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Two- And Three-Dimensional Photogrammetric Mass Estimation Techniques For Two Phocid Species: Halichoerus Grypus And Phoca Vitulina Concolor

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TWO- AND THREE-DIMENSIONAL PHOTOGRAMMETRIC MASS ESTIMATION TECHNIQUES FOR TWO PHOCID SPECIES: *HALICHOERUS GRYPUS* AND *PHOCA VITULINA CONCOLOR*

by

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ABSTRACT

TWO- AND THREE-DIMENSIONAL PHOTOGRAMMETRIC MASS ESTIMATION TECHNIQUES FOR TWO PHOCID SPECIES: *HALICHOERUS GRYPUS* AND *PHOCA VITULINA CONCOLOR*

by

Jennifer Harris

University of New England, January, 2012

Collecting mass measurements of seals is a common technique used to determine health. To determine the mass of Western North Atlantic Grey seal (*Halichoerus grypus*) and Atlantic Harbor seal (*Phoca vitulina concolor*) pups, a researcher must physically measure each animal. This produces stress for both pups and their mothers. Photogrammetric analysis (PGA) (evaluating photographs to obtain characteristics of a subject) has been used to determine physical measurements in a number of marine mammals. The purpose of this study was to develop a nonintrusive method for determining the mass of grey and harbor seal pups. Through this study we developed two- and three-dimensional PGA multiple regression models for predicting body mass of weanlings of both species. Photographs of grey seal pups were taken in the field and harbor seal photographs were taken in a captive setting. Calibration parameters were determined in Matlab and Olympus software, using an object of known measurement as a scale. Three-dimensional stereo-PGA was the most accurate close-range mass estimation technique. The most accurate grey seal model demonstrated significant agreement (p=0.006, r^2=0.913) between predictions and the true population mean at a 95% CI. The harbor seal model with the highest accuracy demonstrated significant agreement as well (p= <0.0001, r^2=0.904). Two-dimensional grey and harbor seal PGA models functioned best when used for distance PGA, predicting mass within 4% - 20% accuracy, at distances up to 22 meters. PGA models were validated through results of models created from physical measurements. For instance, a high correlation, Adjusted r^2=0.885, was seen in harbor seal physical models, however a strong correlation, Adjusted r^2=0.807, was seen in harbor seal PGA models as well. Models built in this study will be useful in future field and captive setting work with both species. Using these models for distance PGA purposes will also limit mother-pup disturbance.

Introduction

History and Applications of Photogrammetric Analysis (PGA)

Research conducted on mammals requires collection of physical data and can reveal valuable characteristics of individuals, such as sex, size, age, mass, and condition (Webster et al. 2010; Breuer et al. 2007; Bennet et al. 2007; Noren et al 2008; Engelhard 2001). Collecting physical data in the field, however, involves capturing and handling animals in ways that are intrusive and can cause unintended stress, such as disruption of feeding patterns, and mortality if animals are sedated improperly (Engelhard 2001; Hall-Martin & Ruther 1979). Also, measuring large animals can be extremely difficult and challenging (Webster et al. 2010). Photogrammetric analysis (PGA), extrapolation of physical data from photographic image analysis, is a safe technique that can aid researchers without compromising their study subjects (Hall-Martin & Ruther 1979; Bell et al. 1997; Brager & Chong 1999). PGA has been used as a tool for collecting physical data of marine mammals, such as dolphins (Brager & Chong 1999), whales (Spitz et al. 2000), and seals (Bell et al. 1997), as well as other mammals such as Western gorillas (*Gorilla gorilla*) (Breuer et al. 2007), African elephants (*Loxodonta Africana*) (Hall-Martin & Ruther 1979), and Alpine ibex (*Capra ibex*) (Bergeron 2007).

Among pinnipeds (seals, sea lions, fur seals and walruses) determining the length, girth and mass of each animal provides information of the individual's overall body condition (Hoff 2005). Fifty-nine years ago Laws (1953) first applied aerial PGA as a technique to estimate the length of a pinniped, the Southern elephant seal (*Mirounga leonina*). Later, close-range terrestrial, PGA was used for determining the mass of phocid species (true seals) hauled out on land (Haley et al. 1991). The utilization of PGA for physical measurements of pinnipeds has saved researchers time and resources (Bruyn et al. 2009). Although PGA initially began in the field for mass determination of adults (Laws 1953), it has since been applied to younger and smaller species in order to decrease the invasiveness of handling time and disturbance (McFadden et al. 2006).

PGA methods are either two-dimensional or three-dimensional. In two-dimensional photographs an object of known measurement is placed near a study subject for scale. If this is impossible, the focal length of the camera, distance to the study subject and camera sensor pixel size can be used to determine the subject's size (Spitz et al. 2000). Two-dimensional techniques are highly accurate and have created such PGA measurements with coefficient of variance's (CV) values of 3.08% and 2.57% (Spitz et al. 2000). This small error in estimation of a specific physical measurement is a respectable value because the smaller a CV the less error in estimation occurs. For example, a CV value of zero is a perfect result because it demonstrates zero variance between real and estimated values (Sokal & Rolf 2001). Other studies have created multiple linear regression models that predict body mass, developing predictions within 13.8% of the actual mass (Ireland et al. 2006).

Three-dimensional stereo-PGA has been demonstrated to be most efficient in mass estimation of active subjects (Brager & Chong 1999). This technique utilizes two cameras that are mounted a known distance apart. These cameras are triggered simultaneously, yielding photographs at slightly different angles. These stereo-images are used to triangulate measurements of the study subject to an accuracy of 1.4 - 1.9% error between estimated and actual lengths (Brager & Chong 1999). Knowing the length is useful in predicting body mass; in phocids (earless seals), the physical length is proportional to the mass in standard body condition calculations (Hoff 2005).

Bruyn et al. (2009) invented a method to collect three-dimensional data using one camera. Multiple photographs of an immobile subject were taken at different heights and angles around the subject. Objects of known size were placed in each image and used as cross references to overlay multiple photographs of the subject. Measurements of the subject, created from these overlaying photographs, yielded highly accurate regression equation models for predicting mass that were within ±1.34 to 3.83% of the actual mass. PGA data can also be collected at large distances, 15 to 50 meters, by mounting laser pointers known distances apart and using their projected points as a scale for each photograph (Bergeron 2007). This technique is accurate within 3.9% of the mean actual length.

Scientists are interested in nonintrusive ways of measuring and comparing the outcomes of maternal investment, especially in phocid mammals that invest heavily in the early stages of pup development (Boness and Bowen 1996). Maternal care is the amount of maternal investment and energy a mother provides and ultimately determines offspring survival, reproductive success and population growth (Hall et al. 2001). Maternal investment can be extrapolated by measuring pup growth during lactation (Redman et al. 2001; Boness et al. 1995). Most phocid mothers exhibit a fasting strategy and lose mass during lactation, transferring this mass to their pups. Mother grey seals prepare themselves for a brief course of lactation (4-50 days) by developing a thick layer of blubber prior to parturition. However, mother harbor seals fast at the beginning of lactation, and soon after make periodic foraging trips away from their offspring in order to maintain the energetic costs of lactation, termed "cyclic foraging" (Boness and Bowen 1996).

Study Objectives

The objective of this study was to create PGA multiple regression models that could predict body mass for use in the field when studying wild grey (*Halichoerus grypus*) and harbor seal (*Phoca vitulina*) pups during, and immediately after, lactation. To date, the use of two- **and** three- dimensional terrestrial PGA techniques to determine body mass of weaned pups of both phocid species has never been conducted. If pup mass could be determined remotely throughout lactation, fragile mother-pup bonds, and other behaviors would remain intact (Boness 1995; *pers. obs.*). Therefore, this study aimed to decrease the amount of interaction and disturbance by humans from making physical measurements directly. Multiple linear regression models, predicting body mass, were created utilizing two- and three-dimensional terrestrial PGA techniques. Predictive models were developed for newly weaned wild grey seal pups on Muskeget Island, MA, and for rehabilitating harbor seal pups of the Marine Animal Rehabilitation Center (MARC) at the University of New England (UNE). Predictive models derived from physical girth and length measurements (physical models), were also created and were used to validate the accuracy of PGA models. Results from this study will be an asset to future phocid studies. Harbor seals and grey seals are abundant in the North Atlantic and many current studies are focused on their health and population growth (Cabezon et al. 2011; Wood LaFond 2009).

Methods

Study Species

Western Atlantic harbor seals (*P.v. concolor*) exist along the south coast of Greenland, around Iceland and from the Arctic to the mid-Atlantic (Thompson & Harkonen 2008). Pupping along the Maine coast takes place mid-May through June (deHart 2002), and occasionally stranded pups are sighted and reported to a local response organization. If a stranded seal requires rehabilitation, and if room is available, seals are sent to the MARC from the stranding response organization; the MARC is the only rehabilitation center in Maine (A. Simpson, *pers comm.*).

There are currently three global populations of grey seals. The western North Atlantic population exists around the Gulf of St. Lawrence along the Atlantic coast. This population is currently growing at such a pace that a population count is not available (NMFS 2010). Breeding occurs at breeding sites extending from Labrador to as far south as Muskeget Island, MA, and New York, USA (Bowen et al. 2003; Rough 1994; NMFS 2010). Muskeget Island in Nantucket Sound, Massachusetts, currently supports the largest western North Atlantic breeding colony of grey seals in the United States. Pupping at Muskeget Island takes place from late November through January (NMFS 2010).

