Ageing Whales from Measurements of Specific Fatty Acids Present in their Outer-blubber Biopsy Tissues

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Background

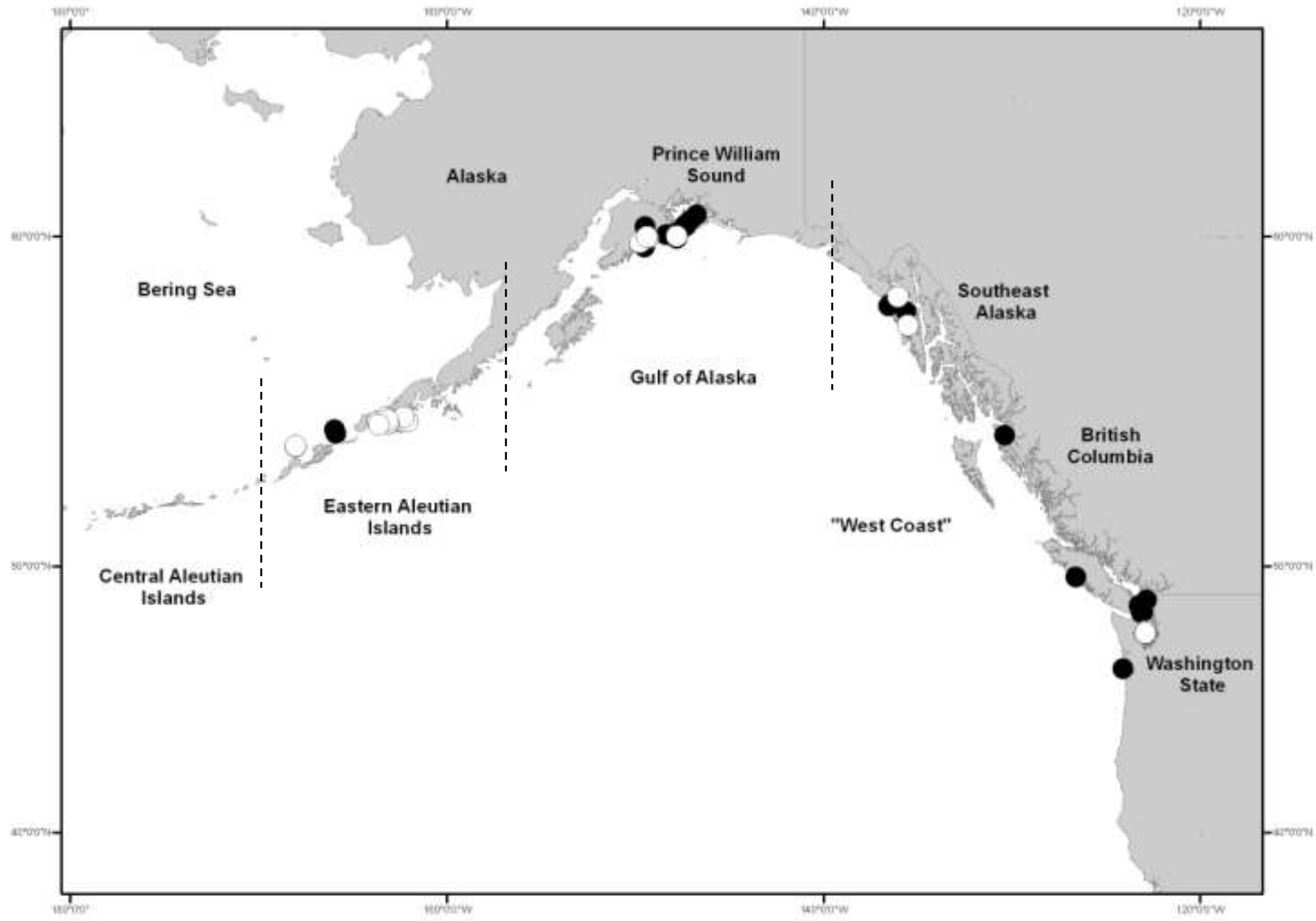
- Ageing of cetaceans is needed to determine
  - Population status
  - Long-term viability
  - Explore a wide range of age-related effects

- Existing methods for ageing cetaceans
  - Necropsy tissues from by-caught, subsistence, stranded whales provide teeth/baleen/ear plugs for growth layer analyses
  - Racemization of amino acids—eye lens tissues
  - Photo-ID—long-term studies difficult to maintain
  - Telomeres (DNA)—skin biopsies (in development)

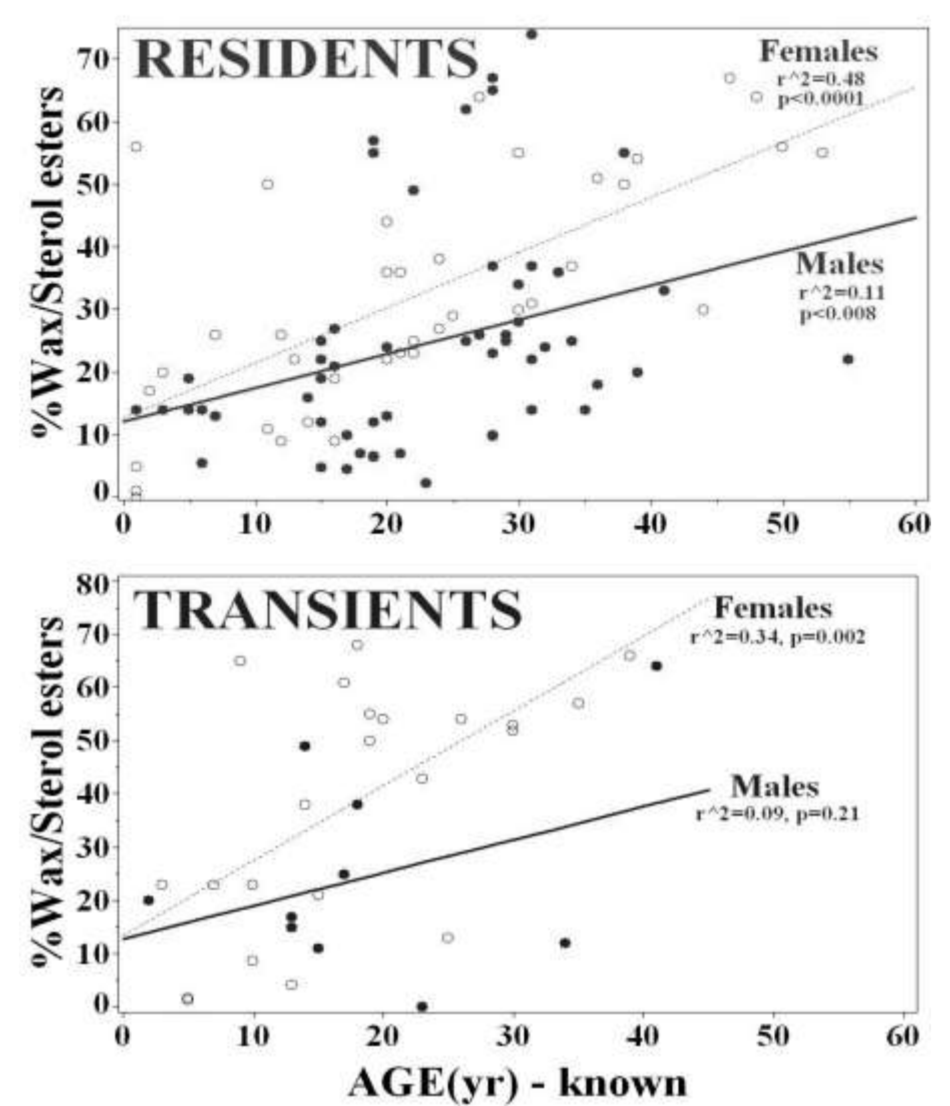
- Age determination from fatty acid ratios in outer-blubber biopsy samples (in Herman et al. 2008;2009)
  - Viable and non-lethal

