may have occurred. The PCR products were not sequenced, so it is uncertain if the different alleles are true homologues within *B. binoculata*.

Egg case number 221 was laid in captivity by female F_0007, who could only have mated with M_1479 or M_2920. Embryos 221C has a genotype consistent with M_1479 as the sire, but embryos 221A and 221D have an allele ($RP44_{164}$) not seen in captive males. Since sperm storage is not a possibility for this female, it is likely that this allele is miscalled (Table 10A).

Female F_6528 produced many embryos (e.g., 227A, 227C, 229A, 232A, etc…) (Table 10B) with alleles not seen in captive males, which could plausibly be derived from stored sperm from mating encounters prior to capture, or from allele calling error.

Discrepancies in the characteristics of the loci (size range and deviation from dinucleotide repeat unit size), as well as offspring genotypes irreconcilable with potential sires suggest caution further interpreting these data. That said, cases from female F_0007 provide evidence of multiple paternity involving known males in the captive group,

discounting possible allele calling error in RP44. Too, if sperm storage accounts for extra alleles found in offspring of F_6258, multiple paternity is also evident.

DISCUSSION

Egg case dimensions and Big Skate productivity

Overall, the egg cases lengths (ECL) and embryos raised from a captive setting were significantly smaller than the wild egg cases. This was expected because the size of embryos is limited by the egg case dimensions (ECL and ECW) which in turn depends on the size of the mother (Howard 2017). A total of three female Big Skates were raised in the captive environment in the MBA exhibit, and two of the females (F_2918 and F_0007) had disc widths of 84 cm and 86 cm, and total lengths of 125 cm and 117 cm respectively. Unfortunately, there are no morphometric data for F_6258. The female sizes from captivity were smaller than the average observed TL female Big Skates caught in the FRAM surveys (TL over 125 cm); and the average embryo numbers (n=2) are fewer in a captive setting than those (n=4) in the wild environment. Howard (2017) observed captive Big Skates attained a smaller sexual maturity and laid smaller egg cases than previously recorded studies suggesting that because of the environment played a role in early reproductive success than their wild counterparts.

Previous studies (Ebert & Davis 2007; Ebert *et al*. 2008b) have suggested that *B. binoculata* may be the most fecund elasmobranch known to date. It has been well-documented that oviparous elasmobranchs such as skates possess the reproductive strategy of producing smaller offspring but at much higher frequencies than their viviparous counterparts (Conrath and Musick 2012), but *B. binoculata* has taken this reproductive strategy to a higher level. Ebert & Davis (2007) and Ebert *et al.* (2008b) estimated a female may lay up to 6000 egg cases during her lifetime (around 350 egg cases per year). This calculation considered that a female would reach an average maturity age of eight years and continue to produce fertile egg cases while attaining a lifespan up to 26 years. Considering the ability to produce 1-8 embryos per egg case, a single female may potentially produce more than 48,000 embryos in her lifetime (Ebert & Davis 2007; Ebert *et al*. 2008b).

However, not all offspring will develop and emerge. Chiquillo *et al*. (2014) observed an average of two embryos per egg case surviving in captivity. With this current information about

offspring survival, the estimated offspring that will survive to emergence from a single female are around 12,000 individuals in her lifetime in captivity. In the wild, excluding predation pressure the offspring number produced is estimated to be around 18,000 to 24,000 individuals, since three and four embryos were observed to be the most common. Producing so many offspring in an egg case offers an insurance for females to ensure that majority of their offspring will develop and emerge successfully. Many avian, amphibian and teleost species follow similar strategies (Forbes 1991). This observation of multiple embryos per egg case was common in fertile egg cases acquired from the FRAM survey. Embryo numbers ranged from 1-6 with four embryos per egg case being most common (47%). However, this study was unable to genetically match whether the egg cases were laid by specific females, which would help further support this hypothesis.

Another explanation why three or four embryos were more common from the FRAM surveys is to time their emergence and limit predation risks. This behavior has been found in Green Sea Turtles (*Chelonia mydas)*. *Chelonia mydas* synchronize their nesting times so their offspring have a higher chance of surviving predation after hatching. Santos *et al*. (2016) were able to observe that an increased group size within a nest reduced predation risk, especially if hatchlings timed their emergence in early evening; the presence of the prey overwhelm the predators causing the attack abatement effect, in which a predator is less likely to attack a group of prey when the potential numbers of prey are much greater (Turner and Pitcher 1986). While the maximum record of eight embryos was not observed in this study, one egg case housing six fully developed offspring had an opening. This observation suggested that there may have been offspring that had emerged earlier before the egg case was collected; implying that there had been more than six embryos in that egg case.

The sample size (n=29) of embryos raised in captivity was smaller, with the most common number of embryos from any one of these egg cases was two. In the captive environment there are more stable conditions within the aquarium exhibit, such as consistent water quality, abundant food, and regular encounters with potential mates. These factors may lead to an earlier sexual maturity and productive success in the adults resulting in smaller egg cases and offspring compared to their wild counterparts (Howard 2017). The differences in the embryo numbers per egg case in these two environments also suggest that the mothers' reproductive conditions may play a more substantial role. Two of the three females (F_2918 and

F_0007) were captive raised themselves, and the third female (F_6528) was caught from the wild. Interestingly, nine of the ten egg cases collected from MBA were from F_6528 (Table 10B) which six of the egg cases were laid while she was in quarantine for 3 months indicating that sperm storage is present in this species. Paternity evidence suggested at least 4 males contributed to this female's offspring which furthers strengthens that Big Skate exhibit sperm storage; two of these males were from the MBA exhibit. This female also produced abnormal amount of egg cases until her death. Autopsy analysis revealed that she was heavily impacted with egg cases exhibiting various degrees of decay (Kelsey Barker, Monterey Bay Aquarium, personal communication). In egg case number 232, laid by F_6528 an unfertilized ovum was found in the egg case along with two embryos, and this observation suggested that not all the eggs will be fertilized.

Forty-three percent of the egg cases from the FRAM surveys were infertile which validates the captivity observations that not all eggs will be fertilized. However, no unfertilized eggs were found among any of the fertile egg cases. Since these egg cases were collected from the wild, it is difficult to know which females laid certain egg cases from the FRAM surveys. The study was unable to discern or observe each female's reproductive condition. On the other hand, the frequent observations of many unfertilized egg cases lead to a question why females would invest in laying infertile egg cases if it is energetically costly. One explanation is that laying infertile egg cases is an indicator that females are in a healthy condition that and they do not need to maintain their energy reserves. This strategy would allow females to produce infertile 'dummy' egg cases among fertile egg cases to relieve predation pressure. Predators would choose infertile egg cases rather than egg cases containing developing embryos. To test this hypothesis, blood and tissue samples are needed to observe female Big Skate health conditions. In addition, monitoring whether egg case fertility status and quantity affect predatorial behavior would be needed as evidence of whether increasing 'dummy' egg cases has a positive correlation with neonate survival rates.

Latitudinal patterns in egg case and embryo numbers

Bergmann's rule explains how body sizes vary among species or population along latitudinal gradients; usually the smaller body sizes and slower growth occur in the southern

(i.e. warmer) range, while larger body sizes and high growth rates occur in the northern (i.e. cooler) range (Ray 1960). In this study, *B. binoculata* egg case sizes and embryo numbers did not exhibit Bergmann's rule; instead responses peaked at 42 degrees North (Figures 13, 14), rather than increasing in sizes and quantity going further north along the Northeast Pacific coast. Bergmann's assumption was that climate gradients were affiliated with latitudinal geographic locations. However, this relationship is not necessarily strongly correlated, as other environmental factors within regions are known to impact the variability in a locality. Along the coast of California there are two distinct biogeographic barriers that show high intense biological activity due to geological oceanographic formations; these areas are Point Conception in the south, and the Gorda Escarpment Basement Ridge (GEBR). Located at 40° 22' 00" N and 125° 10' 00" W, the GEBR was formed by two plates, the North American Plate and the Pacific Plate that move horizontally against each other (Hoover & Trehu 2017). There are documented cases that the GEBR is a reproductive hotspot, especially for egg-brooding deep-sea fish and cephalopods (Drazen *et al*. 2003). Previous studies have suggested that Big Skates frequent water depths no less than 200 meters. Farrugia *et al* (2016b) found that Big Skates at depths of 500 meters are thermally tolerant of temperatures (2^{o} to 18^{o}C). Data from the FRAM surveys also collected several egg cases (n=3) at 530 meters as evidence of the species tolerating these greater depths. In addition, the egg cases and embryo numbers were the greatest at 42 ^{o} N within the species' temperature range (8^{o} C to 10.4^{o}C) However, many of the egg cases collected were at depths shallower than 200 meters, implying the GEBR does not have a large influence on the Big Skates' reproductive strategies such as altering the egg case dimensions and/or embryo numbers.

Oceanographic currents such as the California current system would best explain why at 42° N, egg case length (ECL) and embryo numbers were the greatest. The California Current System (CCS) is an eastern boundary current that moves southward as the Pacific Ocean current from British Columbia to Baja California (Checkley & Barth 2009). This system has three general areas where sea surface temperatures are influenced by seasonal variations: 1) Columbia River plume (around 44° N), 2) Cape Mendocino (40° N), and 3) San Diego (32-34° N) (Checkley & Barth 2009). In the Cape Mendocino area, the winds are the strongest allowing more sea-surface mixing which increases upwelling intensity. Strong

upwelling conditions are known to be very prolific and enhance the marine bio fauna diversity and quantity within that region. Since *B. binoculata* are known to be opportunistic feeders (Bizzarro *et al*. 2014), areas near Cape Mendocino provide the best conditions to feed, reproduce, and provide a nursery habitat so the offspring would be able to hunt once their yolk sac reserves are depleted. This would be the best explanation of why ECL and embryo numbers were the largest and greatest in this region. However, this study did not include the northernmost region of this species range, so future studies would have to include *B. binoculata* egg cases from the Eastern Bering Sea to British Columbia waters to test if Bergmann's rule applies to this species in their northernmost latitude or if other oceanographic features impact the egg case length and embryo numbers. In addition, skate species up in the Eastern Bering sea are known to aggregate and lay egg cases in specific nursery sites coined as "Habitats of Particular Concern" (HAPC) (Hoff 2010). While FRAM trawl surveys in this study did not find more than 18 *B. binoculata* egg cases in a trawl location, off the Gulf of Alaska, scallop dredge surveys have found nursery sites with numerous Big Skate egg cases near Kayak Island, (Farrugia, pers. Communication, Figure 14). These observations suggest that Big Skates in that region or perhaps in the northern most part of their range may exhibit different behavior tactics to maximize their offspring survival rate.

Allometric growth curve

Although the allometric growth curve was a good fit to the data (r^2=0.87), there were outliers and values that are above or below the allometric growth curve. Interestingly, the aquarium-raised embryos' disc widths were longer than their total length compared to the wild embryos (Figure 11). Wider disc width might be beneficial in a captive setting but may not be advantageous in the wild. In addition, neonates raised from captivity were observed to have remaining yolk sacs (> 4mm in diameter) even after emergence, no remaining yolk sacs were found in the skate embryos collected from the FRAM surveys. These observations may indicate that maternal investment and embryo development do differ among individuals within an egg case, among litters, and between the two different environments.