Data Collection

In addition to photographs, physical measurements of individuals were taken, including the axillary girth (girth directly behind front flippers) in centimeters, standard length (distance from nose to tip of tail) in centimeters, and weight in kilograms (using an I-20 W load cell by Ohaus Corp., Florham Park NJ for grey seals on Muskeget Island, MA, and an SR digital scale by SR Instruments Inc., for harbor seals in the MARC). Grey seal measurements were obtained as part of another study. Physical measurements were used to create physical models, which aided in determining the accuracy and reliability of PGA models, through comparison. Measured masses were used to aid in the fitting of PGA models as well. Rehabilitating western Atlantic harbor seals, in MARC at UNE in Biddeford, Maine, USA, were photographed in July 2011 (Number of samples = 29). Wild grey seals were photographed on Muskeget Island, MA, January 12th- 16th 2011 (N=131).

Two-Dimensional PGA

Harbor Seals. Two-dimensional harbor seal photographs were taken from a tripod on a level plane using an Olympus SP 590 UZ camera. The tripod was set so that the camera was 0.286m above the ground during photography. Olympus camera(s) were set at a focal length of 4.6mm during all close-range photography. At all times a ruler was placed near the seal as a source of scale. Tape was applied to the floor to determine seal distance from the camera. If markers on the floor were not in place during photography the actual distance was measured. Photographs were taken perpendicular to the long axis of the seal when the animal was in a prone position (side view photograph), as well as from a cranial-end perspective (head-on view photograph). Radial distortion was corrected using the *Olympus Master 2* software (applying the known parameters of the camera to correct distortion), prior to measurement of photographs.

Grey Seals. Grey seal photographs (N=77), were taken using a Samsung Digimax S500 camera. A focal length of 2.8 – 4.9mm was used during photography. A ruler, or measuring tape, was placed in each frame for scale. Photographs were taken from a side-view and head-on view, while the seal was in as close to a prone position as possible. A

0.305m x 0.305m calibration checkerboard with 10 x10 squares (squares 0.03m x 0.03m), from <http://www.vision.caltech.edu/bouguetj/calib_doc/> through Jean-Yves Bouget, was placed at a known distance (0.914 m) perpendicular to the camera and photographed in six different orientations (Figure 1) using the same Samsung Digimax S500 camera. Six orientations were chosen because this was the fewest number of calibration images that could be used to calibrate a camera using this method (B. Maxwell, *pers comm.*). These orientations did not require specific angles, however they did require consistency. Orientations included: Vertical (A), the horizontal top of the board tilted towards the camera (B), horizontal bottom of the board tilted towards the camera (C), one vertical side tilted towards the camera (D), the other vertical side tilted towards the camera (E), and a position different from all other orientations used (F), ensuring a useable number of significantly different orientations. These orientation photographs were analyzed using the *camera calibration toolbox* provided by Matlab Software, from the same internet location as the checker board, to calibrate the camera and ultimately, correct two-dimensional grey seal PGA measurements. Analysis occurred through a process created by Dr. Zhengyou Zhang, called "corner extraction," in this toolbox to determine intrinsic parameters. Intrinsic parameters (focal length, principal point, skew, pixel error and distortion of the camera lens) relate the properties of an image with the parameters of the camera, and are necessary for camera calibration (Zhang 1998; Remondino & Fraser 2006).

Image Analysis. All photos were downloaded to an Inspiron E1505 Dell computer and analyzed in Image J Analysis Freeware, found at <http://rsbweb.nih.gov/ij/>, created by the National Institutes of Health (NIH). When a seal was not in a prone position it became distorted and accurate PGA measurements were difficult to determine (McFadden et al. 2006). Photographs were categorized based on seal position into three

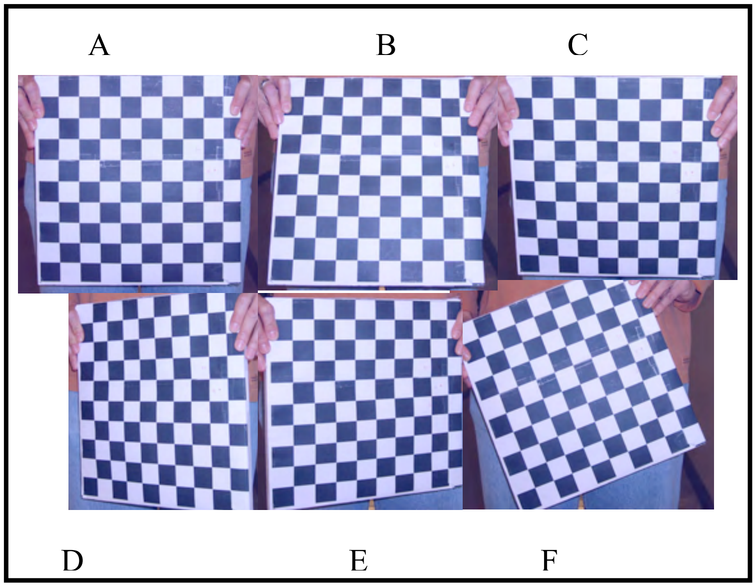

Figure 1. Six calibration board orientations (A, B, C, D, E, F) of the 0.305m x 0.305m calibration checkerboard with 10 x10 squares (squares 0.03m x 0.03m) used for Samsung camera calibration.

categories, with Type 1 being the best and Type 3 being the least desirable and most distorted. Two perspectives, side and head-on view (Figures 2a, b) were evaluated as follows:

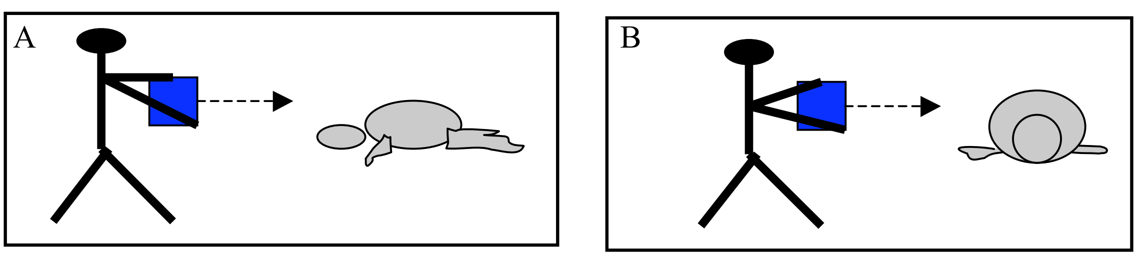

Figure 2. (A) Head-on view, where the seal was facing the camera(s). (B) Side view where the seal was laterally perpendicular to the camera(s).

1. Type 1 Photographs:

    a. Photographs where the seal was perpendicular in side view images or straight in head-on view images, and the camera(s) were level to the ground and the seal.

b. The seal was "as close as possible" to a prone position, prone position meaning the head was down on the ground and not turned towards the camera (in side view), the body was not contorted but in a relaxed state. In head-on view photographs the head was pointed directly towards the camera and the seal's girth (ellipse around the body) was visible.
c. The tail of the animal was visible in side images.
d. The scaling tool in the photograph was close to the seal (0.31 – 0.61m)

2. Type 2 Photographs:
   a. The seal demonstrated an angle less than 45 degrees but greater than 20 degrees from the type 1 position in side and head-on views and was level to the camera.
   b. The seal's head was raised out of a prone position.
   c. The tail of the animal was not visible.
   d. The scaling tool in the photograph was close to the seal.
3. Type 3 Photographs:
   a. The seal was largely removed from the type 1, perpendicular, position and not level to the camera.
   b. The seal's body was not in a prone position.
   c. The tail of the animal was not visible.
   d. The scaling tool in the photograph was not close to the seal in the photograph, but was closer to the camera instead.

Photographs where seals were on their backs or restrained were excluded from the data set (however, this could not be avoided with a small sample of harbor seal photographs with restrainers touching seals included in the data set). Type 1 and type 2 photographs were used in grey seal models (236 photographs, 61.84% of photographs excluded from model building, 45 seals). Type 1 photographs were used in harbor seal models (58 photographs, 29 seals). Two assistants categorized grey seal photographs into Types 1, 2 or 3. Photographic categorization by each assistant differed slightly; one assistant included three additional grey seal observations to two-dimensional models that the other assistant did not include, however the additional observations were used in model building. This method of categorizing photographs was used to generate an un-biased sample in the building of PGA models estimating body mass.