Sampling locations of known-age killer whales

(mammal-eating transients and fish-eating residents)



Correlation of %wax/sterol esters with age for ENP resident & transient killer whales

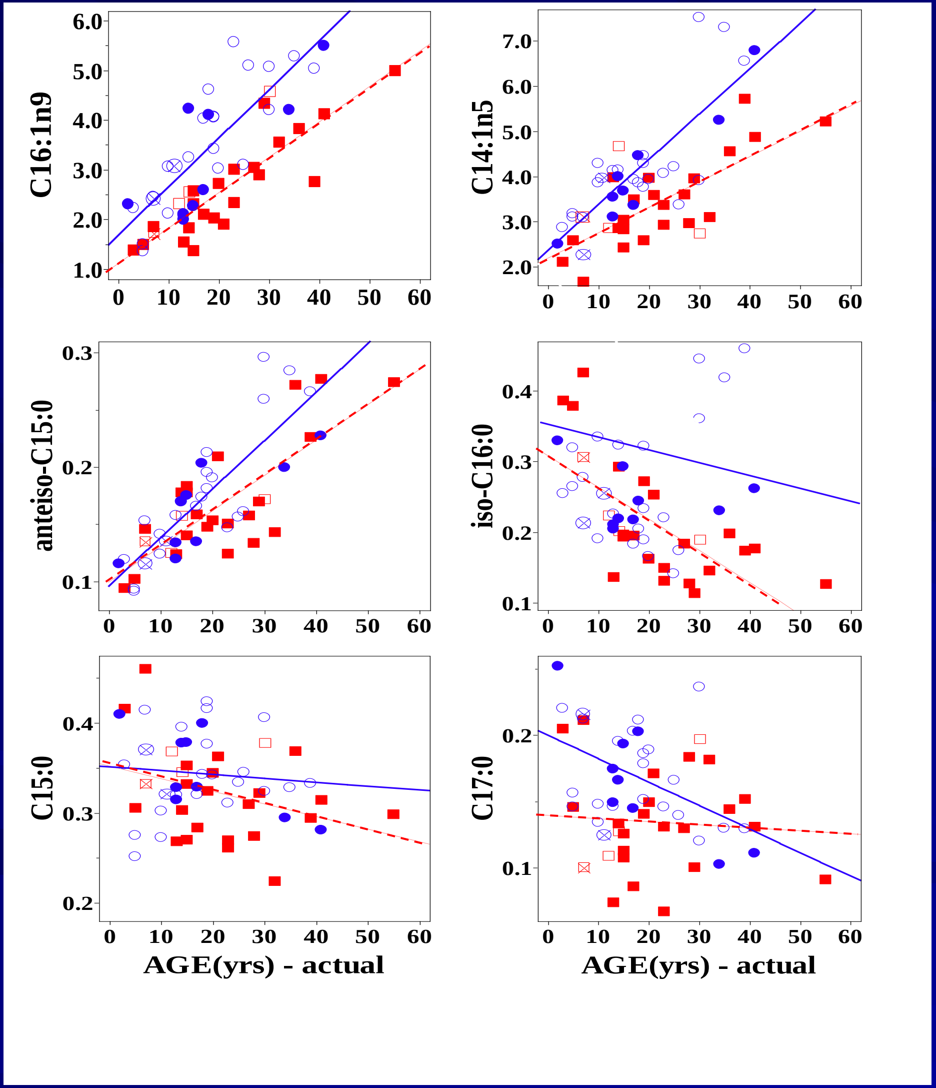


All whales biopsy sampled between 1994 and 2006.

Females (open circles); males (closed circles).

Female residents (n=38; male residents (n=52); female transients (n=21); male transients (n=10).