An example how maternal investment may differ is shown in oophagous viviparous elasmobranchs (Gilmore 1993). Female lamnid sharks invest more in fewer offspring in by providing nutritious yolks for the offspring to consume once their external yolk sac sources are exhausted; only a limited number of individuals survive in the uteri of the mother before birth (Gilmore 1993). In species such as *Carcharias taurus* (Sand-tiger shark), embryos practice adelphophagy, where the largest embryos end up consuming their siblings until there is one offspring left in each uterus, after which the mother provides unfertilized eggs for them until birth (Gilmore 1993). Oophagy has not been observed in oviparous species since most of these species laid single embryos enclosed in a case. In this study, while raising the Big Skate embryos in the captive setting, some individuals in developmental stage 2 were observed to tangle themselves among their littermates' branchial filaments. At this stage 2, the egg case's horns are still plugged preventing seawater from entering; the only way for the embryos to acquire oxygen is to physically move their bodies constantly as their branchial filaments were still external. This stage is also the most vulnerable stage where the most embryos died prematurely in this study. To determine whether maternal investment influences embryonic survival rate, a relationship between embryo growth and yolk consumption will need to be modeled in a time series including all the developmental stages, not just stages 2-5.

Offspring sex ratio (OSR) from FRAM surveys

In both environments, offspring sex ratio (OSR) was found to be more females than males. The variation in the sex ratio of offspring is an evolutionary and life-history strategy in which many taxa have invested to ensure their own fitness. A popular hypothesis from Trivers & Willard (1973) presented a simulation that suggested conditions where mothers should produce more female offspring. They proposed mothers have lower variance in reproductive success than male offspring under poor conditions therefore producing more female offspring is the better reproductive strategy (Trivers & Willard 1973). This is known as the Trivers-Willard Hypothesis (TWH). The TWH is still debatable as many studies and reviews suggest that sex-ratio manipulation depends on a variable number of factors. Cameroon (2004) looked at 422 studies in mammalian sex-ratio studies to see if these studies

were in favor with TWH based on certain condition measures (e.g. range quality, litter size, age, weight of mothers, etc.) and only 34% studies supported TWH. However, this hypothesis only concerned polygynous ungulates, which: 1) have a limited breeding season 2) can only produce limited offspring (n=1-2) and 3) possess one functioning uterus. Ungulates have the conservative K-selected reproductive strategy focusing on a limited amount of offspring to insure a higher survival rate (Leslie *et al*. 1999). Whereas *B. binoculata* reproductive characteristics are very prolific. In elasmobranchs OSR is not known to occur. After looking at 223 liters (n=1005), Charnov (1982) stated that the sex ratio in *Squalus acanthias* (Spiny Dogfish) was around 49%. Ishihara *et al.* (2002) found the sex ratio of *Okamajei kenojei* (Spiny Rasp Skate) from one female in captivity that laid 40 egg cases in that year, of the 30 offspring recorded 17 were males (56.67%) and 13 were females (43.33%). In *B. pulchra,* the OSR were 45.50% for females and 54.40% for males (Kang *et al.* 2013). Therefore, significant deviations from an even OSR may not be prevalent in elasmobranchs, but even though most species are known to segregate among the sexes, especially during breeding seasons.

Genetic relatedness and multiple paternity presence

This study showed that multiple paternity within an egg cases laid after captive mating was plausible if an unexpected allele in one locus is resolved as an allele calling error. Further, a female that may have mated both prior to capture and after introduction to the mating group produced cases with multiple paternal alleles, many of which may derive from sperm storage. DNA sequencing of a sample of microsatellite PCR products for each locus in future studies will clarify allele calling and establish the homology of alleles. If sequencing shows that these loci are not homologous to the microsatellites of *B. pulchra*, then future studies will need newly designed, species-specific primers.

High genetic relatedness is not uncommon in elasmobranchs population studies when sampling areas are limited in range, therefore this increases the chances of sampling related individuals. Larson *et al.* 2011 found that 65-87% of Bluntnose Sixgill Sharks sampled (*Hexanchus griseus)* were either half-siblings or full siblings within Puget Sound (PS), Washington. These close relationships occur because PS has been observed to be a pupping

and nursery ground for this species with siblings remaining in proximity in some areas through tagging and genetic sampling studies (Larson *et al*. 2011).

While there is no current study on whether certain estuaries or bays in California are suitable nursery grounds for Big Skates, Hoff (2010) and Hoff (2016) found multiple nursery sites in two species of skates in the Eastern Bering Sea. These two species, the Alaska Skate (*Bathyraja parmifera)* and the Aleutian Skate (*Bathyraja aleutica)*, use different types of nursery sites. Each nursery site is associated with different geographical features, 1) outer continental shelf/canyon openings for egg deposition, and 2) outer and middle continental shelf for newly emerged juveniles (Hoff 2016). The discovery of these nursery sites in the Eastern Bering Sea was proposed to the North Pacific Fisheries Management Council (NPFMC) to implement protection and monitoring programs for these regions (NPFMC 2018). Future studies should investigate whether Big Skates also use these two geographical features along the coast, which may provide evidence of potential nursery sites for this species that can be monitored and protected.

Although there are only two oviparous species of elasmobranchs that are known to exhibit multiple paternity, this strategy is present in many viviparous elasmobranchs as well as other taxa (Jones & Coleman 2011; Conrath & Musick 2012). Clearly, this reproductive strategy is not a rare occurrence. However, because *B. binoculata* is one of only two species that exhibit multiple embryos within an egg case, out of more than 300 recognized species, the presence of MP from this study is a novel finding in the family Rajidae. One male (M_1479) fathered more offspring than the other present male, this may be due to because of where they were raised. M_1479 was caught from the wild while M_2920 was captive raised. Studies have suggested that captive-raised individuals due to being in a more relaxed and less competitive sexual selection pressure environment than their wild counterparts may carry high fitness in captivity, but low fitness in wild environments which may affect developmental phenotypic plasticity among individuals and these traits would be passed to future offspring (Evans *et al*. 2014).

Christie *et al*. (2012) suggested that while first generation (parents were wild) captive-raised animals spawned in captivity may have a greater lifetime reproductive success rate than their wild counterparts, their offspring performed the worst in the wild with many of these

offspring unable to return to spawn. This inability was found in hatchery steelhead (*Oncorhynchus mykiss)*. The Oregon Department of Fish and Wildlife have been trying to restore natural steelhead populations (which are listed as threatened under the US Endangered Species Act) in the Hood River in Oregon through captive breeding programs. Christie *et al*. (2012) was able to provide evidence that captive-breeding programs may not be successful for certain species. O'Regan & Kitchner (2005) found that effects of captivity on wild mammals have changed their morphology including bone structure in skull shape differences, brain size reduction, postcranial adaptations and digestive tract changes. Behavioral changes were also observed as well as sexual dimorphism. Therefore, if such breeding programs for Big Skates were to be undertaken, precautions must be taken in effect so that 1) offspring survival rates will not be altered once they are released in the wild 2) behavioral changes from captive skates will not decrease their reproductive success, and 3) genetic diversity within this species remains robust.

Will breeding programs replenish wild skate populations?

Aquariums such as the Monterey Bay Aquarium are part of captive breeding programs primarily focusing on replenishing wild stocks of certain endangered species. However, if captive-raised offspring indeed perform worse in the wild, then precautions must be considered if the goal is to replenish wild stocks. For instance, the Mottled Skate (*B. pulchra*) populations in the Yellow and East seas have drastically declined due to overfishing. It is a species that is fished commercially as an important fish species in Korea and has been overexploited. *Beringraja pulchra* is now considered 'Vulnerable' under the IUCN criteria, but past studies have recorded drastic catch declines of 90% over the past 10 years in Korea (Ishihara *et al*. 2009). These declines have prompted recovery efforts to begin studying the spawning characteristics by artificially propagating the species. The conclusion of this study suggests that artificial reproduction might be the way to recover this species, and to focus on producing females due to their faster growth rates and being more commercially desirable (Kang *et al.* 2013). This method of restoration will be difficult to implement as Im *et al.* (2017) has found evidence of low genetic diversity in this species. Therefore, artificial propagation or captive breeding programs may not be beneficial even with a species that also possess the same ability of producing multiple embryos (n=9) as *B.*

binoculata (Kang *et al.* 2013). Continued monitoring and imposing harvesting restrictions during peak spawning season (especially in April to June) (Kang *et al.* 2013) are the current solutions for protecting *B. pulchra.* These case studies should serve as an indicator to fishing management on the Eastern Pacific to pay attention and implement monitoring programs to ensure that *B. binoculata* does not share the same predicament as its overfished cousin.

REFERENCES

Bizzarro, JJ, Broms, KM, Logsdon, MG, Ebert, DA, Yoklavich, MM, Kuhnz, LA, Summers, AP. 2014. Spatial Segregation in Eastern North Pacific Skate Assemblages. PloS ONE*,* **9** *(10): e109907* DOI: 10.1371/journal.pone.0109907

Blackburn TM, Gaston KJ, Loder N. 1995. Geographic gradients in body size: a clarification of Bergmann's rule. Diversity and Distributions **5**:165-174.

Boomer JJ, Harcourt RG, Francis MP, Walker TI, Braccini JM, Stow AJ. 2013. Frequency of multiple paternity in Gummy shark, *Mustelus antarcticus*, and, *Mustelus lenticulatus*, and the implications of mater encounter rate, postcopulatory influences, and reproductive mode. Journal of Hereditary **104**(4):371-379. Doi: 10.1093/jhered/est010

Cameroon EZ. 2004. Facultative adjustment of mammalian sex ratios in support of the Trivers-Willard hypothesis: evidence for a mechanism. Proceedings of the Royal Society of London B.**271**:1723-1728. Doi: 10.1098/rspb.2004.2773

Chabot CL. 2015. Microsatellite loci confirm a lack of population connectivity among globally distributed populations of the tope shark *Galeorhinus galeus* (Triakidae). Journal of Fish Biology **87**:371-385. Doi: 10.1111/jfb.12727

Charnov EL. 1982. The theory of sexual allocation. Princeton University Press, Princeton, New Jersey. Pp 355.

Checkley DA, Barth JA. 2009. Patterns and Processes in the California Current System. Progress in Oceanography **83**: 49-64. Doi:10.1016/j.pocean.2009.07.028

Chevolot M, Reusch TBH, Boele-Bos S, Seam WT, Olsen JL. 2005. Characterization and isolation of DNA microsatellites primers in *Raja 34anicul* L. (Thornback Ray, Rajidae). Mol Ecol Notes.**5**:472-429.

Chevolot M. 2006a. Assessing Genetic structure of Thornback ray, *Raja 35anicul*: a thorny situation? Ph.D. thesis. University of Groningen: Netherlands. 148 pp.

Chevolot M, Hoarau G, Rijnsdorp AD, Stam WT, Olsen JL. 2006. Phylogeography and population structure of thornback rays (*Raja 35anicul* L., Rajidae). Molecular Ecology **15**: 3693-3705.

Chevolot M, Ellis JR, Rijnsdorp AD, Stam WT, Olsen JL. 2007a. Multiple paternity analysis in the thornback ray *Raja 35anicul* L. Journal of Heredity **98**:712–5.

Chevolot M, Wolfs PHJ, Pálsson J, Rijnsdorp AD, Stam WT, Olsen JL. 2007b. Population structure and historical demography of the thorny skate (*Amblyraja radiata*, Rajidae) in the North Atlantic. Mar Biol **151**:1275–1286.

Chiquillo KL, Ebert DA, Slager CJ, Crow KD. 2014. The secret of the mermaid's purse: Phylogenetic affinities within the Rajidae and the evolution of a novel reproductive strategy in skates. Molecular Phylogenetics and Evolution (75): 245–251. DOI: 10.1016/j.ympev.2014.01.012

Christie MR, Marine ML, French RA, Blouin MS. 2012. Genetic adaptation to captivity can occur in a single generation. PNAS **109**(1):238-242. https://doi.org/10.1073/pnas.1111073109

Cicia AM, Driggers WB, Ingram GM Jr, Kneebone J, Tsang PCW, Koester DM, Sulikowski JA. 2009. Size and age estimates at sexual maturity for the little skate *Leucoraja erinacea* from the western Gulf of Maine, U.S.A. Journal of Fish Biology **75**: 1648-1666. Doi: 10.1111/j.1095-8649.2009.02392.x

Coleman SW, Jones AG. 2011. Patterns of multiple paternity and maternity in fishes. The Linnean Society of London, Biological Journal of the Linnean Society **103**:735-760.