Pixel coordinates (X, Y) outlining seal PGA measurements of each photograph were determined (Figure 3). The Euclidian distance(s) between the pixel coordinates in each photograph were determined using the Euclidean distance formula (distance between two points) then scaled to size.

$$\text{Euclidean Distance} = \sqrt{[(x - x_0)^2 + (y - y_0)^2]} \qquad [1]$$

Seal side height, *SH* (height from ventral to dorsal side directly behind the front flippers), snout-tail length, *WTL* (length from nose to tail of the animal), and dorsal height, *DH* (the height at 60% of the standard length from the nose towards the tail) were each determined (Figure 4) (Bell et al. 1997; Ryg et al. 1988). Grey seal front-end elliptical circumference, *End Girth,* was determined by measuring the major and minor axes, as well as foci distance of the ellipses generated by head-on view photographs of each seal (Figure 5). Harbor seal *End girth*, the complete lateral side perimeter of a seal

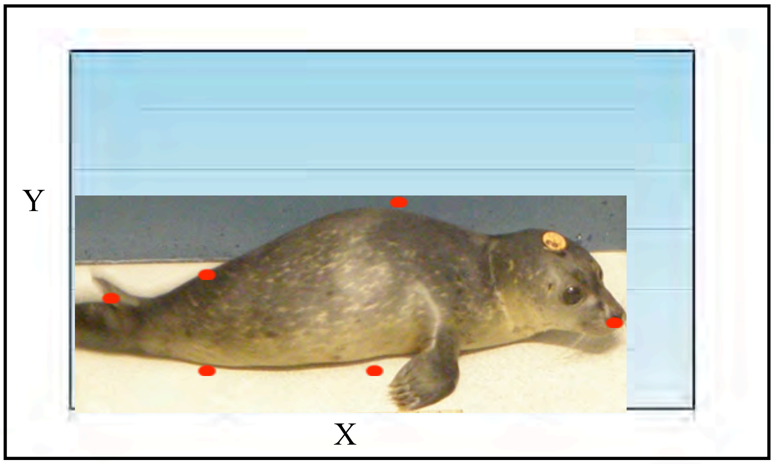

Figure 3. Example of X,Y coordinates outlining points on a photograph to determine seal PGA measurements.

not including the flippers (*SP*), and the complete lateral side area of a seal not including the flippers (*SA*) (McFadden et al. 2006), were drawn around the seal and measured in Image J Analysis Freeware, using the segmented line, polygon and elliptical selections (these are selections that enable a user to trace an object or line and discover the object's length and area). Camera calibration techniques influenced the types of measurements taken of each photograph. The software used to calibrate harbor seal photographs was able to calibrate not only x,y coordinate measurements, but also polygon selections. Whereas the software used to calibrate grey seal photographs could only calibrate x,y coordinate measurements.

Additional PGA measurements were calculated using previously mentioned PGA measurements, and were included in PGA models (Table 1). These PGA measurements were chosen as variables for PGA model building because they were used in previous studies for estimating seal body mass (Ireland et al. 2006; Bell et al. 1997; Hoff et al. 2005). Other variables, such as flipper length (the longest part of the hind, or pectoral, flipper from the base to the tip of the flipper), were only weakly correlated with body mass and were not included (Pearson product moment correlation: Hind flipper r = 0.440,

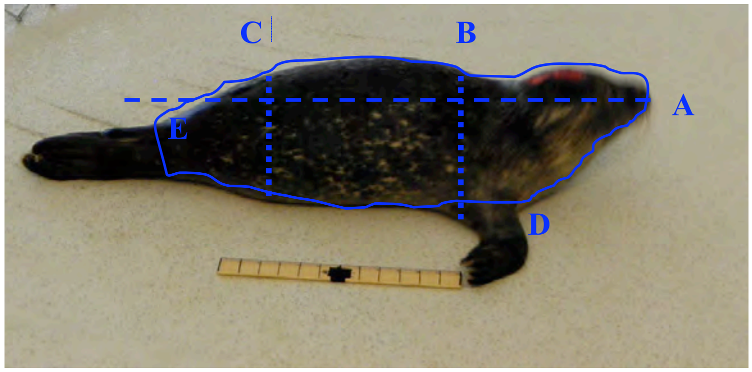

Figure 4. Harbor seal juvenile in MARC parallel to mounted cameras demonstrating PGA measurements obtained from a side view. (A) Standard length, *WTL*; (B) Side height, *SH*; (C) Dorsal height, *DH*; ruler placement for scaling; (D) Side perimeter; *SP*; and (E) Side area, *SA*.

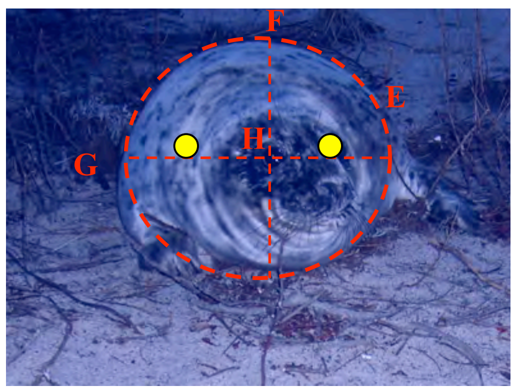

Figure 5. Grey seal pup in the field demonstrating PGA measurements obtained from a head-on view. Front end circumference, (E) *End Girth*, which is derived from (F) the Minor axis of the ellipse; (G) Major axis of the ellipse; and (H) the distance between two foci points on the major axis.

Pectoral flipper r = 0.38, N=76).

Two grey seal models and four harbor seal models were developed. Harbor seal models 1-4 (Table 2) were created in SAS (9.2) using the *MAXR* function. SAS (9.2) was not always used because it was not always accessible through UNE; R (2.12) was used in its place. However, SAS (9.2) was the preferred program because it out performed R

(2.12) in model building when more than five variables were used. Grey seal models 1-2 (Table 4) were developed in R (2.12) using the *Fit Model* and *Generalized linear model*

Table 1. All PGA variables used for model construction are presented.

PGA variable	Description	Calculation
1. End girth	Elliptical head on girth from head on photograph	
2. WTL	Length from nose to tail from side photograph	
3. SP	Side Perimeter: Complete lateral side perimeter, not including flippers, from side photograph	
4. SH	Side Height: Height from dorsal to ventral side, behind front flippers, from side photograph	
5. DH	Dorsal Height: Height from dorsal to ventral side, 60% of the WTL towards the rear of the seal from a side photograph (Ryg et al. 1988).	
6. SA	Side Area: Complete lateral side area, not including flippers, from side photograph	
7. SH girth	Side Height girth: Circular girth created using SH as the diameter	2π *(SH/2)
8. DH girth	Dorsal Height girth: Circular girth created using DH as the diameter	2π*(DH/2)
9. TVEG (See Figure 6)	Total Volume End Girth: The volume created from the addition of two volumetric cones, treating the End Girth as the base of both cones (the symbol r represents radius). The larger cone was created using a height that was 60% of the WTL and the smaller cone was built using a height that was 40% of the WTL. The DH sits at 60% of the WTL towards the rear of the animal, splitting the animals' dimensions into two parts (60% and 40%). In grey seal models 101.59 was added to the End girth radius for TVEG measurements. This was the average measured girth of animals in the sample and was used as a scaling factor.	((1/3*π* End girth +101.59r^2)*0.6WTL) + ((1/3*π*End girth+ 101.59r^2)*0.4WTL)
10. TVSG (See Figure 6)	Total Volume Side Girth: The volume created from the addition of two volumetric cones, treating the SH girth as the base of both cones. TVSG in grey seal models 1 and 2 (Table 4) was created using the radius of SH girth + 1.825, the average SH of all animal measurements in the sample. This was used as a scaling factor.	((1/3*π*SH girth r^2)*0.6WTL) + ((1/3*π*SH girth r^2) *0.4WTL)
11. Total Area (See Figure 6)	The area created from the addition of two planar triangles. The height of the larger triangle was created from 60% of the WTL and the base was created from the SH of the animal. The smaller triangle was created from a height of 40% of the WTL and a DH base.	[(0.5*SH)*(0.6WTL) + (0.5*DH)*(0.4WTL)]

Table 1. All PGA variables used for model construction are presented (cont).

PGA variable	Description	Calculation
12. LG^2	Standard phocid body condition calculation. In models lacking end girth components SH girth was substituted for End girth to create LG^2.	WTL * End $Girth^2$
13. Major Axis	Major axis of ellipse (horizontal axis)	
14. Minor Axis	Minor axis of ellipse (vertical axis)	
15. End Area	Area of End Girth	
16. Vol * Density	TVSG or TVEG, depending on model, multiplied by the average density of a healthy mammal: 1.01(kg/m^3)(Durnin et al. 1974). Bruyn et al. (2009) found this density to be more useful than blubber to lean-mass density ratio for seals of different sexes and ages.	TVSG *1.01(kg/m^3) TVEG *1.01(kg/m^3)

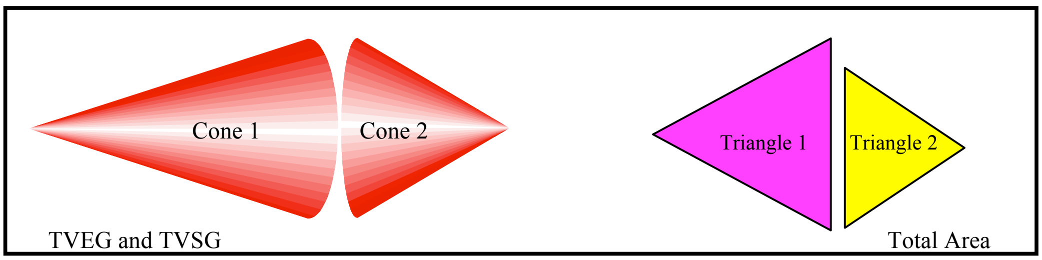

Figure 6. The above figure demonstrates how the addition of two cones was used to create TVEG and TVSG. It also demonstrates how the addition of two triangles was used to create Total Area.

functions of the R Commander package. Models that had the greatest significance were chosen using a minimum alpha of 0.05, and greatest r^2 (as well as Adjusted r^2) value. Models were created from a combination of the variables described above, with the following exceptions: models developed that omitted head-on view photographs did not utilize *End girth* or *End Area,* and utilized *SH girth* instead (in order to provide models that could be easily used in the field, a number of these models were designed from side-view photographs only). Harbor seal two-dimensional models contained the additional variables *SA, SP* and *Vol * Density*, lacking *Total Area* as a variable.