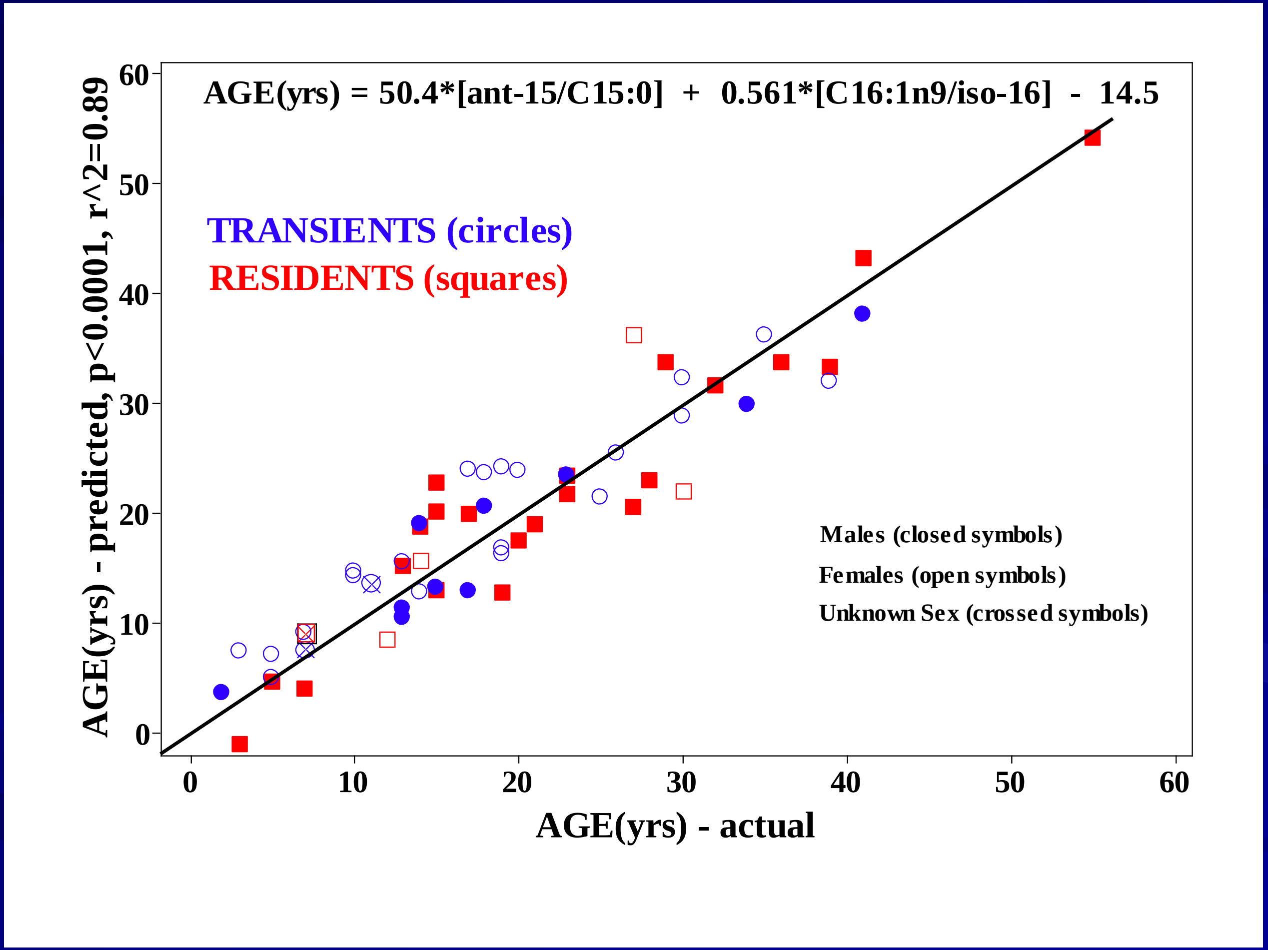
Correlation of individual fatty acids with age for ENP killer whales



Correlation of individual short-chain mono-unsaturated-(*top*), branched-(*middle*), and odd-chain (*bottom*) fatty acids (wt% composition) with known age (yrs) for ENP resident (red, n=27) and transient (blue, n=32) killer whales. Males (closed symbols); females (open symbols)

NOTE: No single fatty acid alone will predict the ages of both transient and resident KWs from a single equation

Linear relationship between known killer whale ages and ages predicted via the FA ratio model



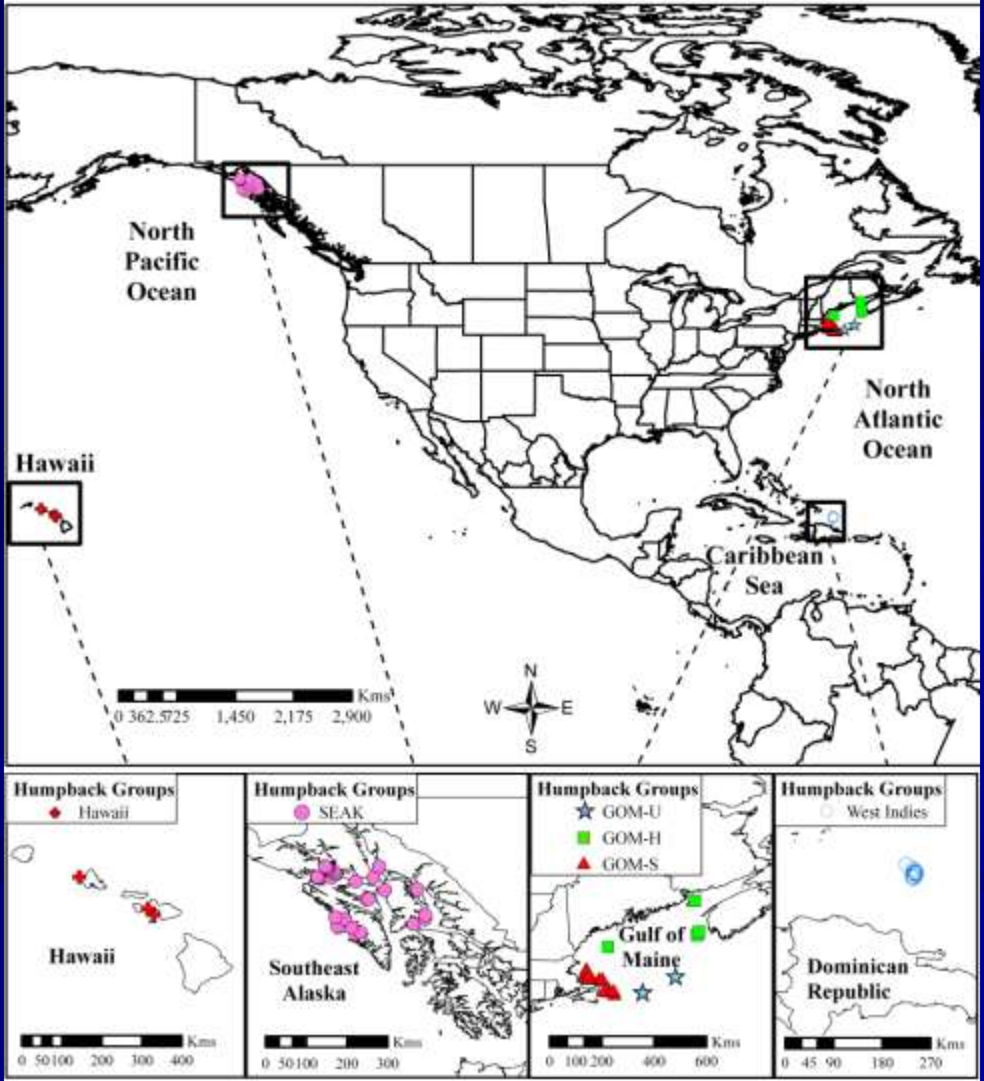
Linear relationship ($p<0.0001;r^2=0.89$) between the actual, known ages of 59 resident and transient killer whales (*Orcinus orca)* and the ages predicted from their outer blubber fatty acid compositions using the KW(age)- FA model (Equation top of figure)

Precision is ± 3.8 years.