Compagno LJV. 1990. Alternative life-histories of cartilaginous fishes in time and space. Environmental Biology of Fishes **28**:33-75.

Conrath CL, Musick JA. 2012. Reproductive biology of Elasmobranchs. In Carrier JC, Musick JA, Heithaus MR. (Eds). Biology of Sharks and Their Relatives II. CRC Press, New York. Pp 291-306, pp 633.

Daly-Engel T, Grubbs R, Feldheim K, Bowen B, Toonen R. 2010. Is multiple mating beneficial or unavoidable? Low multiple paternity and genetic diversity in the shortspine spurdog *Squalus mitsukurii*. Mar Ecol Prog Ser **403**:255–267.

DiBattista JD, Feldheim K, Gruber SH, Hendry AP. 2008a. Are indirect genetic benefits associated with polyandry? Testing predictions in a natural populations of lemon sharks. Molecular ecology **17**:783-795.

DiBattista JD, Feldheim K, Thibert-Plante X, Gruber SH, Hendry AP. 2008b. A genetic assessment of polyandry and breeding-site fidelity in lemon sharks. Molecular ecology **17**:3337–51.

Drazen JC, Goffredi SK, Schlining B, Stakes DS. 2003. Aggregations of egg-brooding deep-sea fish and cephalopods on the Gorda Escarpment: a reproductive hot spot. The Biological Bulletin 205:1-7. DOI: 10.2307/1543439

Ebert DA. 2003. Sharks, Rays, and Chimeras of California. University of California Press, Berkeley, California, 284 pp.

Ebert DA. 2005. Reproductive biology of skates, *Bathyraja* (Ishiyama), along the eastern - Bering Sea continental slope. Journal of Fish Biology 66:618-649. Doi:10.1111/j.1095-8649.2005.00628.x

Ebert DA, Compagno LJV. 2007 Biodiversity and systematics of skates (Chondrichthyes: Rajiformes: Rajoidei) Environmental Biology of Fishes (80):111-124.

Ebert DA, Compagno LJV, Cowley PD. 2008a. Aspects of the reproductive biology of skates (Chondrichthyes: Rajiformes : Rajoidei) from southern Africa. ICES Journal of Marine Science. **65**:81–102.

Ebert DA, Smith WD, Cailliet GM. 2008b. Reproductive biology of two commercially exploited skates, *Raja binoculata and R. rhina*, in the western Gulf of Alaska. Fisheries Research **94**:48–57.

Ebert DA, Davis CD. 2007. Descriptions of skate egg cases (Chondrichthyes: Rajiformes: Rajoidei) from the eastern North Pacific. Zootaxa **1393**:1–18.

Ebert DA, Sulikowski JA. 2007. Preface: Biology of Skates. In DA Ebert & JA Sulikowski (eds). Biology of Skates. Pp 1-18, pp 243.

Ebert DA, Ho HC, White WT, Carvalho M De. 2013. Introduction to the systematics and biology of sharks, rays, chimaeras (Chondrichthyes) of Taiwan. Zootaxa. **3752** (1):005–019. http://dx.doi.org/10.11646/zootaxa.3752.1.3

Evans ML, Wilke NF, O'Reilly PT, Fleming IA. 2014. Transgenerational effects of parental rearing environments influence the survivorship of captive-born offspring in the wild. Conservation Letters **7**(4): 371-379. Doi: 10.1111/conl.12092

Faraway JJ. 2016. Extending the linear model with R: Generalized linear, with mixed effects and nonparametric regression models, 2nd Eds. Chapman and Hall/CRC, New York, New York, pp 59, 399.

Farrugia, TJ, Goldman, KJ, King, JR, Ormseth, OA. 2016a. *Beringraja binoculata*. The IUCN Red List of Threatened Species 2016:

e.T44183A80679344.http://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS.T44183A80679344.en. Downloaded on **10 August 2018**.

Farrugia TJ, Goldman KJ, Tribuzio C, Seitz AC. 2016b. First use of satellite tags to examine movement and habitat use of Big Skates *Beringraja binoculata* in the Gulf of Alaska. Marine Ecology Progress Series **556**:209-221.

Feldheim KA, Gruber SH, Ashley MV. 2004. Reconstruction of parental microsatellite genotypes reveals female polyandry and philopatry in the lemon shark *Negaprion brevirostris*. Evolution **58**(10): 2332-2342.

Feldheim KA, Stow AJ, Ahonen H, Chapman DD, Shivji M, Peddmors V, Wintner S. 2007. Polymorphic microsatellite markers for studies of the conservation and reproductive genetics of imperiled sand tiger sharks (*Carcharias taurus*) Molecular Ecology Notes*,* **7** *(6): 1366–1368* DOI: 10.1111/j.1471-8286.2007.01888.x

Fitzpatrick JL, Kempster RM, Daly-Engel TS, Collin SP, Evans JP. 2012. Assessing the potential for post-copulatory sexual selection in elasmobranchs. Journal of fish biology **80**:1141–58.

Forbes LS. 1991. Insurance offspring and brood reduction in a variable environment: the cost and benefits of pessimism. OIKOS **62**:325-332.

Gburski CM, Gaichas SK, Kimura DK. 2007. Age and growth of Big Skate (*Raja binoculata*) and Longnose skate (*R. rhina*) in the Gulf of Alaska. Environmental Biology of Fishes **80**:337–349.

Gilmore GR. 1993. Reproductive biology of lamnoid sharks. Environmental Biology of Fishes 38: 95-114.

Gosselin T, Sainte-Marie B, Bernatchez L. 2005. Geographic variation of multiple paternity in the American lobster, *Homarus americanus.* Molecular Ecology **14**:1517–25.

Griffiths AM, Jacoby DM P, Casane D, McHugh M, Croft DP, Genner MJ, Sims DW. 2011. First analysis of multiple paternity in an oviparous shark, the small-spotted catshark (*Scyliorhinus canicular L.*). The Journal of heredity, **103**(2), 166–73.

Haas D. 2009. 5: Skates and Rays. In California Fish and Game: Status of the Fisheries Report 2008, pp 5-1:5-17.

Hancock JM. 1999. Microsatellites and other simple sequences: genomic context and mutations mechanisms. In Goldstein DB, Schlötterer. (Eds). Microsatellites: Evolution and Application. Oxford University Press, Inc., New York. Pp 1-9, pp 341.

Hitz CR. 1964. Observation on Egg Cases of the Big Skate (*Raja binoculata* Girard) Found in the Oregon Coastal Waters. Journal of the Fisheries Research Board of Canada. **21**(4): 851-854.

Hoff GR. 2010. Identification of skate nursery habitat in the eastern Bering Sea. Mar Ecol Prog Ser **403**:243-254

Hoff GR. 2016. Identification of multiple nursery habitats of skates in the eastern Bering Sea. Journal of Fish Biology. **88**(5): 1746-1757. https://doi.org/10.1111/jfb.12939

Hoover, S.M. and A.M. Trehu, 2017, Uplift, Emergence, and Subsidence of the Gorda Escarpment Basement Ridge Offshore Cape Mendocino, CA, Geochem. Geophys. Geosyst., doi: 10.1002/2017GC007128.

Howard MJ. 2017. Chapter 44: Fecundity, egg case sizes, and neonate morphometrics of big skate *Beringraja binoculata* (Girard, 1855). In The Elasmobranch Husbandry Manual II: Recent advances in the care of sharks and their relatives, In Smith M, Warmolts D, Thoney D, Hueter R, Murray M, Ezcurra J (eds). Ohio Biological Survey, INC, Columbus, pp 451-458, pp 504.

Im Y-J, Jo H-S, Ji H-S, Myoung S-H, Kim J-K. 2017. Geographic variations of the mottled skate, *Beringraja pulchra* (Liu, 1932) (Rajidae) in the Yellow and East seas based on molecular and morphometric data. Journal of Applied Ichthyology. 33:950–956. https://doi.org/10.1111/jai.13408

Ishihara H, Mochizuki T, Homma K, Taniuchi T. 2002. Reproductive strategy of the Japanese common skate (Spiny Rasp Skate) *Okamejei kenojei*. In Elasmobranch biodiversity, conservation and management: Proceedings of the International Seminar and Workshop, Sabah, Malaysia, July 1997, In Fowler SL, Reed TM, Dipper AF (eds). IUCN, Oxford, UK. Pp 236-240, pp 275.

Ishihara, H., Wang, Y., Tanaka, S., Nakaya, K. & Jeong, C-H. 2009. *Beringraja pulchra*. The IUCN Red List of Threatened Species 2009: e.T161669A5476757. http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T161669A5476757.en. Downloaded on **20 August 2018**.

Ishihara H, Treloar M, Bor HFP, Senou H, Jeong CH. 2012. The Comparative Morphology of Skate Egg Cases (Chondrichthyes: Elasmobranchii: Rajiformes). Bull Kanagawa prefect Mus (Nat Sci) 9–25.

James KC, Ebert DA, Natanson LJ, Cailliet GM. 2014. Age and growth characteristics of the Starry Skate, *Raja stellulata*, with a description of life history and habitat trends of the central California, U.S.A., skate assemblage. Environ Biol Fish **97**: 435-448.

Jones OR, Wang JL. 2010. Colony: a program for parentage and sibship interference from multilocus genotype data. Molecular Ecology Resources **10**:551-555.

Kang HW, Jo JR, Kang DY, Jeong GS, Jo HS. 2013. Spawning Characteristics and Artificial Hatching of Female Mottled Skate *Beringraja pulchra* in the West Coast of Korea. Dev. Reprod. **17**(3):247-255.dx.doi.org/10.12717/DR.2013.17.3.247.

Kang J-H, Park J-Y, Jo H-S. 2012. Rapid Development of Microsatellite Markers with 454 Pyrosequencing in a Vulnerable Fish, the Mottled Skate, *Raja pulchra*. International journal of molecular sciences **13**:7199–211.

Kashi Y, Soller M. 1999. Functional roles of microsatellites and minisatellites. In Goldstein DB, Schlötterer. (Eds). Microsatellites: Evolution and Application. Oxford University Press, Inc., New York. Pp 10-23, pp 341.

King JR, McFarlane G. 2010. Movement patterns and growth estimates of Big Skates (*Raja binoculata*) based on tag-recapture data. *Fisheries Research*, *101*, 169–178.

King JR, Helser T, Gburski C, Ebert DA, Cailliet G, Kastelle CR. 2017. Bomb radiocarbon analyses validate and inform age determination of longnose skate (*Raja rhina*) and big skate (*Beringraja binoculata*) in the north Pacific Ocean. Fisheries Research **193**: 195-206.

Last PR, White WT, Carvalho MR De, Seret B, Stehmann MFW, Naylor GJP. 2016a. Rays of the world. CSIRO Publishing, Australia, pp 218-223; 790.

Last PR, Weigmann S, Yang L. 2016b. Changes to the nomenclature of the skates (Chondrichthyes: Rajiformes). In book: Rays of the World – supplementary information, Chapter: 2, Publisher: CSIRO Australian National Fish Collection, Editors: Peter R. Last, Gordon K. Yearsley, pp.11-34

Larson S, Christiansen J, Griffing D, Ashe J, Lowry D, Andrews K. 2010. Relatedness and polyandry of Sixgill sharks, *Hexanchus griseus*, in an urban estuary. Conservation Genetics **12**:679–690.

Leslie DM Jr, Bowyer TR, Kie JG. 1999. Life-History Strategies of Ungulates. Journal of Mammalogy **80**(4):1067-1069.