The accuracy of the models was verified by a 95% confidence interval and R-squared correlation. Confidence intervals at 95% were built for each model from the estimated body masses generated by each model using the standard formula (Sokal & Rolf 2001):

$$CI = x +/- t^* (s/\sqrt{n}) \quad [2]$$

Where x represents the sample mean, t represents the t statistic from a student's t-table at a 0.05 significance level, s represents the standard deviation and n represents the number of samples in the model equation.

The percent (%) difference between the bounds of the confidence interval and the true population mean was then determined.

Three-Dimensional PGA Using Stereo-PGA

Three-dimensional observations of grey seals (N=45) and harbor seals (N=28) were taken using two Olympus SP 590 UZ cameras mounted on an aluminum bar, cut to fit their dimensions, and secured to a tripod 0.838 meters above the ground. Cameras were set at a distance of 0.406m from middle of lens to middle of lens. Both cameras were operated simultaneously using remote control cables (remote cable RM-UC1) and the bar was leveled each time photographs were taken (Figure 7a). This design (Figure 7b) was a downscaled version of that used by Hall-Martin & Ruther (1979).

Harbor Seals. The same photography set-up used in two-dimensional photography was used for three-dimensional photography, except that two photographs were generated for each seal (Figure 8) (these images are referred to as stereo-pairs). All stereo-pairs were downloaded and analyzed in Image J Analysis Freeware. Stereo- pairs were measured and pixel coordinates (X,Y) were determined. Pixel coordinates of from each photograph outlined the *SH, DH* and *WTL* of each animal (as they did in two-dimensional photography), using segmented lines and multipoint selection options. The multipoint

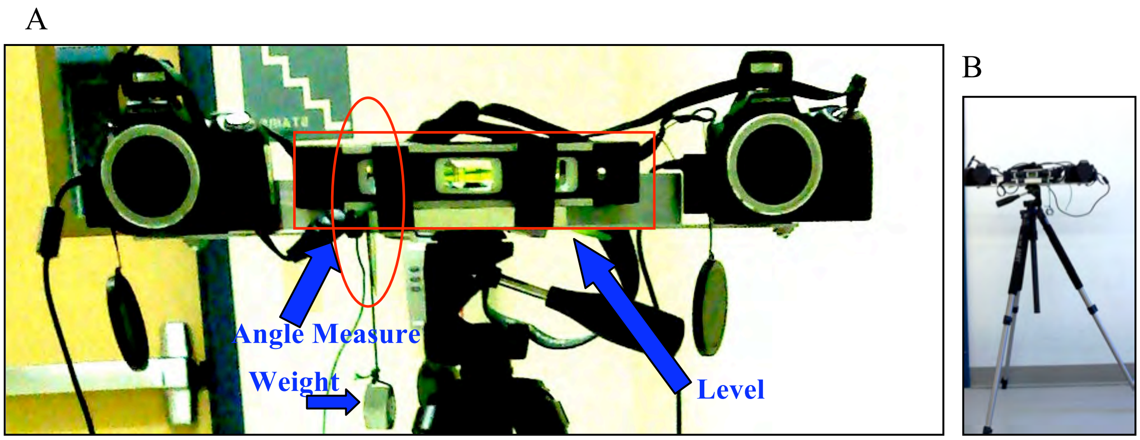

Figure 7. (A) Set-up of two Olympus cameras and their remote controls with a level in between and an angle measure with a weight to determine angle to the horizontal of each camera (outlined in red). (B) The entire set-up, including the tripod.

Figure 8. A set of camera stereo-pair images of a harbor seal pup.

selection allows a user to select multiple points within a photograph and measure their locations on a two-dimensional axis. The major and minor axes of the elliptical circumference from a head-on view photograph were also measured from four points placed around the seal to determine *End Girth*. The two-dimensional pixel points were then put through the *stereo-calibration* program of the Matlab *calibration toolbox* to determine each point's three-dimensional (X,Y,Z) location (Figures 9a, b). The program computed, for each stereo-pair, the intrinsic parameters of both cameras combined, as well as the extrinsic parameters (rotation and translation vectors) of both cameras. Extrinsic parameters are required for stereo-photography, and relate the properties of a camera to real-world dimensions, taking into account camera orientation and distance to

A B

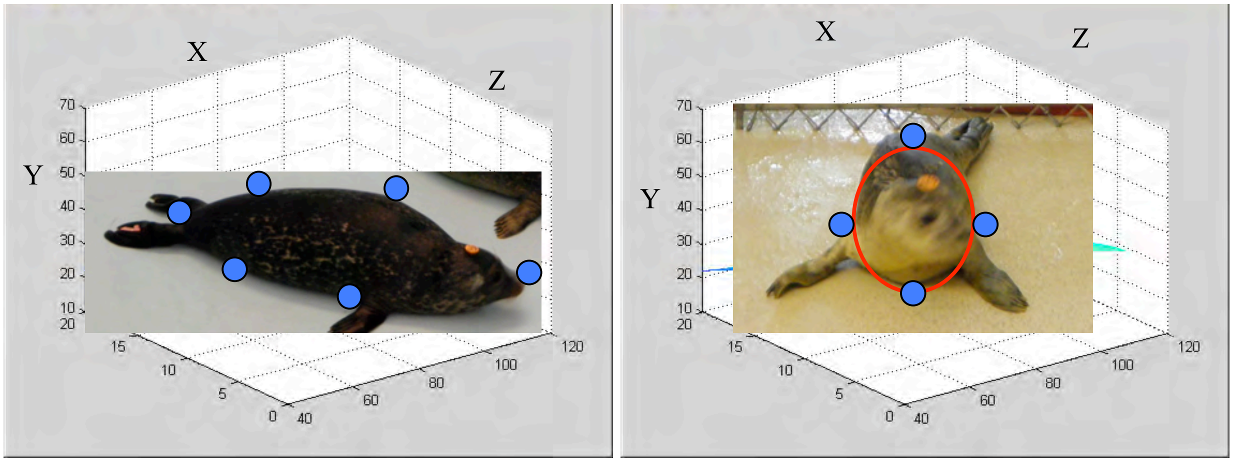

Figure 9. (A) Model of three-dimensional side photograph points determining *WTL, SH, DH* of a prone harbor seal. (B) Model of three-dimensional head-on photograph points determining *End girth* of a prone harbor seal.

the object being photographed. After photography, cameras were calibrated using a combination of both a 0.305m x 0.305m board and a similar 0.914m x 0.914m board with 9 x 9 squares (squares 0.098 x 0.098m), the same method used to calibrate the Samsung camera in two-dimensional grey seal photography. Again, six different orientations of the calibration boards were photographed. Each set of calibration images was taken with cameras at the same angle to the ground, and distance to seals, as they were during photography. Calibration was performed after photography because the location of each seal changed during photography and was unpredictable.

Stereo-pairs of the calibration board in the vertical position (A), taken at the same angle and distance to the cameras as the seals, were processed in Image J. The pixel coordinates of the points located at the furthest opposite corners of the horizontal bottom of each board (Figure 10) were determined. These points were then uploaded to Matlab and run through the *stereo-calibration* program of the *calibration toolbox* to determine each point's three-dimensional (X, Y, Z) location. Once the Euclidian distance between these points was determined (known length of the horizontal bottom of the calibration board: 0.914m), these measurements became the scales for the stereo-pairs of seal images.

Figure 10. Points outlining the horizontal bottom of the large calibration board from stereo-pair images (0.914m x 0.914m board with 9 x 9 squares (squares 0.098 x 0.098m)) in the A position. Points were triangulated using the Matlab stereo-calibration program, and the Euclidian distance in pixels (known length 0.914m) was used to scale seal photographs.

Grey Seals. The distance of the tripod to each seal was measured for every photograph, as well as the angle of the cameras to the ground. Calibration images were analyzed using the same methods as harbor seal photographs. All variables described in the harbor seal two-dimensional methods section were measured. In contrast to harbor seal measurements, six points around the front-end circumference of the seal were measured to determine the *End Girth.* The Euclidian distance between these points was calculated and the elliptical *End Girth* of each seal was determined using the minor axis and foci distance of each ellipse.

Image Analysis. Two research assistants categorized the grey seal photographs, using the same descriptions outlined in the two-dimensional categorization process (Grey seal photographs = 229, Type 1 and 2 were used to build models; N = 45) and there was no significant difference in their categorization results. This categorizing scheme differed from the two dimensional categorization, in that the angle of the cameras to the ground was not relevant and did not need to be taken into account when categorizing photographs because it had been recorded for each photograph and was incorporated into camera calibrations. Type 1 photographs were used in harbor seal models (Harbor seal photographs = 56). Grey seal models 3-6 (Table 4) were built using SAS (9.2). Harbor seal models 5-7 (Table 2) were built in R (2.12).

Two-Dimensional Distance PGA

Five grey seal pups were photographed laterally, meaning only side view photographs were taken, using an Olympus SP 590 UZ camera on Muskeget Island in January 2011 at distances ranging from 19-35 meters, using a range of camera focal lengths, 62.19 – 119.6mm. Distance with correct elevation inclination, was determined to each seal using an infrared laser range finder (Bushnell Scout 1000) with ± 3 meter accuracy. Radial distortion of photographs was corrected using the *Olympus Master 2* software. Measurements used to determine body mass, including *SH, DH, STL*, were determined using Image J Analysis Freeware. These measurements were then scaled using the internal properties of the camera, such as sensor size, dimensions of the photograph, distance to the seal and focal length of the camera in the following equation (Spitz et al. 2000):

$$l_o = (D_o + l_i) / (f \times sp) \qquad [3]$$

Where D_o *is the distance to the animal in each photo,* l_i *is the initial pixel measurement,* f *is the focal length (mm) and* sp *is the sensor pixel size (mm/pixel).* l_o *is the measurement result, representing the photographic measurement that takes distance into account.*

Body mass was then estimated using a grey seal model (model 2, Table 4), derived using the variables *WTL, SH, SH girth, DH, DH girth, Total area, TVSG* (these variables were determined using the measurements from side view photographs). This grey two-dimensional model was chosen because it generated body mass predictions with the least error. Grey seal three-dimensional models were not applicable to distance photography.