Conclusions—killer whale ageing using endogenous fatty acids

- Empirical killer whale age-FA model developed
  - Robust – independent of ecotype, sex, geographical range and diet
  - Predicts ages with good precision (s ± 3.8 yrs)
- Can be used to predict age distributions in ENP killer whale populations where ages are unknown
- No clear understanding of underlying biological mechanisms at present
- Results published in Marine Ecology Progress Series; Herman et al. 2008, MEPS 372:289-302

North Atlantic and North Pacific sites for biopsy sampling of 70 humpback whales



Blubber biopsy samples from "known-age" humpback whales were collected from:

- *Gulf of Maine*

Feeding grounds: n=28

- *West Indies*

non-feeding/breeding: n= 11

-*Southeast Alaska*

Feeding grounds: n=28

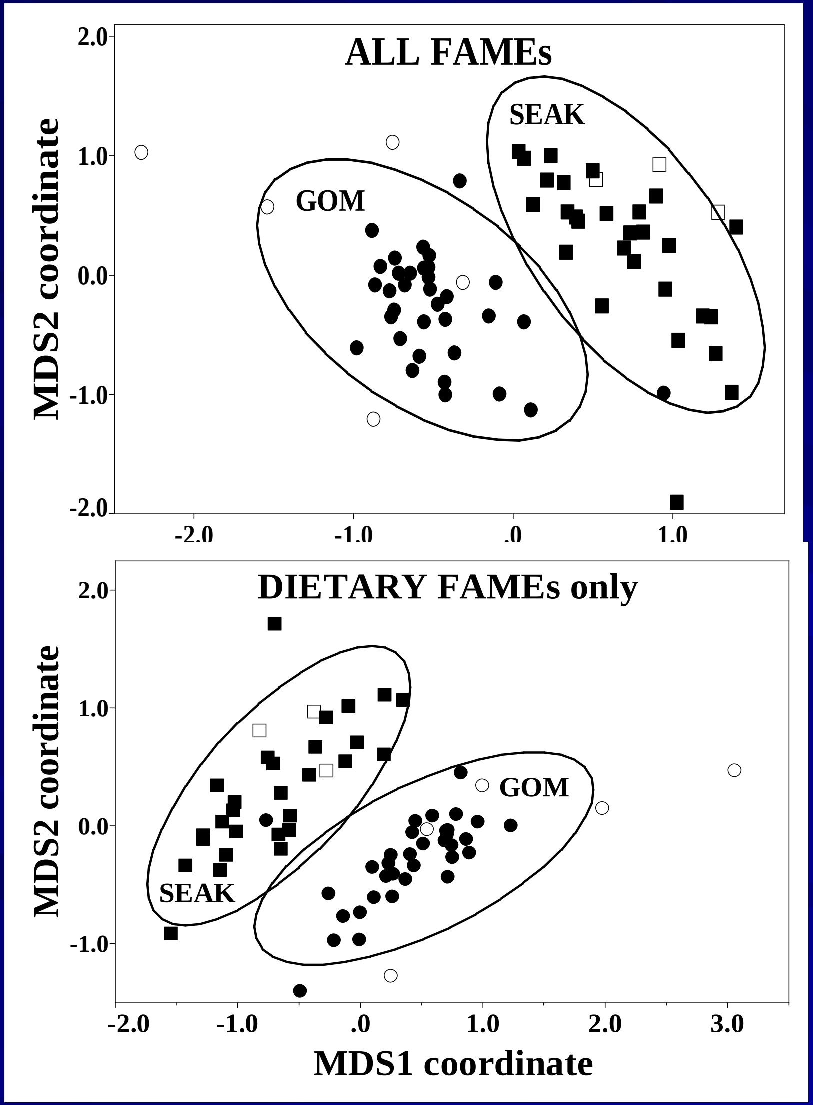
- Hawaii

non-feeding/breeding: n=3

Samples included both sexes, wide age range (0-40+), different diets, feeding status.

Ages: "exact" vs "minimum" based on photo ID

Multidimensional Scaling Analysis of fatty acids from GOM and SEAK humpback whales