Luer CA, Gilbert PW. 1985. Mating behavior, egg deposition, incubation period, and hatching the Clearnose skate, *Raja eglanteria*. Environmental Biology of Fishes (13):161-171.

McFarlane G, King JR. 2006. Age and growth of Big Skate (*Raja binoculata*) and Longnose skate (*Raja rhina*) in British Columbia waters. Fisheries Research **78**:169–178

McKnight D. 2011. 18: Longnose skate *Raja rhina*. In California Fish and Game: Status of the Fisheries Report 2011, pp 18-1:18-8

Musick JA, Ellis JK. 2005. Reproductive Evolution of chondrichthyans. In Hamlett, WC (Ed.), Reproductive Biology and Phylogeny of Chondrichthyans. Science Publishers, Enfield, NH, pp.45-79.

Nagar AE, McHugh M, Rapp T, Sims DW, Genner MJ. 2010. Characterisation of polymorphic microsatellite markers for skates (Elasmobranchii: Rajidae) from expressed tags. Conservation Genetics **11**:1203-1206. Doi:10.1007/s10592-009-9919-8

Naylor GJP, Caira JN, Jensen K, Rosana KAM, White WT, LAST PR. 2012. A DNA sequence-based approach to the identification of shark and ray species and its implications for global elasmobranch diversity and parasitology. Bulletin of the American Museum of Natural History, 367: 262 pp., 102 figures, 5 tables

North Pacific Fishery Management Council. 2018. Habitats of Particular Concern- HAPC. North Pacific Fishery Management Council https://www.npfmc.org/habitat-protections/habitat-areas-of-particular-concern-hapc/. Accessed 20 December 2018.

O'Regan HJ, Kitchener AC. 2005. The effects of captivity on the morphology of captive, domesticated and feral mammals. Mammal Review **35***(3-4)*:215-230

Portnoy DS, Piercy AN, Musick J, Burgess GH, Graves JE. 2007. Genetic polyandry and sexual conflict in the sandbar shark, *Carcharhinus plumbeus*, in the western North Atlantic and Gulf of Mexico. Molecular ecology **16**:187–97.

R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Ray C. 1960. The application of Bergmann's and Allen's Rules to the poikilotherms. Journal of Morphology **106**:85-108. DOI:10.1002/jmor.1051060104

Raymond M, Rousset F, 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Heredity, 86:248-249

Robinson HJ, Cailliet GM, Ebert DA. 2007. Food Habits of the longnose skate, *Raja rhina* (Jordan and Gilbert, 1880), in central California waters. In Biology of Skates Ebert DA, Sulikowski (eds.), pp 59-73, pp 243. Doi: 10.1007/978-1-4020-9703-4-5.

Salinas-de-León P, Phillips B, Ebert D, Shivji M, Cerutti-Pereyra F, Ruck C, Fisher CR, Marsh L. 2018. Deep-hydrothermal vents as natural egg-case incubators at the Galapagos Rift. Scientific Reports 8:1788. Doi: 10.1038/s41598-018-20046-4.

Santos RG, Pinheiro HT, Martins AS, Riul P, Bruno SC, Janzen FJ, Ioannou CC. 2016. The anti-predator role of within-nest emergence synchrony in sea turtle hatchlings. Proc. R. Soc. B 283: 20160697. http://dx.doi.org/10.1098/rspb.2016.0697

Schulke M. 2000. An economic method for the fluorescent labeling of PCR fragments: A poor man's approach to genotyping for research and high-throughput diagnostics. Nature Biotechnology **18**:233-234.

Selkoe KA, Toonen RJ. 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecology letters **9**: 615-629. doi: 10.1111/j.1461-0248.2006.00889.x

Sugg DW, Cheeser RK. 1994. Effective Population Sizes with Multiple Paternity. Genetics of Society of America. **137**:1147-1155.

Trivers RL, Willard DE. 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science **179***(4068)*:90-92.

Turner FG, Pitcher JT. 1987. Attack abatement: a model for group protection by combined avoidance and dilution. The American Naturalist **128**(2): 228-240.

Veríssimo A, Grubbs D, McDowell J, Musick J, Portnoy D. 2011. Frequency of multiple paternity in the spiny dogfish *Squalus acanthias* in the western north Atlantic. The Journal of heredity **102**:88–93.

Wood SN. 2006. Generalized additive models: An introduction with R. Chapman & Hall/CRC, New York, New York. Pp 225-226, 392.

Wang JL. 2011. COANCENSTRY: a program for simulating, estimating, and analyzing relatedness and inbreeding coefficients. Molecular Ecology **11**: 141-145

Webster MS, Reichart L. 2005. Use of Microsatellites for parentage and kinship analysis in animals. Methods in Enzymology **395**: 222-238. doi: 10.1016/S0076-6879(05)95014-3

Winton MV, Natanson LJ, Kneebone J, Cailliet GM, Ebert DA. 2013. Life History of *Bathyraja trachura* from the eastern Bering Sea, with evidence of latitudinal variation in a deep-sea skate species. Journal of the Marine Biological Association of the United Kingdom: 1-12. doi: 10.1017/s00253154130001525

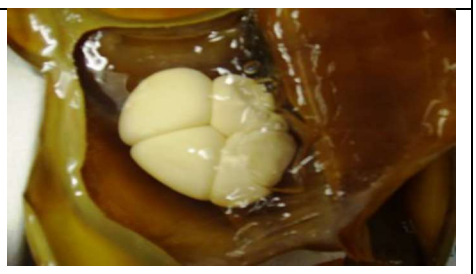
Wilson AB. 2009. Fecundity selection predicts Bergmann's rule in syngathids fishes. Molecular Ecology **18**:1263-1272. Doi: 10.1111/j.1365-294X.2009.04084x

Zeiner SJ, Wolf PC. 1993.Growth characteristics and estimates of age at maturity of two species of skates (*Raja binoculata* and *Raja rhina*) from Monterey Bay, California. In: Branstetter, S. (Ed.), Conservation Biology of Elasmobranchs. NOAA Technical Report, NMFS 115, pp. 87-

Table 1. Life history of sexual maturity and longevity of *B. binoculata*

	Females maturity (TL, Total Length)	**Males maturity**	**Life span (yrs)**	**Documentation**
Gulf of Alaska	125.8 cm-148.6 cm	119.2 cm-124 cm	15	Ebert *et al*. 2008b; Gburski *et al*. 2007;
British Columbia	120 cm	90 cm	26	King and McFarlane 2010; McFarlane and King 2006;
California	130 cm	100 cm-110 cm	12	Zeiner and Wolf 1993

Table 2. Big Skate development from Hitz 1964

Stage	Descriptions	Image *
1	Yolk may have blood vessels present; yolk sacs are fully separated among each other; but no physical embryo present	
2	Embryo present; gill filaments present, no wing development; embryo is physically moving back and forth for oxygen intake	
3	Wings have developed; Gill slits have formed; embryo still moving for oxygen intake	
4	Wings have developed eye spots and coloration; sex appendages have formed (claspers); embryos are actively pumping water through their gills	
5	Wings fully developed; embryo has developed orbital spines for emergence; yolk sac is 90% absorbed	

* Stage 1 image credited to Kang *et al*. 2013

Table 3. Egg cases and their corresponding embryos for paternity analysis

Egg Case Number	Number of Embryos
201	4 (2) *
202	3
204	4
221	4 (3)
227	3 (2)
228	3
229	2
232	2
234	2
235	2

* Numbers in parentheses indicate samples used for genetic analysis

Table 4. Microsatellites with the M-13 primer tail sequence with updated annealing temperatures and corresponding fluorescent tags (modified from Kang *et al*. 2012)

Locus	**Dye**	**Primer sequence Forward (5'-3')**	**Primer sequence Reverse (5'-3')**	**Motif**	**Annealing Temp (C^O)**	**Allele size (bp)**	**GenBank Accession Number**
Rp 30	NED	TGTAAAACGACGGCC AGTCGTGTATATGTATGTGTGCATGT	GCAGAAGCACTACAGAATGTTT	$(TG)_{11}$	59	216-230	JQ433562
Rp 39	VIC	TGTAAAACGACGGCC AGTGCTTGGTTTTCTGAAATCAGTG	ATAAAATTGCAGGGAGAATGC	$(AT)_{13}$	61	150-166	JQ433565
Rp 43	VIC	TGTAAAACGACGGCC AGTCTCCTGCCTTTGCTATGTGT	GACTTTTCAGCGACAGTCTTCT	$(TG)_{15}$	61	154-162	JQ433566
Rp 44	VIC	TGTAAAACGACGGCC AGTACATGGTCACGAGTAGAATGTG	TTCAGACCCTATTCAAAATGCT	$(CA)_{16}$	64	149-161	JQ433566

Table 5. Number of egg cases collected from FRAM surveys (Fertile and Infertile)

	Fertile	**Not Fertile**	Total
2008	18	13	31
2014	29	18	47
2015	10	10	20
2016	2	3	5
Total	59	44	103

Table 6. Generalized additive model (GAM) estimates between egg case length (ECL) and latitude, edf: estimated degrees of freedom, ref. df: estimated residual degrees of freedom, Dev. Expl: the proportion of the null deviance explained by the model. Variables: ECL: Total Length; Lat: Latitude

ECL~ Lat						
	Estimate	Std. Error	T-value	P-value		
Intercept	25.9937	0.3644	71.34	<2e-16		
Response variable	edf	Ref. df	F	P- value	R-squared	Dev. Expl
s(Lat)	2.306	2.84	7.566	0.000371	0.255	28.20%

Table 7. Generalized additive model (GAM) estimates between embryo counts and latitude, edf: estimated degrees of freedom, ref. df: estimated residual degrees of freedom, Dev. Expl: the proportion of the null deviance explained by the model. Variables: Embcounts: Embryo numbers; Lat: Latitude; s(Lat): response variable

Embcounts~ Lat						
	Estimate	Std. Error	T-value	P-value		
Intercept	3.587	0.127	28.25	<2e-16		
Response Variable	edf	Ref. df	F	P- value	R-squared	Dev. Expl
s(Lat)	1.984	2.362	2.769	0.0591	0.0907	12.00%

Table 8. Observed embryo morphometrics during developmental stages (2-5)

	2	**3**	**4**	**5**
Total Length (cm)	2.5-9 (6.29) * ± 1.58	5-13.4 (9.74) ± 1.83	10.5-20.1 (15) ± 2.72	8.5-21 (17.13) ± 2.20
Disc width (cm)	0.4-4.5 (1.06) ± 0.88	1-5 (2.61) ± 1.11	3.1-12.8 (7.21) ± 2.98	7-14 (11.03) ± 1.43
Yolk Diameter (cm)	3.3-5.7 (4.48) ± 0.59	2.4-5.4 (4.21) ± 0.59	1.3-5.2 (3.04) ± 0.95	0.3-3 (1.11) ± 0.62
Yolk Volume (cm^3)	18.82-96.97 (47.08)	7.24-82.45 (39.07)	1.15-73.62 (14.71)	0.014-14.14 (0.72)

*: Values in parentheses denotes average

Table 9. Number of embryos collected from FRAM surveys; UD: sexes were not applicable

	F	**M**	**UD**	Total
2008	17	16	34	67
2014	25	15	68	108
2015	6	6	26	38
2016	0	0	6	6
Total	48	37	134	219

Table 10. Genotype of the 31 captive *B. binoculata* individuals. A) Genotypes of potential sires with F_0007 offspring; B) Genotypes of potential sires with F_6528 offspring, (* indicates egg cases laid during while this female was in quarantine for 3 months)

A.

Sample	RP30		RP39		RP43		RP44	
M1479	166	250	166	174	125	135	175	179
M2920	170	230	130	166	121	125	165	175
F0007	158	222	130	166	125	145	126	160
221A	166	222	130	166	125	135	160	165
221C	166	222	0	0	125	135	160	179
221D	170	222	166	174	125	130	160	165

B.