<u>Additional Analysis</u>

The efficiency of the PGA models was validated using physical data collected from the same animals as those photographed. This data was collected for another study that allowed this study to have access to the data. Standard length and axillary girth were measured using meter tape to the nearest half centimeter and used to create models in SAS (9.2) for both harbor and grey seals (models 1-2, Table 3; models 1-4, Table 5). To determine if gender had a significant influence in building PGA models, non-parametric Wilcoxon two sample tests ($\alpha = 0.05$) (Sokal & Rohlf 2001), were applied to the differences between PGA measurements and physical measurements in grey seal two- and three-dimensional models (N = 37, 22) using SAS (9.2). There was no significant difference between genders in two- and three-dimensional *End girth* results (two sided, p= 0.511, 0.326) and standard length (*WTL*) results (two sided, p= 1.000, 0.562). Non-parametric paired sample Wilcoxon signed rank tests were also run in R (2.12) to determine gender influence in harbor seal two-dimensional models (N= 29). There was no significant difference between males and females in PGA *WTL* and *End girth* measurements (two sided, p= 0.326, 0.585). As with gender, there was no significant effect of the presence of lanugo, white hair present on grey seal pups when they are born, in two-dimensional PGA measurements (N=38**;** two sided, *p*= 0.683). The sample size was too small for comparison of three-dimensional models.

Error in PGA measurement methodology was determined by conducting a paired Student's T-test. Out of a sample size of 45 seals, 5 seals' photographs were randomly chosen and multiple *WTL* measurements were made of each photograph in Image J Analysis Freeware (C. Tilburg, *pers comm.*). Results demonstrated *WTL* measurements did not significantly differ between photographs (α =0.05, *p*= 0.391).

Influence of the stereo-PGA set-up over results, created by camera angle to the ground and distance to each object from the set-up, was determined by conducting a two-way ANOVA of all three-dimensional grey seal data (N= 34) used to build three-dimensional grey seal models. Results indicated that the angle of the cameras had a significant relationship to body mass estimation in models built using PGA *End girth* measurements (*p*= 0.024, F=4.095) and distance did not have a significant relationship (*p*= 0.379, F=1.162). The interaction of both variables was not significant (*p*= 0.866, F=0.030).

In order to better understand the significant influence angle of the cameras to the ground had over body mass predictions, a Styrofoam seal was constructed from a grey seal pup's dimensions. The percent (%) difference of PGA measurements and the Styrofoam seal's measurements were determined at angles ranging from 90 – 150°. A greater angle of 160° was not used because at this angle a full view of the Styrofoam seal's girth in a head-on view photograph was not possible.

To further determine the efficiency of PGA measurements, the difference between physical measurements, axillary girth and standard length, were compared to the PGA measurements, *End girth* and *WTL*. These measurements were compared for each seal included in each set of models incorporating these measurements. The following standard percent (%) error equation was used to determine the percent (%) difference:

$$\frac{\text{PGA measurement} - \text{Physical measurement}}{\text{Physical measurement}} * 100 \qquad [3]$$

The average percent (%) difference and standard deviation were then computed.

Cross Validation (CRV) was performed using a random 10% sample of the grey seal physical data, not included in grey seal physical model building. Body masses of the 10% not included in model building were estimated using physical models. The percent (%) difference between estimated and actual body masses was determined.

Results

Models Predicting Body Mass (Harbor Seals)

Pearson product moment correlation tests (denoted using *r* as a measurement of correlation, and *p* as a measurement of significance; α =0.05) were performed to determine PGA variables with the greatest correlation to body mass in each model. Two-dimensional harbor seal PGA variables (models 1-4, Table 2) included *SH* (*r*=0.569, *p*=0.001), *SH girth* (*r*=0.569, *p*=0.001), *LN(SH)* (*r*=0.542, *p*=0.002), *LN(SA)* (*r*=0.540, *p*=0.002), *SA* (*r*=0.541, *p*=0.002), *End girth* (*r*=0.506, *p*=0.005), *LN(End Area)* (*r*=0.503, *p*=0.005), *LN(End girth)* (*r*=0.533, *p*=0.005), *LN(LG^2)* (*r*=0.506, *p*=0.005), and *LN(TVEG)* (*r*=0.506, *p*=0.005). Three-dimensional harbor seal PGA variables (models 5-7, Table 2) included *End girth* (*r*=0.777, *p*=<0.0001), *LN(DH)* (*r*=0.597, *p*=0.001), *LN(End girth)* (*r*= 0.710, *p*=<0.0001), Major axis of *End girth* (*r*=0.747, *p*=<0.0001), Minor axis of *End girth* (r=0.798, p=<0.0001), *SH* (r=0.750, p=<0.0001) and *SH girth* (r=0.750, p=<0.0001). In harbor seal physical models (models 1-2, Table 3), only axillary girth (r= 0.846, p=<0.0001), demonstrated a significant correlation to mass. Further analysis of individual regression slopes of each PGA variable to determine significance revealed no individual variable to be highly significant in three-dimensional model building. However, in harbor seal two-dimensional model 4 (Table 2), LG^2 (p=0.017) was significant.

Predictive model equations were selected based on r^2 correlation, Adjusted r^2 correlation, p-value and confidence intervals. All harbor seal models had r^2 values greater than 0.65, and Adjusted r^2 values close to or > 0.65, making these models credible and significant (McFadden et al. 2006). Models with the greatest predictive strength from Table 2 were as follows:

1) Two-dimensional model including *End girth* components: model 1
2) Two-dimensional model lacking *End girth* components: model 3
3) Three-dimensional model including *End girth* components: model 5
4) Three-dimensional model lacking *End girth* components: model 7

Models Predicting Body Mass (Grey Seals)

Grey seal PGA variables with the greatest correlation to mass in three-dimensional models (models 3-4, Table 4) included *LN(TVEG)* (r=0.705, p=0.0003) *LG^2* (r=0.6791, p=0.0005), *LN(Girth + 101.59)* (r=0.612, p=0.002), *End girth* (r=0.603, p=0.003), *(Girth + 101.59)* (r=0.604, p=0.003), *LN(LG^2)* (r=0.701, p=0.003), and *Total Area* (r=0.498, p=0.018). In physical models, (Table 5) grey seal female variables standard length (r=0.714, p=<0.0001) and axillary girth (r=0.756, p=<0.0001)

Table 2. Harbor seal PGA models using two- and three-dimensional techniques. r^2 shows predictability of each model, the adjusted r^2 is included as ($Adj\ r^2$). CI demonstrates the percent difference from the sample mean of each model's bounds and p represents the exact p value.

Two-Dimensional Models (N=29)	r^2	$Adj\ r^2$	Lower	Upper CI	p
Equations with End girth components					
1. Mass = -561.950 +2.715(WTL) +2.744(DH) + 10.834(SH) -2.239(SP) -0.002(SA) -0.1867(End girth + 58.455) -0.002(End Area) -176.965(LN(WTL)) -31.154(LN(DH)) -173.097(LN(SH))+ 295.411(LN(SP)) + 23.916(LN(SA))+ 20.165(LN(End Area))	0.777	0.584	-6.832	7.362	0.006
2. Mass = -494.116+ 2.603 (WTL)+ 2.716(DH) + 10.676(SH) -2.169(SP) -0.001(SA) -0.002(End Area) -0.000031(LG^2) -165.027(LN(WTL)) -30.507(LN(DH)) -170.315(LN(SH)) + 285.927(LN(SP)) + 14.639(LN(SA))+ 18.114(LN(End Area))	0.776	0.582	-7.533	6.699	0.006
Equations without End girth components					
3. Mass = -702.899+ 2.687(WTL + 74.826) +3.2448(DH) - 4.019(SP) - 0.003(SA) - 0.520(SH girth + 58.455) + 0.00009(LG^2) -41.858(LN(DH)) + 439.646(LN(SH)) + 544.512(LN(SP)) + 47.254 (LN(SA)) -248.333 (LN(LG^2))	0.716	0.533	-6.413	7.209	0.006
4. Mass = -708.001 + 2.903(WTL + 74.826) +2.769(DH) -3.998(SP) -0.003(SA) + 0.00007(LG^2) -34.995 (LN(DH)) +410.783(LN(SH)) +539.821(LN(SP)) + 49.407 (LN(SA)) -250.323(LN(LG^2))	0.714	0.555	-7.168	6.456	0.003
Three-Dimensional (Stereo-photogrammetry) Models (N=27)	r^2	$Adj\ r^2$	Lower	Upper CI	p
Equations with End girth components					
5. Mass = 92.72 + 0.6745(DH) + 0.375 (End girth) +0.00003.649(LG^2) -0.450(LN(DH)) -0.281(LN(SH)) + 155.6 (LN(End girth)) -80.80(LN(LG^2))+69.64(LN(Total Area)) -0.513(Major) + 0.596(Minor) +0.629(SH) +0.290(WTL) -0.0296(Total Area)	0.904	0.807	-7.833	7.871	<0.0001
6. Mass = 89.49 + 0.917(DH) + 0.470(End girth) +0.00003617(LG^2) -33.55(LN(DH)) -24.58(LN(SH)) +121.8(LN(End girth)) - 65.75(LN(LG^2)) +54.56(LN(Total Area)) +0.629(SH) + 0.334(WTL) - 0.035(Total Area)	0.891	0.811	-5.744	9.787	<0.0001
Equations without End girth components					
7. Mass = -378.9+ 0.173(DH) -0.00000003.080(LG^2) -65.87(LN(DH)) +12.67(LN(SH)) +38.58(LN(LG^2)) -35.83(LN(Total Area)) +4355(SH) -45.46(SH girth*30.48) +0.124(WTL) -0.007(Total Area)	0.726	0.554	-1.536	11.272	0.005

Table 3. Harbor seal physical models. r^2 shows predictability of each model, Adjusted r^2 (*Adj* r^2) is included, CI demonstrates the percent difference away from the sample mean of each model's bounds and the exact *p* value.