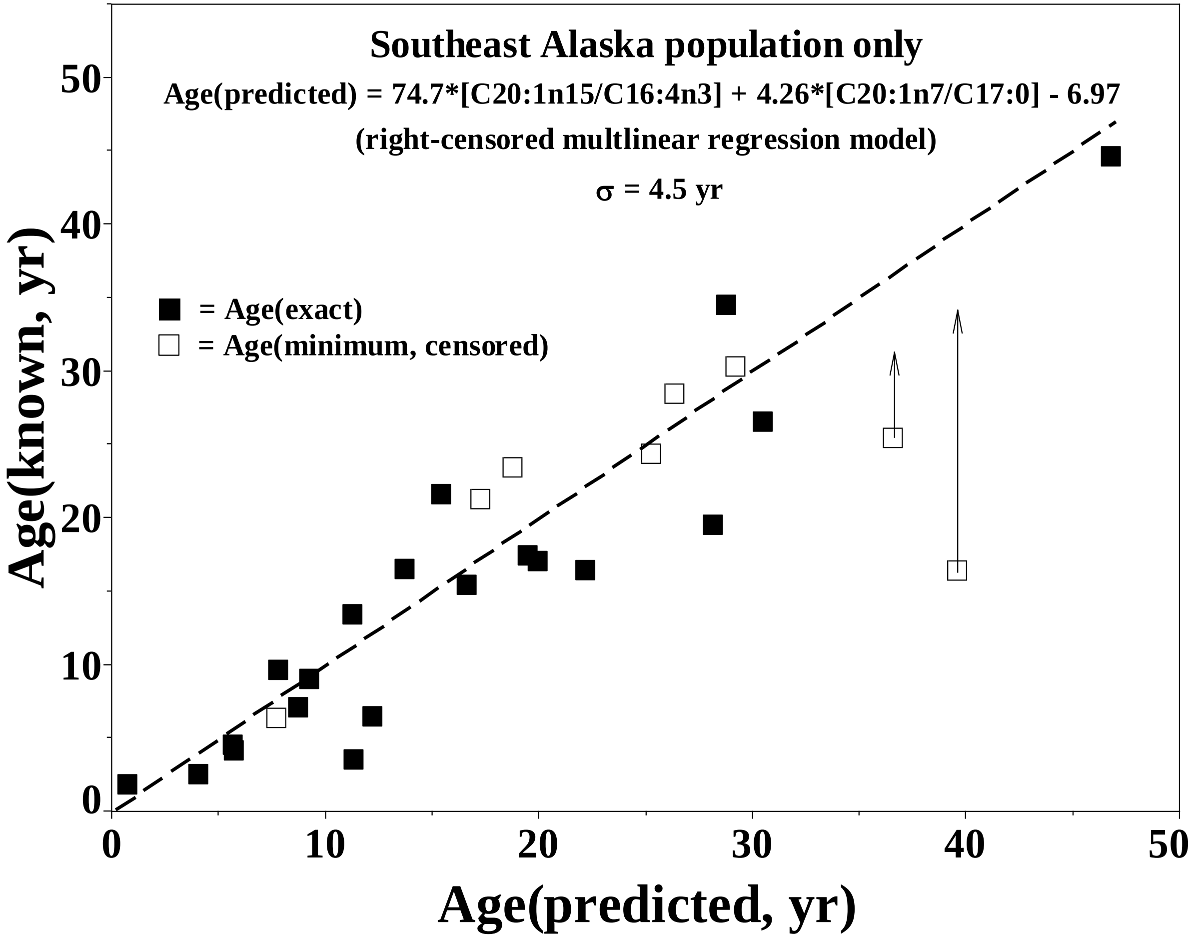
Multidimensional Scaling Analysis (MDS) of the outer-blubber FA compositions of the GOM and SEAK humpback whales.
Upper panel: all 64 quantifiable FAs (dietary and non-dietary)

Lower panel: FA predominantly dietary in origin.

Shows that diets of the two populations are different.

Outliers (open symbols) tend to be young animals (still nursing), but those are easily aged.

Linear relationship between photo-ID ages (exact and minimum) and ages predicted from FA compositions for 28 SEAK humpback whales

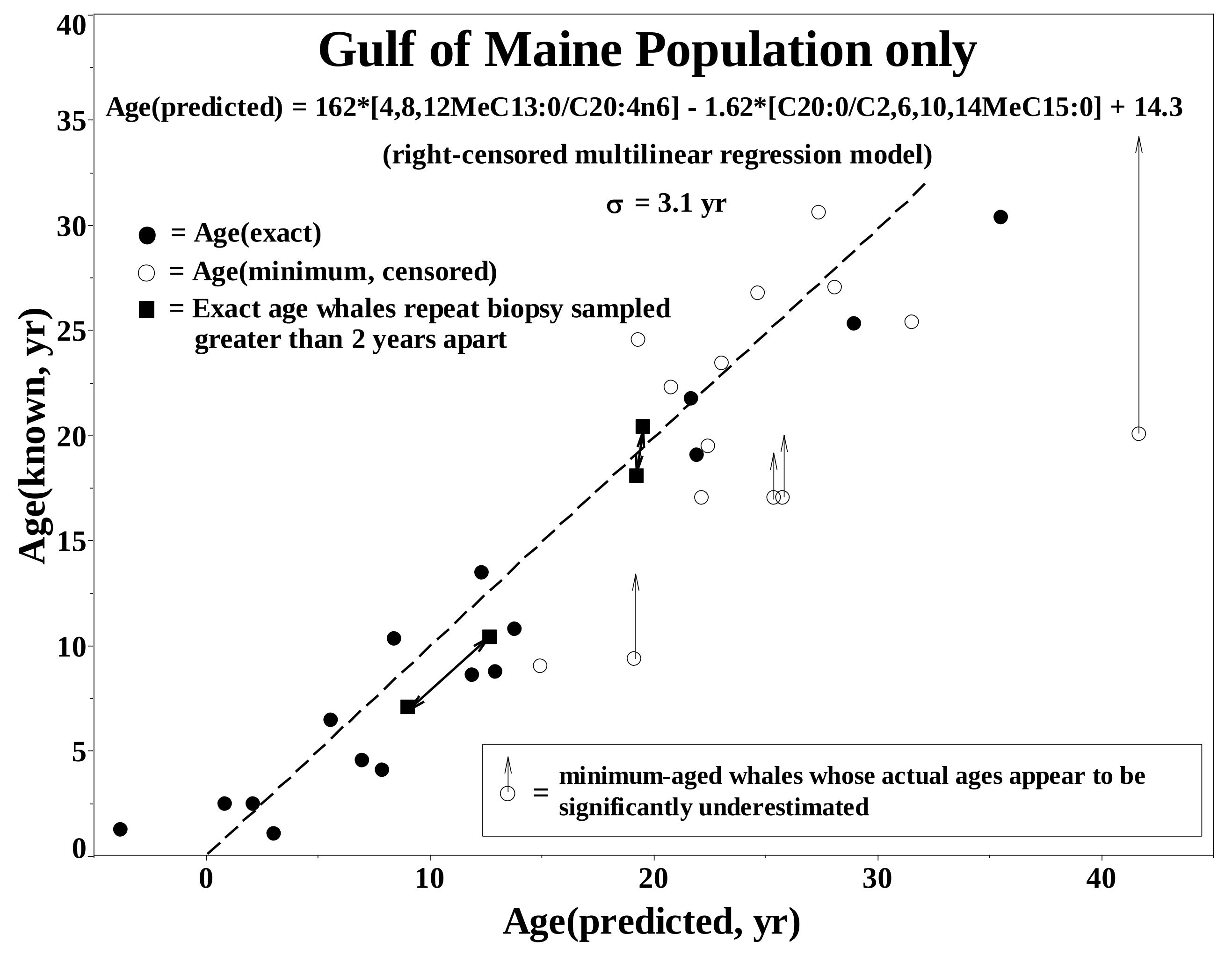


This FA-age model was derived using FA data from both exact and minimum-aged whales from SEAK population, employing the Tobit right-censored multilinear regression.