Sample	RP30		RP39		RP43		RP44	
M1479	166	250	166	174	125	135	175	179
M2920	170	230	130	166	121	125	165	175
201B	166	224	166	174	125	135	165	179
201D	166	224	166	174	125	135	165	179
202A	170	224	158	166	125	155	165	179
202B	166	224	158	166	125	135	165	175
202C	166	224	130	166	125	155	165	179
204A	224	250	130	166	125	135	165	179
204B	224	250	142	166	125	135	135	165
204C	224	250	166	174	125	135	165	179
204D	224	250	150	166	125	135	165	179
227A *	0	0	160	166	125	135	165	179
227C *	0	0	160	166	125	135	165	179
228A *	166	224	166	166	121	125	165	165
228B *	166	224	166	174	125	125	165	165
228C *	170	224	166	166	135	139	121	165
229A *	166	224	130	166	125	155	165	165
229B *	166	224	166	166	125	135	165	179
232A *	222	224	130	166	135	155	121	165
232B *	0	0	150	166	121	135	165	165
234A *	224	250	0	0	125	135	165	179
234B *	224	240	0	0	125	135	165	165
235A *	0	0	0	0	125	135	165	179
235B *	224	240	0	0	125	135	165	165

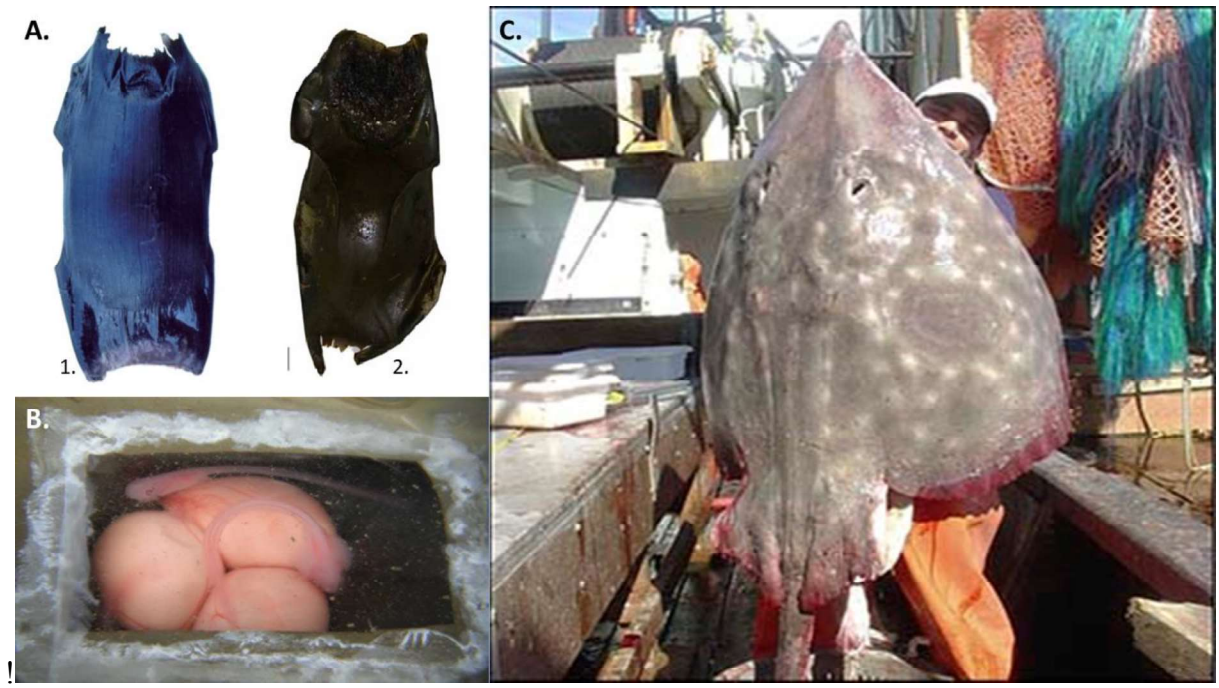


Figure 1. Distinct traits of the *Beringraja* genus; A. Egg case morphology (1: *B. pulchra*) (2; *B. binoculata*); scale bar: 20 mm. Modified from Ishihara *et al.* 2012 and Ebert & Davis 2007; B. Multiple embryos; C. Size comparison of a Big Skate; Photo credit: Joe Bizzarro

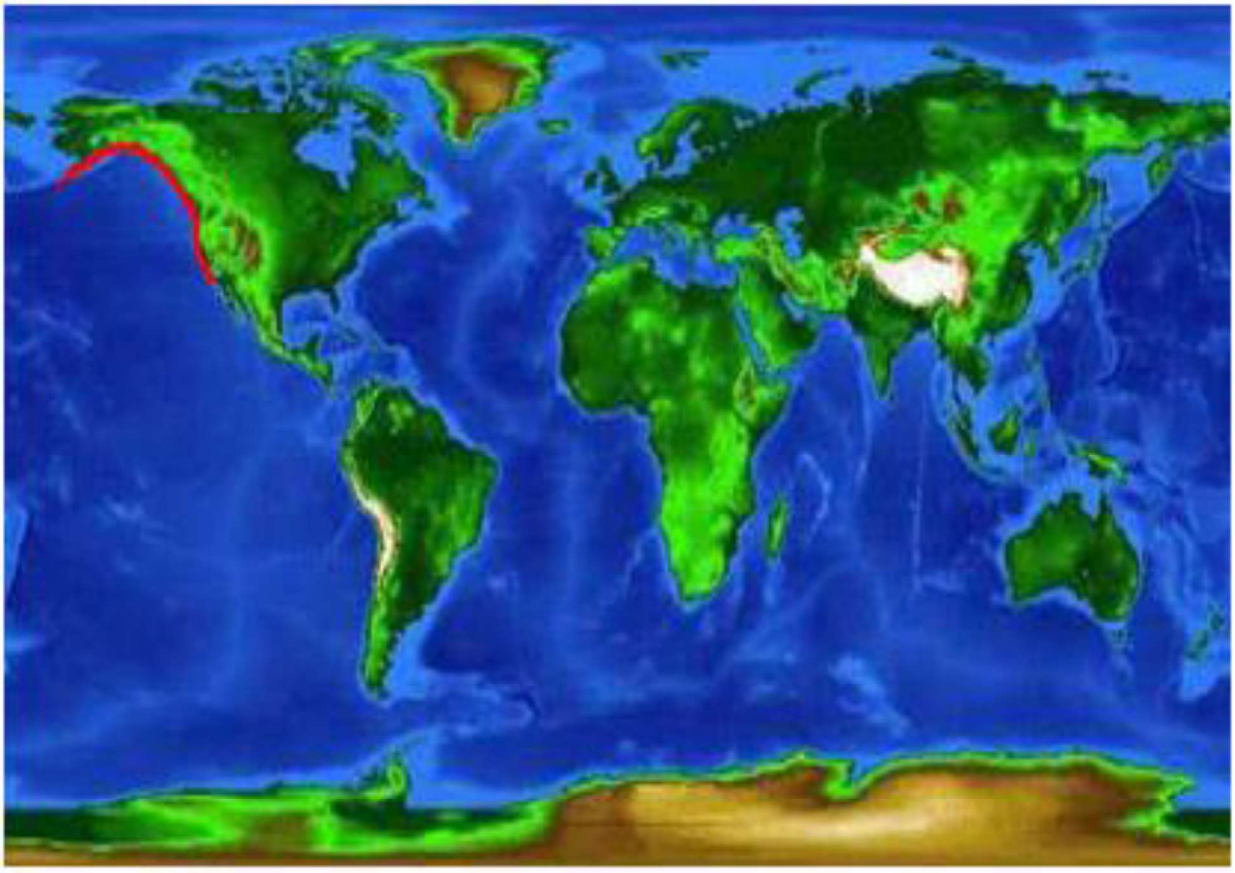


Figure 2. Geographic range of *B. binoculata*, (shown in red) acquired from www.flmnh.ufl.edu.

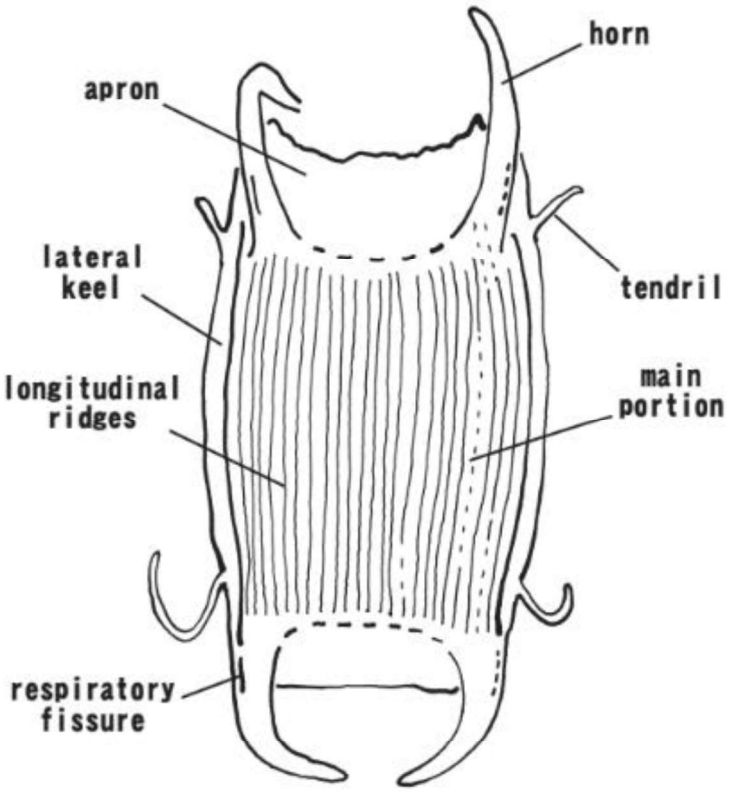


Figure 3. General shape of a skate egg case with the different components labeled (Ishihara *et al*. 2012)

A. B.

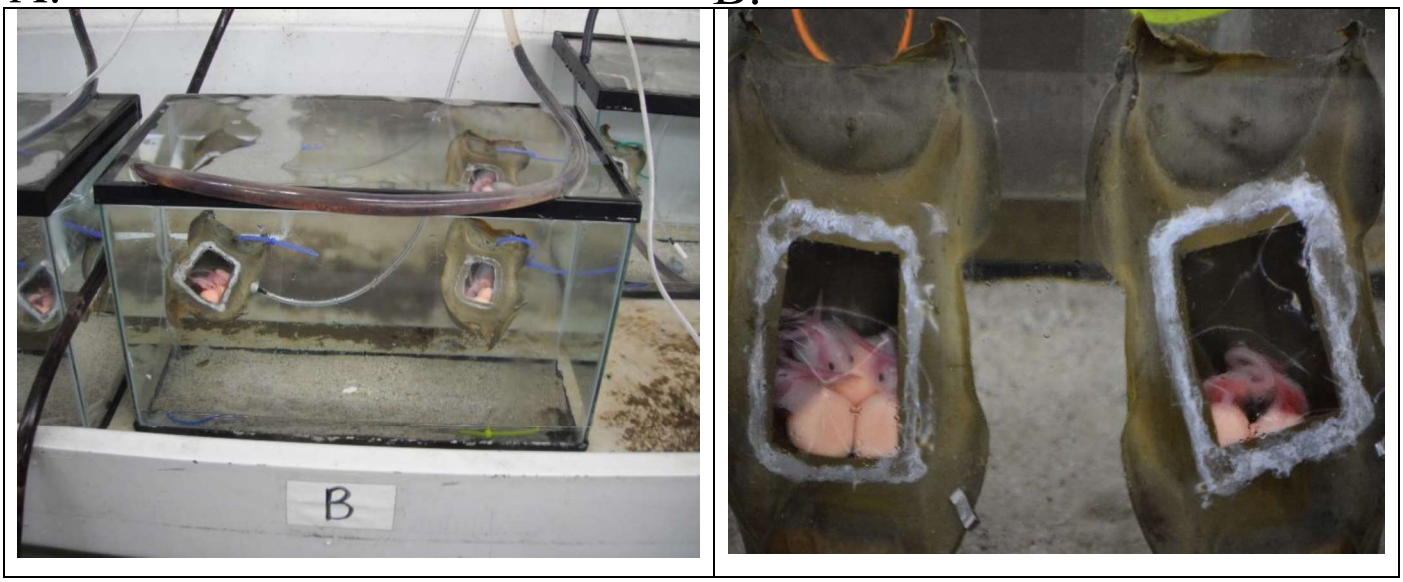


Figure 4. Tank setup for rearing Big Skate embryos and studying the stages of development (A) Aeration devices help oxygenate the water; (B) Magnified view of the embryos through the egg case windows