Harbor Seal Physical (N=29)	r^2	*Adj* r^2	*Lower*	*Upper* CI	*p*
1. Mass = -18.176 + 0.307 (Axillary girth) + 0.159(Standard Length)	0.893	0.885	-7.756	7.757	<0.0001
2. Mass = -2.131 + 0.236(Axillary girth)	0.716	0.705	-6.940	6.943	<0.0001

demonstrated high correlation to mass; variables in male physical models were not highly correlated to mass. Further analysis of regression slopes of each PGA variable was conducted. In grey seal model 3 (Table 4) variables created using *SH* (*SH girth p*=0.003, *LN (SH) p*=0.007, *Total Area p*=0.014)*, DH* (*DH p*=0.039, *DHgirth p*=0.036)*,* and *End girth* (*LN (End girth + 101.59) p*=0.006) were significant. Grey seal model 4 (Table 4) had significant variables created from *SH* (*LN (SH)) p*=0.0019, *SH girth p*=0.001), *DH* (*DH p*=0.018, *DH girth p*=0.017) and *End girth* (*LN(End girth + 101.59) p*=0.003). Grey seal PGA models with the highest predictive strength from Table 4 were as follows:

1. Three-dimensional models incorporating *End girth* components: model 3
2. Three-dimensional models that did not include *End girth* components: model 5

Grey seal physical models of both genders produced significant models for predicting body mass. Gender influenced physical models, as standard length, but not axillary girth, significantly and differentially influenced the physical model data (Wilcoxon Two-Sample test, α of 0.05, two sided, *p*= 0.0002, 0.088 respectively; N = 60Males, 71Females). Four physical grey seal models were created based on these results, two for males and two for females. The female model with the highest predictive strength was the model 1 from Table 5 (N=64). The male model with the highest predictions strength was model 3 from Table 5 (N=54).

Table 4. Grey seal PGA models using two- and three-dimensional techniques. r^2 shows predictability of each model, Adjusted r^2 (*Adj* r^2) is included, CI demonstrates the percent difference away from the sample mean of each model's bounds and p represents the exact p value.

Two-Dimensional Models (N=45)	r^2	*Adj* r^2	Lower	Upper CI	p
1. Mass = -2720 -7.744 (WTL + 3.62) +722.2 (DH + 1.8247)-396(DH girth) -1017(DH) + 0.00042(LG^2) + 507(LN(SH)) +10723(LN(SH +1.8247)) -844.5(LN(TVSG))+573(SH +3.287) -570(SH girth)) + 427.7((WTL+3.62)^0.5) -0.726(Total Area)	0.448	0.240	13.744	17.982	0.041
2. Mass = -3481.557 +-17.502 (WTL + 3.62)-1113.069(DH) -342.238 (DH girth) + 398.545(LN(SH)) 1182.927(LN(SH +1.825)) -856.742(LN(TVSG))-281.649(SH+1.825))+817.311(SH +3.287) -533.251(SH girth) + 130.364(LN(DH)) + 693.947 (DH +1.825) + 506.888((WTL+3.62)^0.5)	0.445	0.237	-3.411	0.329	0.043
Three-Dimensional (Stereophotogrammetry) Models	r^2	*Adj* r^2	Lower	Upper CI	p
Equations with End girth components (N=22)					
3. Mass = -6102.497- 1.1677(WTL) + 315.566(LN(WTL)) + 1404.360(LN(SH)) - 18.816(SH girth) + 495.859(DH) - 23.113(LN(DH)) - 159.791(DH girth) - 2.476(End girth) + 1336.554(LN(End girth+101.59)) -97.201((WTL+111.66)^0.5) + 0.375(Total Area) 0.00002694(LG^2) - 336.196(LN(TVEG)	0.913	0.773	-9.990	0.194	0.006
4. Mass = -5826.884 + 304.335(LN(WTL)) + 1430.372(LN(SH)) - 19.020(SH girth) + 506.6946(DH) -30.19259(LN(DH)) - 163.10408(DH girth) - 2.47596(End girth) + 1344.690LN(End girth+101.59)) – 122.0622 ((WTL+111.66)^0.5) + 0.366(Total Area) - 0.00002627(LG^2) - 342.015 (LN(TVEG))	0.913	0.797	-10.067	0.124	0.002
Equations without End girth components (N=34)					
5. Mass = 48354 + 22.603(WTL) + 14.455(SH girth) - 1188.798(DH) + 370.171(DH-114.24) 31.244(WTL + 110.8) + 35.975((WTL+110.8)^0.5) + 0.660(Tot Area) + 2379.168(Log(Total Area)) -0.00044(WTL*(LG^2) - 886.873(LN(TVSG))	0.511	0.299	-2.660	3.741	0.040
6. Mass = 52033 + 15.322(SH girth) - 1353.511(DH) + 422.239(DH-114.24) - 8.710(WTL + 110.8) + 31.786((WTL+110.8)^0.5) + 0.690(Total Area) + 2475.080(Log(Total Area)) 0.00047 (LG^2) - 930.239(LN(TVSG))	0.502	0.315	- 4.279	2.124	0.026

Table 5. Grey seal physical models. r^2 shows predictability of each model, Adjusted r^2 (*Adj* r^2) is included, Cross Validation (CRV) was performed with 10% of the seal data left out of production of each model (this was the percentage determined that could be left from the model without sacrificing it's accuracy). CI demonstrates the percent difference away from the sample mean of each model's bounds and the exact *p* value.

Female Physical Models (N=64)	r^2	*Adj* r^2	CRV (% Difference)	Lower	Upper CI	*p*
1. Mass = -50.459 + 0.466(Standard Length) + 0.383(Axillary Girth)	0.675	0.664	4.708 ± 5.036	-4.034	4.030	<0.0001
2. Mass = -18.210+ 0.567(Axillary Girth)	0.572	0.565	8.059 ± 4.654	-3.960	3.962	<0.0001
Male Physical Models (N=54)	r^2	*Adj* r^2	CRV (% Difference)	Lower	Upper CI	*p*
3. Mass = -66.299 + 0.256(Standard Length) + 0.791(Axillary Girth)	0.834	0.828	0.249 ± 3.470	-2.949	2.950	<0.0001
4. Mass = -50.751 + 0.921(Axillary Girth)	0.805	0.802	8.721 ± 5.312	-2.716	2.714	<0.0001

A comparison was conducted between PGA measurements *End girth* and *WTL*, and physical measurements, axillary girth and standard length. Results revealed the largest % difference between PGA and physical measurements occurred in harbor seal three-dimensional measurements (girth: 28.41 ± 10.03%, length: 3.59 ± 38.39%). The smallest % difference occurred in grey seal three-dimensional measurements (girth: 12.52 ± 11.24%, length: 15.11 ± 6.68%).

Sources of Error

Use of cameras that could not accurately be calibrated (inadequate for this type of research) incurred error in two-dimensional grey seal models. A Styrofoam seal was built using the dimensions of a juvenile grey seal and was photographed using the Samsung Digimax camera. The Samsung camera did not have a un-distort program and a large amount of error was incurred by this camera set-up, causing poor grey seal models to be created (models 1-2, Table 3). The *Olympus Master 2* software is a un-distort program meant for the Olympus cameras and was used for two-dimensional Olympus camera calibrations in this study. Comparing the PGA measurements of the Styrofoam seal to its physical measurements, the Samsung camera functioned best at 90°(Figure 10). In angles ranging from 90 – 110° the % difference of PGA versus physical measurements, *SH, DH,* and *End girth*, ranged from 0.158 – 19.23 ± 4.114 – 5.103%. The largest % difference (72.161%) occurred in length measurements, *WTL*, at 90°.

However, when distortion corrections were applied through the *Olympus Master 2* software to photographs of the Styrofoam seal taken in the lab using the Olympus cameras, less two-dimensional error occurred between PGA and physical measurements from 90 to 130°. The average % differences in *WTL, SH* and *End girth* measurements ranged from 3.375 – 13.553% ± 0.647 – 4.807% at all angles. The largest % differences (33.444 ± 6.664%) occurred at 150° and were seen in *DH* measurements. All measurements demonstrated considerably greater accuracy than those generated by the Samsung camera.

The three-dimensional error incurred by the angle of the cameras attached to the stereo-rig during grey seal photography was also determined using the Styrofoam seal. Comparing PGA and physical measurements, the smallest % difference (*WTL:* 0.7%, *SH*: 5.82%, *DH*: 35.587%, *End girth*: 8.56%) was incurred at 90°. The largest % difference in measurements (*WTL*: 5.31%, *SH*: 89.429%, *DH*: 77.482%, *End girth*: 67.647%) occurred at 140°. The measurement displaying the largest percent (%) difference was again the *DH* measurement.