Objective was to determine whether precision is better with a single population.

Precision is ± 4.5 years.

Linear relationship between photo-ID ages (exact and minimum) and ages predicted from FA compositions for 34 GOM humpback whales

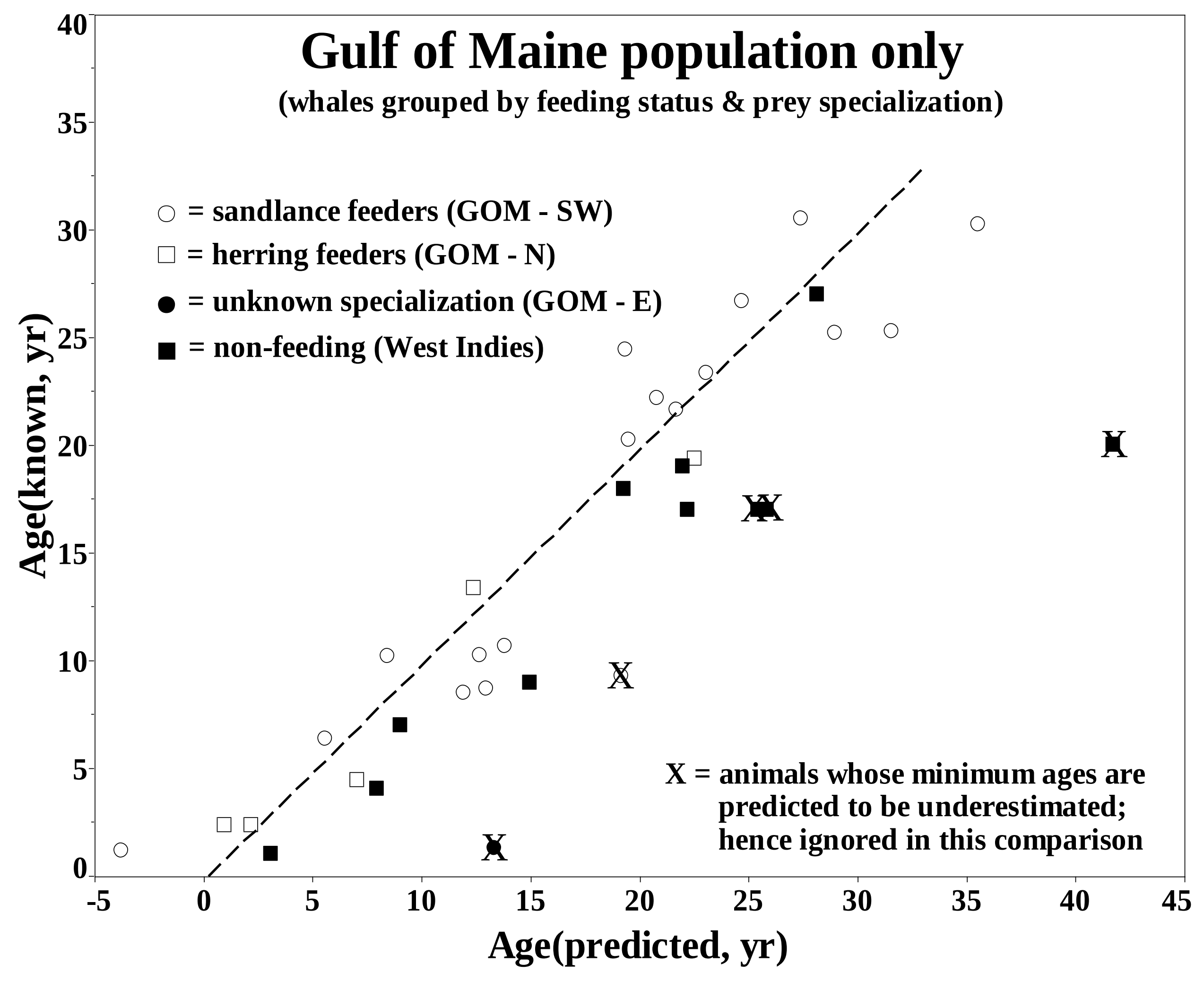


This FA-age model was derived using the FA/age data from both exact and minimum-aged whales from GOM, employing the Tobit right-censored multilinear regression.

Points below the line — probably much older than minimum.

Precision is ± 3.1 years. Two exact-aged whales were repeat biopsy sampled at >2 year intervals.

GOM humpback whales grouped by presumed location-dependent prey preferences & feeding status