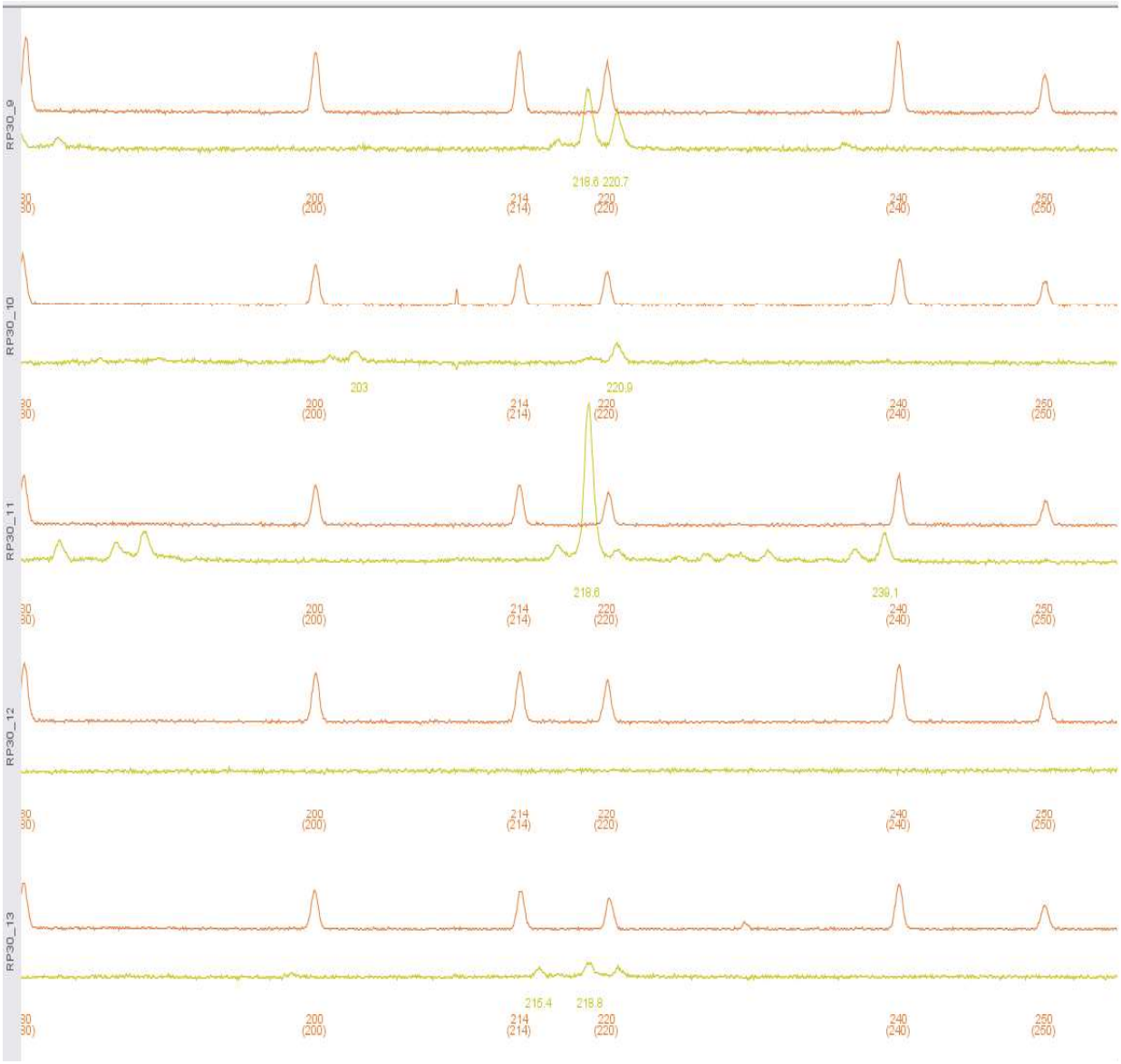


Figure 5. Chromatogram of scoring microsatellites; Orange peaks are size standards, whereas the yellow peaks are the microsatellites (RP 30) of interest

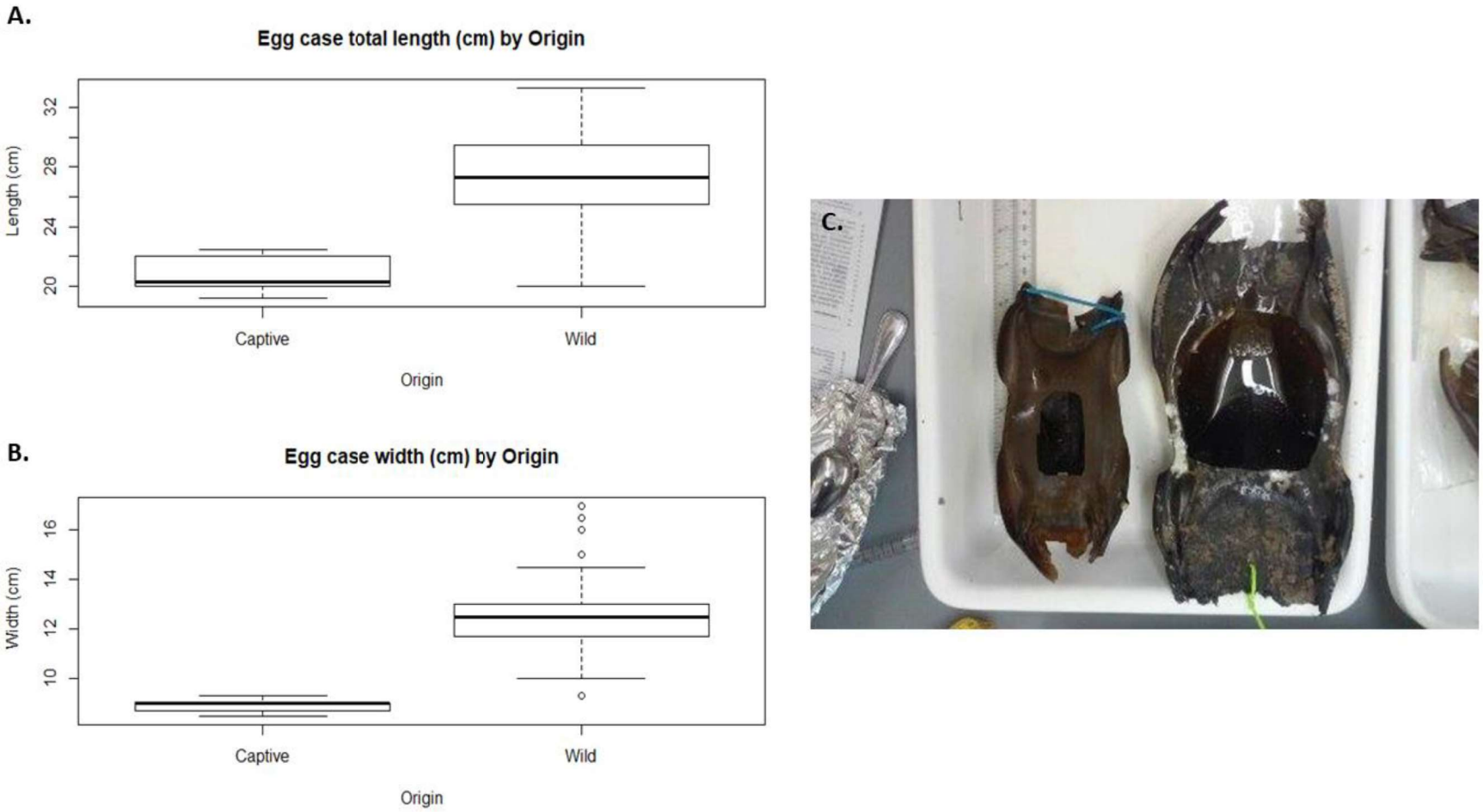


Figure 6. Egg case dimensions among captive and wild environments; A. Big Skate egg case length (ECL) range among captive and wild; B. Big Skate egg case width range among captive and wild C. Visual comparison of egg cases among the two environments

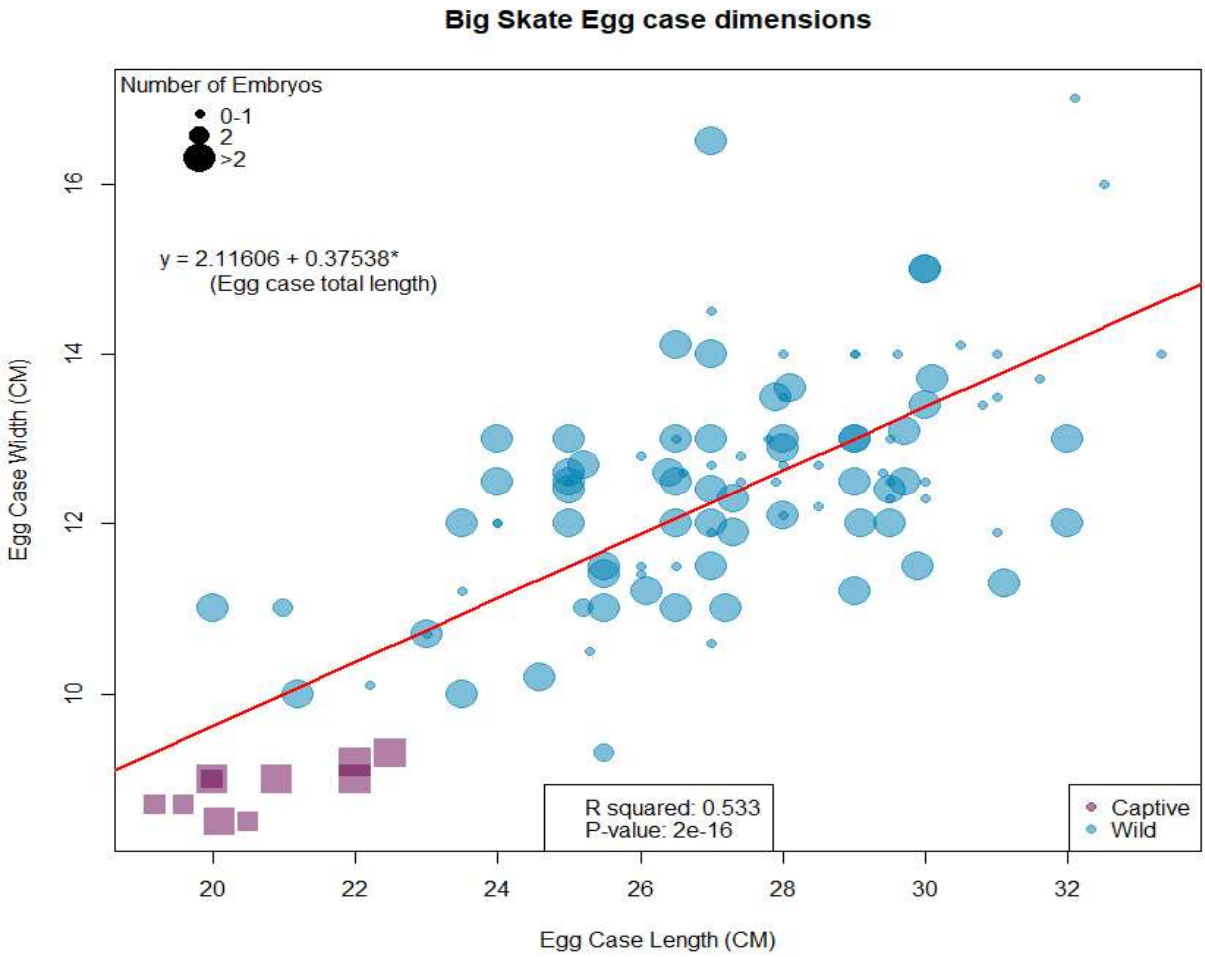


Figure 7. Regression relationship between egg case dimensions from captive and wild environments. Note the size of the points indicate the number of embryos found (0-1, 2, and >2)