<u>Distance PGA</u>

Two-dimensional mass estimates were determined from side PGA measurements of grey seals 19-22 meters away from the photographer; using a focal length of 119.6mm. Values were then applied to grey seal model 2 because this model accurately represented

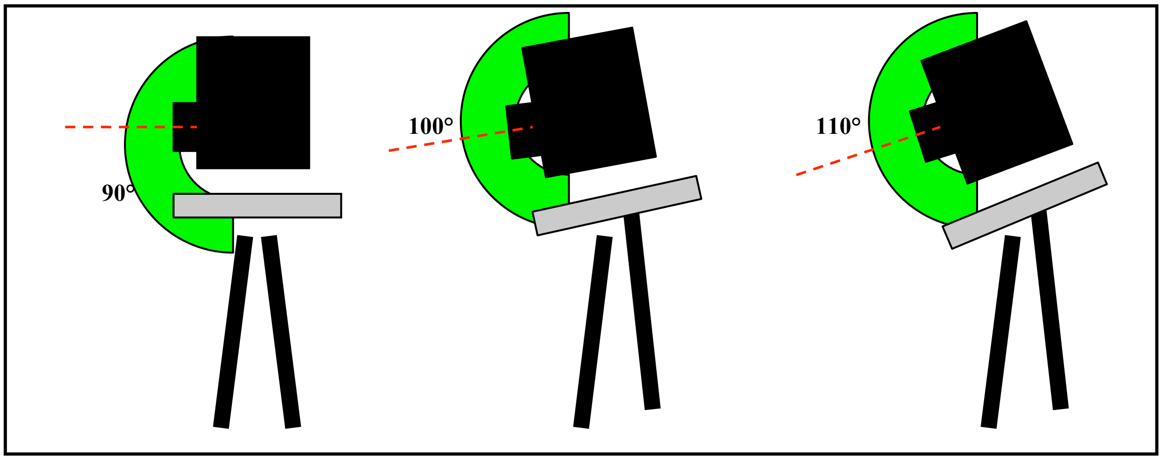

Figure 10. This figure demonstrates three examples of camera angles in which stereo-photography took place. Notice that camera angle increases as the lens points closer towards the ground, which is measured by the green angle measure attached to the mount.

the samples collected in this stage of the distance PGA project (i.e. seals of the appropriate mass that were modeled using two-dimensional PGA, lacking head-on photographs) (Table 4). Results demonstrated estimated masses within 4.01% - 16.42% accuracy of the actual mass (N=3).

The same Styrofoam seal, constructed from measurements of a grey seal weighing 23.55kg used in set-up error testing, was used for distance PGA tests due to the small sample size of grey seals photographed in the field. The seal was photographed between 18m and 34m (at 18, 22, 25, 30 and 34m) using a 119.6mm focal length. The predicted mass was within 3% accuracy of the measured mass at 18m, and 20% accuracy at 22m using harbor seal model 4 (Table 2). A harbor seal model was used because it worked best with the Styrofoam seal, which was created from dimensions similar to those used to create harbor seal models. Accuracy decreased with distance and at 30m mass estimation was within 38% of the measured mass.

Discussion

PGA Models

Hall et al. (2001) discovered that mass at weaning was positively correlated with first year survival probability in grey seal juveniles. However, collecting physical data to determine mass and survival can be intrusive. Therefore, PGA models predicting body mass of un-sedated phocid pups may be created to alleviate this issue. Such models are uncommon because pups are difficult to photograph at the correct angles because of their high mobility, increasing the risk of errors in photographic measurements (Ireland et al. 2006; McFadden et al. 2006). The PGA models built in this study have been shown to be relatively accurate in predicting body mass for both captive and wild seal pups (Adjusted r^2: harbor seal, 0.807 (model 5, Table 2); grey seal, 0.773 (model 3, Table 4)).

These models involve many essential steps to estimate body mass; such as calculating complex variables like *TVEG* and *Total Area* from such measurements as *SH*, *DH*, *STL* and *End girth*. The strength of each model is improved with the addition of strong predictive variables; however, a simplistic model is preferred in model building because it demonstrates the high prediction power of the variables included. This is seen in physical models built within this study with high predictive strength and fewer variables than PGA models.

The Shapiro Wilk's test determines the normality of variables used in a model. If variables are found to be non-normal in distribution they may be transformed with log base 10 transformation, or natural log transformations. In some instances a square root transformation is used over "count" data (Gotelli & Ellison 2004). McFadden et al. (2006) and Bruyn et al. (2009) performed Shapiro Wilk's tests for normality over all of their variables to determine if they required transformation, before model building. This study utilized the same method. However, we found that including a version of a variable that was transformed and untransformed in the same model ultimately improved the strength of the model. The addition of both versions of a variable was acceptable for model building (J. Holly, *pers comm.*). If the inclusion of a version of a variable in the transformed state added little to the strength to a model, it was not included. It is possible that a degree of multi-colinearity may have occurred. Model normality was not influenced by the addition of non-transformed variables based on Normal Q-Q diagnostic plot tests. Also the degree to which the number of variables used in a given model influenced the predictive strength (r^2) was revealed in the Adjusted r^2 results, making the Adjusted r^2 results more reliable statistics for PGA models. These results show the predictive strength of the models with a minimum number of required variables. Two-dimensional harbor seal models and models lacking head-on photographs were the weakest, as reflected in low Adjusted r^2 values. However, inference investigation of each variable revealed that all models could not be reduced without damaging predictive strength. Therefore, incorporation of new data is the only way to improve the models. As these PGA models are used by other studies and larger sample sizes and more data are incorporated, better models will be generated and their complexity should decrease. For example, PGA studies of Southern elephant seals (*Mirounga leonina*) have been conducted since the 1950s and since then their use and incorporation of new PGA data has become widespread (Laws 1953). Initial models have been improved upon by later studies. Bruyn et al. (2009) only used two variables, volume and density of a healthy mammal, in building successful models for predicting body mass of Southern elephant seals. Bruyn et al. (2009) also used terrestrial PGA methods with one digital camera, whereas Laws (1953) used aerial photography and a highly technical camera. Use of specific density values (specific density of each phocid weanling) in PGA models will also benefit each model's predictive ability, due to the slight differences in fat gain during lactation between species. Weanlings of both species experience a difference in mass gain throughout lactation, created by different lactation strategies. Whereas grey seal pups average a fat gain of 1.15±0.147 kg of fat/day during lactation, harbor seal pups gain 0.41 kg of fat/day, on average (Iverson et al. 1992; Bowen et al. 1992).

Variables used in PGA models presented here were derivations of four basic, variables, *WTL, SH, DH* and *End girth*. Pearson product moment correlation results conducted over each model revealed the strength and importance of these variables. In three-dimensional grey seal models the use of head-on photographs for calculating *End girth* was one of the most useful tools in determining the strength of models. *End girth* and variables derived from it, demonstrated high correlation to actual body mass. However, in combination with *WTL* the relationship was even stronger. In two-dimensional harbor seal PGA models, variables with the strongest correlation to body mass were derived from *End girth* and *SH*.

Two of the four fundamental PGA variables in this study, *End girth* and *WTL,* have been noted in PGA body mass estimation literature as being among the most valuable, thus their importance in this study was to be expected (McFadden et al. 2006; Bell et al. 1997; Trites et al. 1998). In this study all models lacking *End girth* component counterparts demonstrated weaker correlations (harbor seal models 3, 4, 7 from Table 2 and grey seal models 4 - 6 from Table 4). McFadden et al. (2006) found girth perimeter

(*GP)* (similar to *End girth* in this study) to be among the most useful variables for building juvenile Monk seal (*Monachus schauinslandi*) PGA models. They also found lateral perimeter (*LP*) (similar to *SP* in this study) to be among the most useful, whereas this study did not. Bell et al. (1997) suggested that a head-on photograph would improve body mass estimation of adult phocid seals in general. They incorporated both head-on photographs and side photographs into their mass estimation models of Southern elephant seals. Ireland et al. (2006) agreed with this assessment while estimating body masses of mother and pup Weddell seals (*Leptonychotes weddellii*), using a more photographically robust approach than Bell et al. (1997), adding that it would be useful if animals' spines remained "un-curved" and good quality photographs were used.

Trites et al. (1998) stressed the importance of assessing PGA length (*WTL*) to estimate body mass and that this procedure may be applied to not only pinnipeds, but also cetaceans (Mysticetes and Odontocetes). Models in this study demonstrated that *WTL* was also useful in the creation of *Total Area* and *TVEG* in grey seal three-dimensional PGA models. In contrast, *WTL* was not a valuable variable in harbor seal models. This may have been due to the harbor seal *WTL* variable demonstrating 12.12 ± 35.64% difference from the measured standard length. This was mostly created by two seals' photographs, revealing inadequately estimated PGA measurements. Removed from the model, the percent (%) difference dropped to 9.99 ± 10.73%.

The variable, *SH*, is not an entirely new variable. Bell et al. (1997) named this variable H3 and used it to estimate the body mass of adult elephant seals. It was later used by Ireland et al. (2006) throughout their study and proved to be an important variable in estimating the mass of Weddell mothers and pups. Ireland et al. (2006) also had a variable dubbed H5 that was measured in a similar location as *DH* (60% of the *WTL* from the snout towards the hind flippers). However, *DH* was officially created by Ryg et al. (1990) who found this location to be most varying in blubber thickness of Ringed seals (*Phoca hispida*) and used it in estimating blubber content, as percent of body mass, in their study.