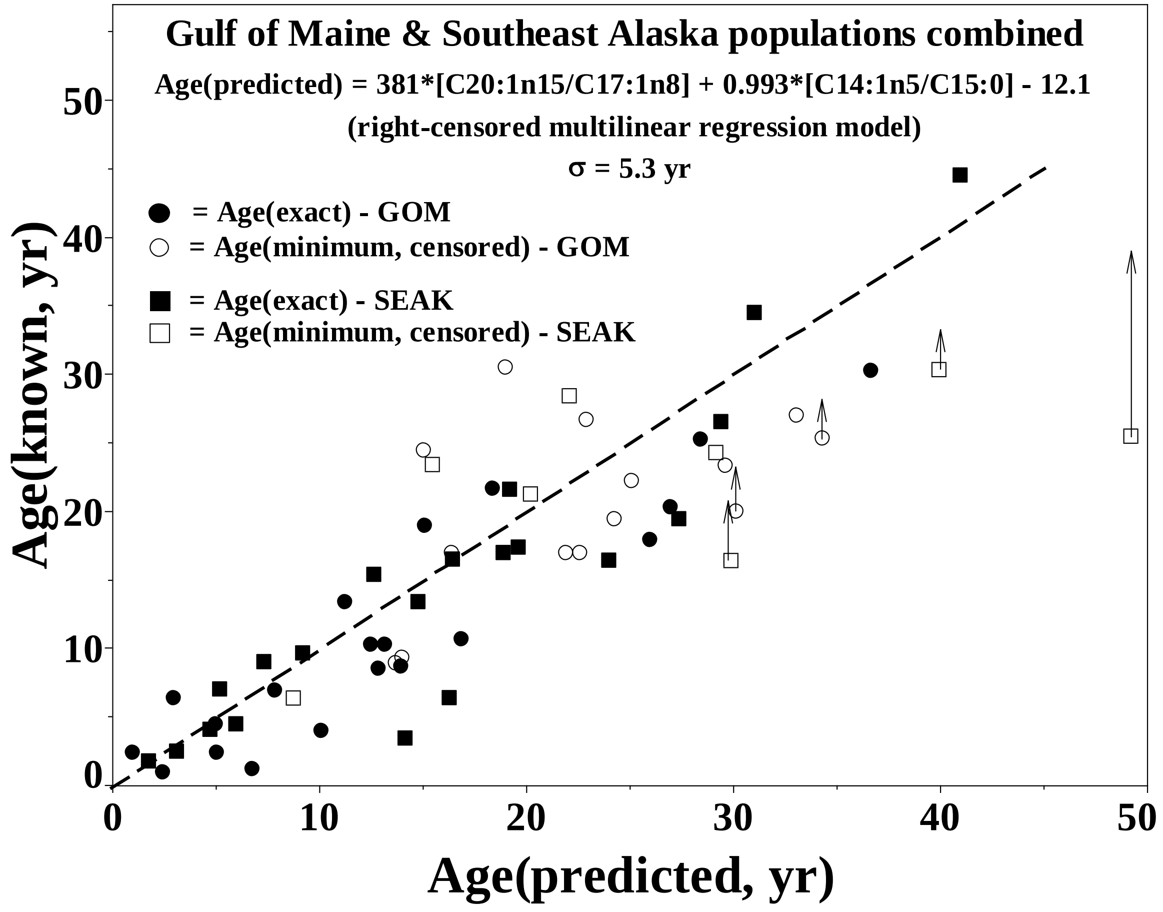


Same as previous model.

Presumed sandlance feeders (*open circles*); presumed herring feeders (*open squares*); unknown prey specialization (*closed circles*); non-feeders in the West Indies (*closed squares*).

Non-feeders fall along the same line, so model is moderately independent of feeding status.

Linear relationship between photo-ID ages (exact and minimum) and ages predicted from FA compositions of 62 GOM and SEAK humpback whales



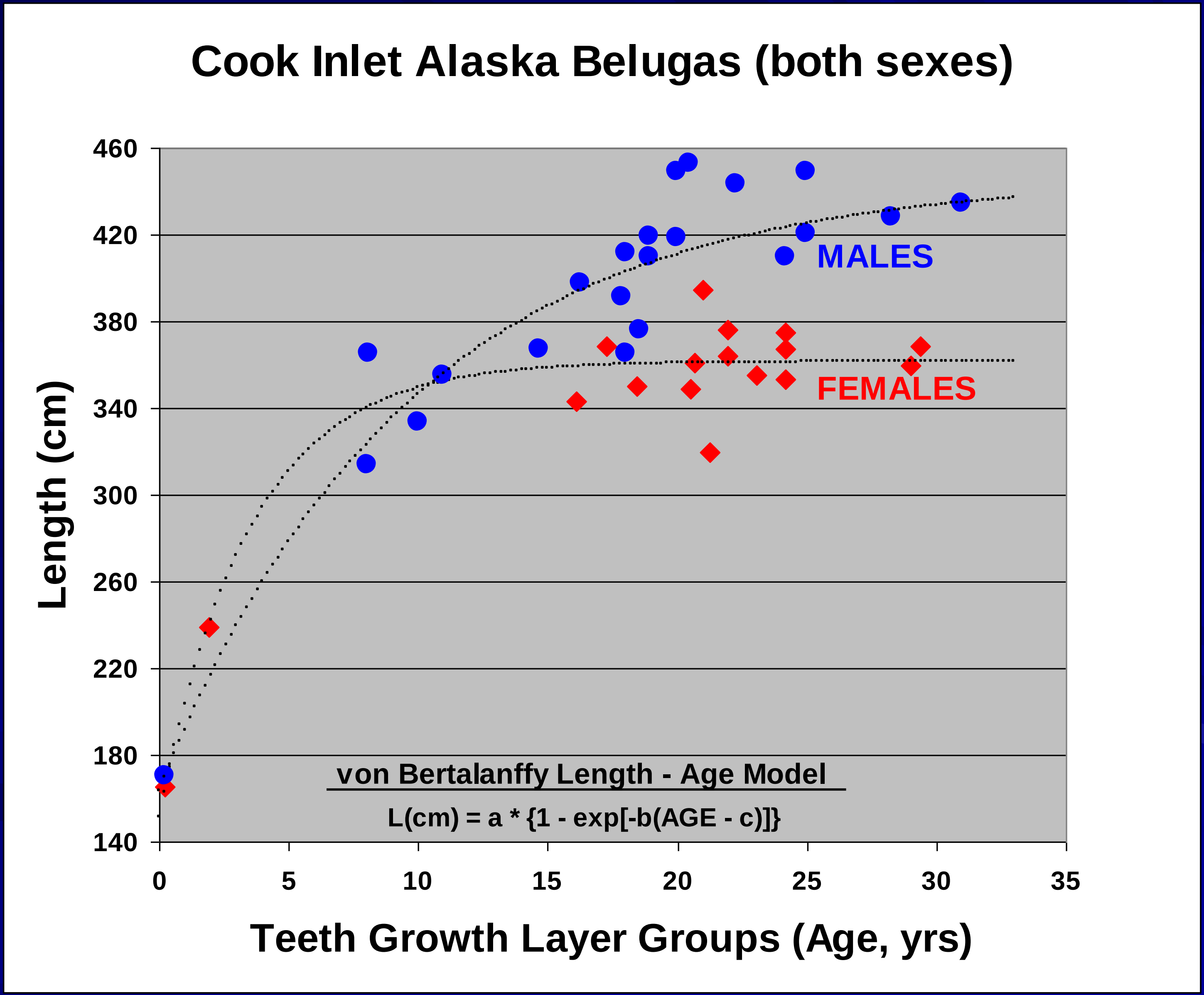
This FA-age model was derived using photo-ID ages (exact and minimum) to refine the previous model (by increasing sample size and age range). The Tobit right-censored multilinear regression procedure was used (less weight to minimum aged whales). Model is robust because it combines two different populations with different diets & age ranges.