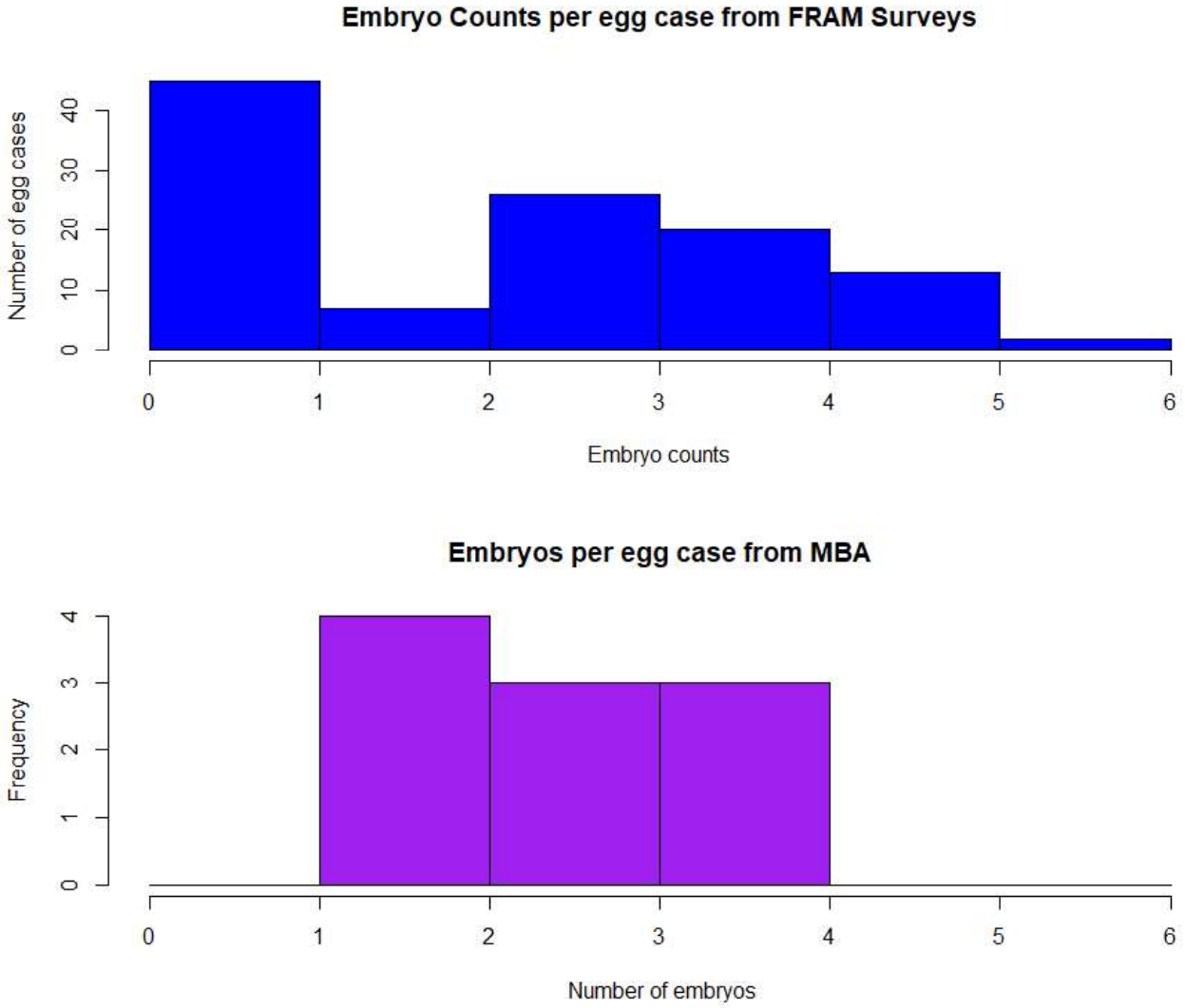


Figure 8. Histograms depicting embryo numbers per egg case from samples collected from the wild (top) and captive specimen from the Monterey Bay Aquarium (bottom)

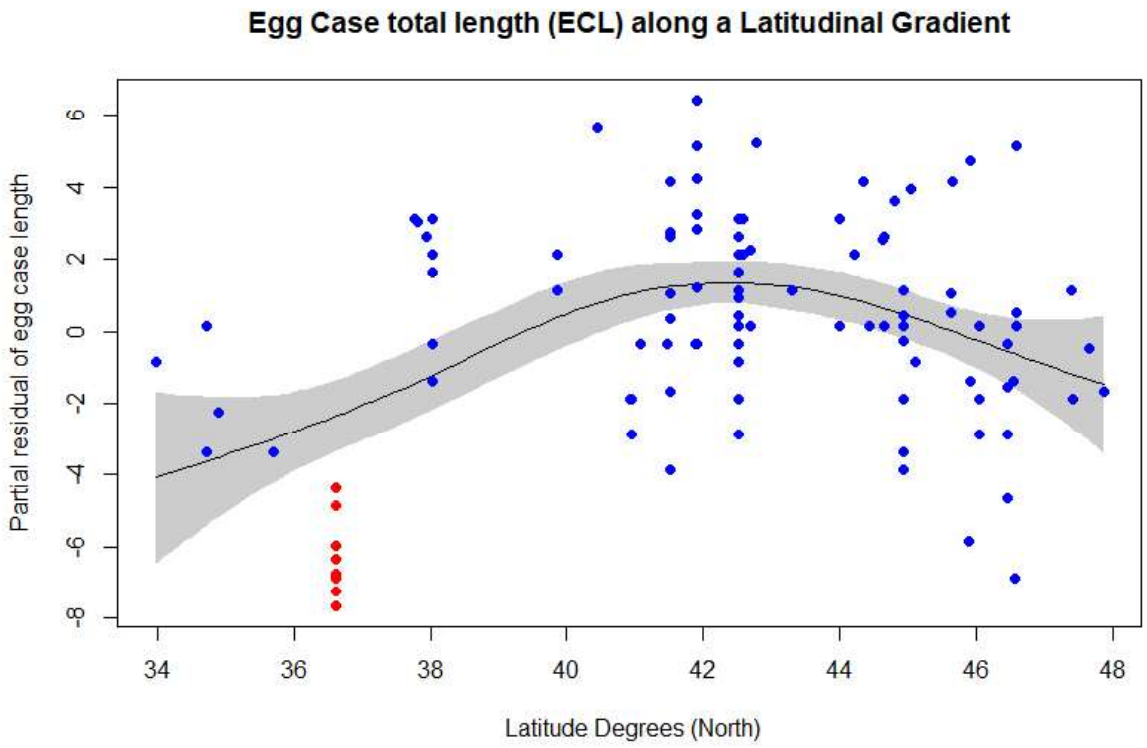


Figure 9. GAM plot of egg case length (ECL) versus latitude; ECL show a peak at 42 degrees North; Y-axis: Partial residuals of the relationship between ECL and a cubic regression spline of latitude (df=2.89); gray shaded areas are the 95% CI; (Colors indicate origin where egg cases were collected from [blue: wild, red: captive])

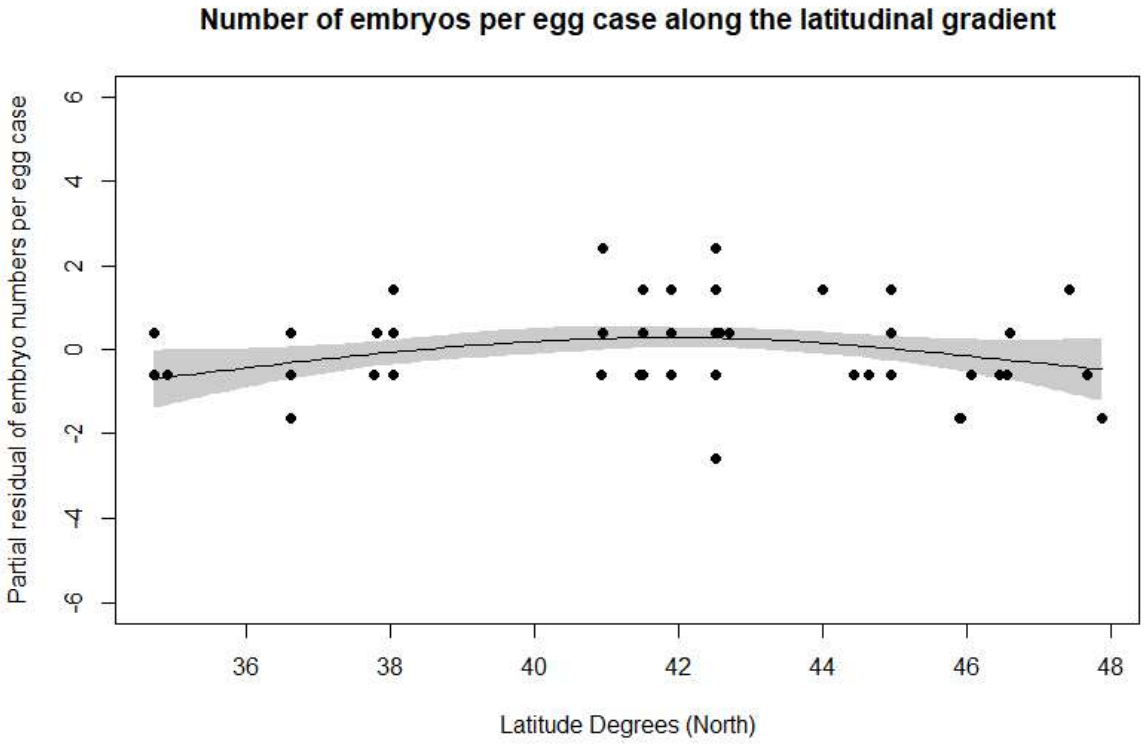


Figure 10. GAM plot of number of embryos per egg case versus latitude; the average number of embryos per egg case peaked at 42 degrees North; Y-axis: Partial residuals of the relationship of the number of embryos per egg case and a cubic regression spline of latitude (df=1.89); gray shaded areas are the 95% CI