Further analysis of individual harbor seal three-dimensional models revealed no individual PGA variable to be highly significant in three-dimensional model building. The lack of significant variables for model building may have been due to the large differences between PGA and physically measured variables (girth and length). This difference was most likely created by the small size of the seals used and the fact that PGA measurements become increasingly more difficult to measure, and lose accuracy, as subjects become smaller and there are fewer pixels within each measurement to work with (N. Bruyn, *pers comm.*). The best grey seal models, models 3 and 4 (Table 4) revealed significant influence of certain PGA variables in model building. These variables were most likely useful in model building because grey seal PGA variables did not demonstrate large differences from physically measured variables.

Comparison of Models

Models created from physically derived measurements have been the most accurate predictors of body mass in previous phocid studies (Bell et al. 1997; Castellini & Calkins 1993). In this study, grey seal physical models where cross validation (CRV) could be performed (Table 5), demonstrated accuracy of mass prediction within an average of 5.434 ± 4.618% of actual body mass. However, sample sizes for remaining models were too small to perform CRV, therefore to validate the accuracy of our strongest PGA models we compared them with physical models. Three-dimensional PGA models' rivaled prediction strength of physical models in several instances. For example, in grey seal models, three-dimensional PGA model 3 (Table 4) Adjusted r^2 results, CI's and significance were comparable to the most accurate physical model (male model) 3 (Table 5). Harbor seal PGA model 5 (Table 2) demonstrated an Adjusted r^2 and *p*-value close to that of physical model 1 (Table 3). A comparison of these data collection techniques shows that while physical models created from physical measurements have high accuracy in predicting body mass, PGA methods created from data collected non-intrusively can be just as effective.

PGA Methodology

Photographing each seal within this study took only 5 to 7 minutes in the field, and one photographer. In the lab it took 5 minutes to analyze each photograph. In contrast, collecting physical measurements took 10 to 15 minutes in the field, and required three handlers. Bruyn et al. (2009) demonstrated that not only are PGA techniques efficient in data collection but they also minimize time spent maneuvering equipment in treacherous and isolated rocky terrain. The equipment we used in this project may be used in isolated areas as well, because it is easy for one person to handle and carry.

The use of two computer programs to analyze photographs made processing photographs within this study difficult and time consuming. Although Image J (a free program) and Matlab are reasonably priced, analyzing photographs in one expensive program, such as PhotoModeler, has its advantages. The ability to move data within one program, versus transporting it from program to program, decreases the risk of incorrectly translating and losing data. Data was not lost in this study, however the potential for loss of data was present when it was transferred from program to program. Therefore, use of a program where data does not have to be transported and may be analyzed within one program alone, such as PhotoModeler, should not be overlooked, if it can be afforded. Also a Matlab interface for marking points on an image may be created in coordination with the calibration toolbox in future studies. If researchers use Matlab for camera calibration then setting the camera at the same angle to the ground throughout photography and at the same distance to a study subject is advisable due to the amount of calibration images and analyses required for correct calibration in the program. The amount of flexibility to do so however is dependent of the mobility of the study subject. Ultimately, a photographer must devise a strategy that will limit camera calibrations for their project, which will ensure their calibration consistency.

The greatest error in photography occurred at larger angles between 130 and 150°. This was most likely due to an inability of the person measuring the photograph to correctly view the animal's measurements at such angles, influencing calibrations. At these angles either the *End girth* in head-on view photographs could not be measured correctly or the *WTL* could not be determined from side view photographs. During the calibration procedure assistants held the calibration boards above the ground near the center of the image, not on the floor where seals were located during photography. Holding the calibration board so that it took up the majority of the field of view of the camera was best achieved if the calibration board was held at or near the center of the image. In camera calibration the entire field of view must be analyzed in order to correctly calibrate the camera. Therefore, having a calibration target take up the entire field of view allows for the peripheries and center of the image (the principal point, which is a parameter necessary for camera calibration) to be properly analyzed in the image plane (Clarke et al. 1998).

Unfortunately, successfully using stereo-pair images to predict body mass at distances greater than those used in this study was not possible with our set up. The calibration methodology functioned well if both cameras were set at the same focal length during photography and calibration. However, body mass predictions were drastically inaccurate. A probable source of inaccuracy was the distance both cameras were placed apart on the aluminum bar. Hall-Martin & Ruther (1979) demonstrated the ability to take stereo-pair images of elephants at greater distances (5-30m) than those presented in this study. The distance between cameras was four times the length of the one used for this study. If a wider baseline in triangulation between two cameras was used within this study then the ability to mathematically predict the triangulated coordinates at greater distances would have improved.

Conclusions

Application of the PGA and physical models built in this study will aid in increasing the pace, safety and effectiveness of data collection in the field in future studies of the grey seal colony on Muskeget Island, MA. The stereo-PGA technique was most useful in estimating pup body mass of juvenile grey seals and harbor seals, which corroborates other findings in stereo-PGA works of highly mobile marine mammals (Brager & Chong 1999). However, a better two-dimensional PGA grey seal model may have been achievable if there had been better quality photographs taken and better data. The use of a camera (Samsung Digimax S500) that could not be properly calibrated negatively influenced results as well. This was demonstrated through the creation of harbor seal two-dimensional models with greater predictive abilities from type 1 photographs, taken using Olympus Cameras (SP 590 UZ).

This study also demonstrated that distance PGA is applicable to mobile animals that are hard to capture and may be located in inaccessible study sites. Two-dimensional mass estimation techniques developed from this study demonstrated reasonable accuracy at 18-34 meters using a fixed focal length of 119.6mm on an Olympus SP 590 UZ camera (the largest focal length for this model camera). Further use of this method will significantly decrease the amount of disturbance to grey seal breeding colonies and mother-pup bonds.

There are many applications of these methods in circumstances when handling time and disturbance to focal animals should be minimized. Rehabilitating captive harbor seals, such as those in the MARC, can be easily monitored with minimal human contact. Scientists can also study adult and juvenile grey seals during the breeding season using these methods and experience increased safety for both researchers and seals. Applying these methods to a study of healthy juvenile harbor seals in the field would improve harbor seal PGA models within this study. Additional studies could also increase the number of two-dimensional quality photographs in the current data base of juvenile grey seal close-range and distance photographs. Three-dimensional distance application can be improved upon through use of a larger stereo-photography system.

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PERMITS

UNE UNIVERSITY OF NEW ENGLAND

Institutional Animal Care and Use Committee
Renee LeClair, Chair

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IACUC Protocol Number: 20101221HAR

TO: Jennifer Harris, B.S.
Kathryn Ono, Ph.D., Faculty Advisor

FROM: Renee LeClair, Ph.D.

DATE: December 21, 2010

RE: Protocol Approval

IACUC Review - APPROVAL

Your protocol entitled "Does male harassment influence maternal success in grey seals (Halichoerus grypus)" has been reviewed by the UNE Institutional Animal Care and Use Committee (IACUC). Your project has been approved with the following conditions:

1. You are approved to conduct this research only during the period of approval cited below.
2. You will conduct the research according to the plan and protocol you submitted.
3. You will immediately inform the IACUC of any injuries or near injuries to researchers or animal handlers that occur in the course of your animal care or use.
4. You will immediately inform the IACUC of any adverse events that arise in the course of your research including but not limited to animal illness or unexpected animal death.
5. You will immediately request approval from the IACUC for any proposed changes in your research. You will not initiate any changes until they have been reviewed and approved by the IACUC.
6. If your research is anticipated to continue after **12/20/2011**, you must submit a continuing review form at least 30 days prior to this date. A complete *de novo* review is required on a triennial basis at least 60 days prior to the expiration date of **12/20/2013**.

7. You are reminded that the IACUC requires animals that would otherwise experience severe or chronic pain or distress that cannot be relieved will be painlessly killed at the end of the procedure or, if appropriate, during the procedure.
8. You will follow all IACUC approved euthanasia procedures.
9. You will follow all IACUC approved procedures for the disposal of carcasses.
10. You will notify the IACUC if you terminate the study before completing it, or upon concluding it.

General Safety Requirements:

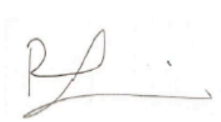
1. Accidents, injuries or illness resulting from the use of toxic, biological, or radioactive substances must be reported to the IACUC and the UNE Environmental Health and Safety department immediately.
2. Any injuries or near injuries to researchers or animal handlers that occur in the course of your animal care or use must also be immediately reported to the IACUC.
3. Appropriate protective equipment and procedures for use and handling of toxic, biological, or radioactive substances must be maintained at all times.
4. Appropriate ABSL's and/or BSL's will be maintained at all times, including the use of appropriate biosafety cabinets.

The University appreciates your efforts to conduct research in compliance with the federal and state regulations that have been established to ensure the protection of animal subjects in research, teaching and testing.

The IACUC wishes you well with your research. Please feel free to contact Jennifer Hutchinson, Research Compliance Specialist, if you have any questions about the IACUC process or continuing review procedures at 602-2244, or by email at jhutchinson2@une.edu

Approval Period: 12/21/2010-12/20/2013
Continuing Review required before: 12/20/2011
Complete *de novo* Review required before: 12/20/2013
Funding Source: UNE's Graduate Research Fund, Lerner-Gray Memorial Fund Committee

Sincerely,



Renee LeClair, Ph.D.
IACUC Chair