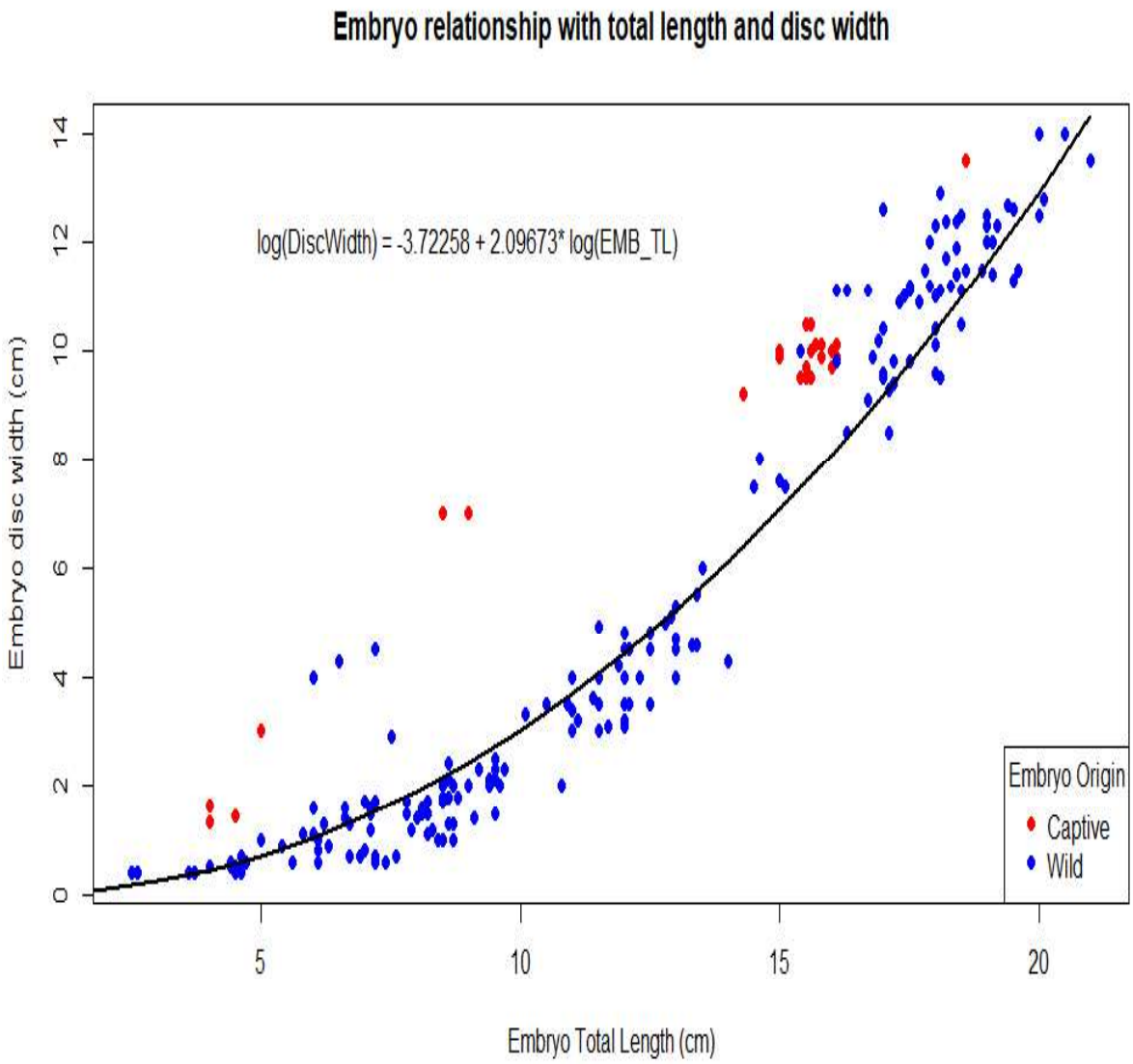


Figure 11. Allometric growth relationship in *B.binoculata* development among wild (blue) and captive (red) embryos;

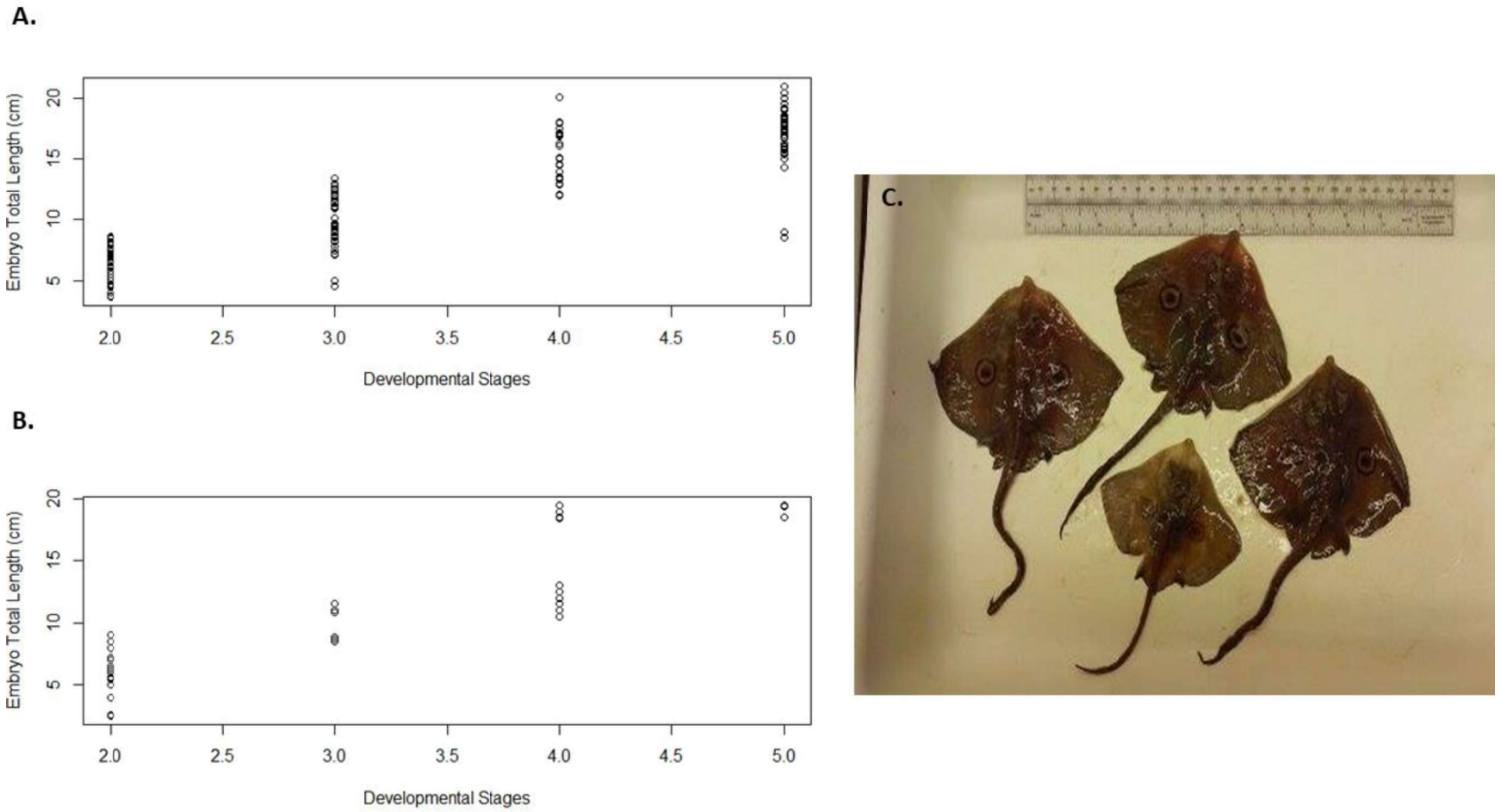


Figure 12. Embryo sizes in captive and wild; A. TL of wild Big Skate embryos among the development stages; B. TL of captive Big Skate embryos among the development stages; C. Visual comparison of Big Skate embryos: 3 wild (top) and one captive (bottom)

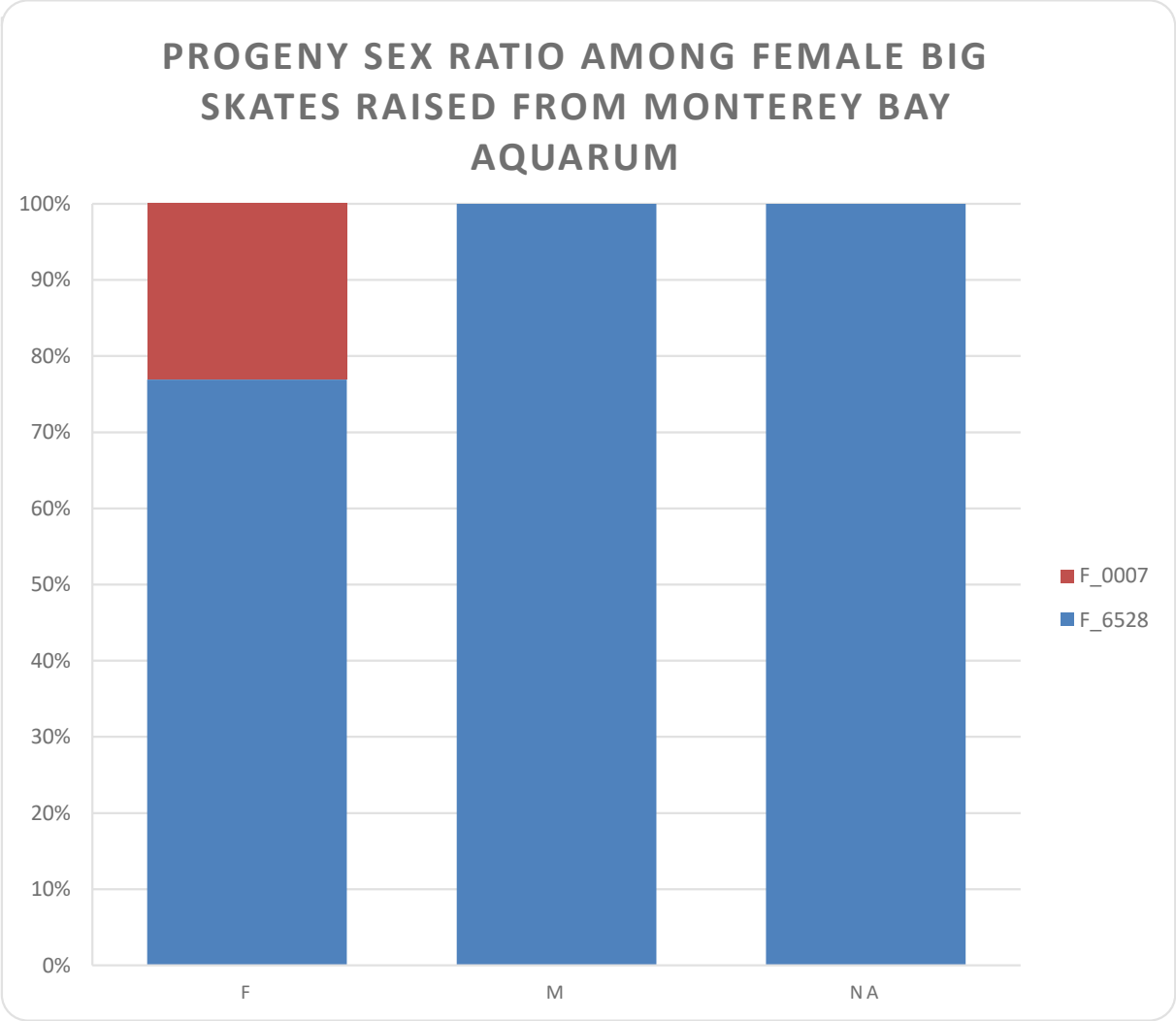


Figure 13. Offspring sex ratio produced by the two females from MBA; F: females, M: males, and NA: not applicable

Figure 14: Large quantity of Big Skate egg cases collected off Kayak Island; this amount suggests that Big Skates may have a different reproductive tactic in their most northern range. Photo credit: T. Farrugia