Precision is ± 5.3 years.

Conclusions – humpback whales

- Empirical age/FA model developed for humpback whales
  - Based on “exact” and “minimum” age humpbacks
  - Robust model that allows ages to be predicted to within (s = ± 5.3 yrs), and is largely independent of sex, diet and feeding status
  - Age prediction uncertainties improved using single population models: (GOM only: s = ± 3.1 yrs; SEAK only: s = ± 4.5 yrs)
  - Humpback whale results published in Marine Ecology Progress Series; Herman et al. 2009, MEPS 392:277-293
- No clear understanding of underlying biological mechanisms at present
- In the future, replace minimum age data with known-age as known photo-ID whales age further
- This technique likely can be extended to other humpback populations

Allometric relationships between CI beluga body lengths and ages derived from teeth growth layer groups (Vos 2003)



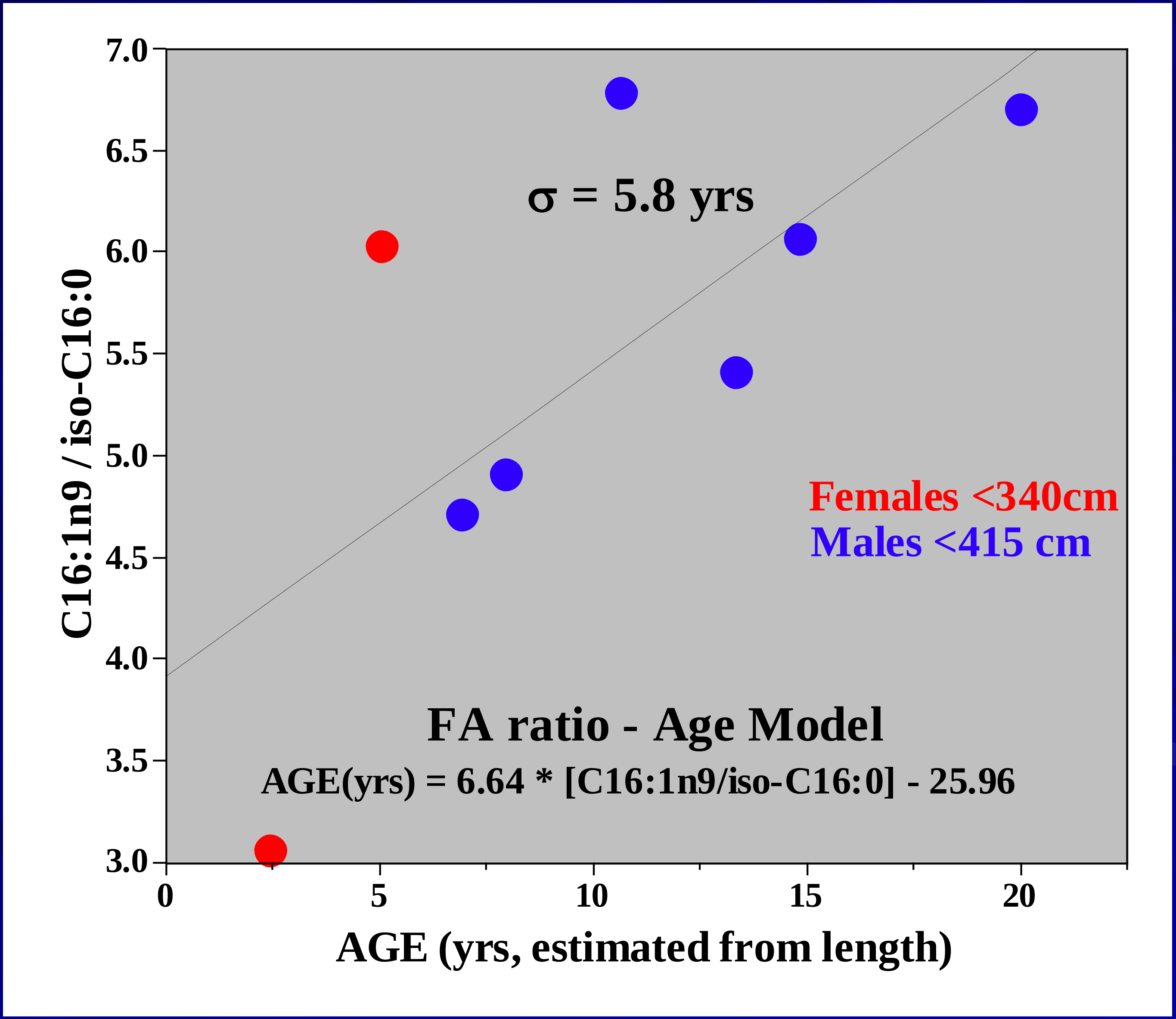
Summary of all Cook Inlet beluga outer-blubber blubber samples collected and analyzed for fatty acids as part of this study

Field ID#	Sex	Date Collected	Sample Type[a]	Body Length(cm)	Age(yrs)[b]
CI-01-01	**F**	8/10/2001	T(B)	**257**	**2.5**
CI-01-03	**F**	8/12/2001	T(B)	**312**	**5.1**
CI-01-04	F	8/13/2001	T(B)	340	*≥8*
CI-02-02	F	7/30/2002	T(B)	340	*≥8*
BLKA-073	F	7/28/2001	N	345	*≥8*
CI-01-05A	F	8/13/2001	T(B)	362	*≥8*
CI-02-03	F	7/31/2002	T(B)	366	*≥8*
692-BLKA-081	F	10/11/2006	N	370	*≥8*
CI-02-07	F	8/3/2002	T(B)	373	*≥8*
CI-02-04	F	8/1/2002	T(B)	378	*≥8*
CI-01-06	F	8/15/2001	T(B)	401	*≥8*
Birchwood K Arm	**M**	9/2/2005	N	**310**	**7.0**
CI-01-02	**M**	8/11/2001	T(B)	**323**	**8.0**
CI-02-06	**M**	8/3/2002	T(B)	**353**	**10.7**
CI-02-08	**M**	8/4/2002	T(B)	**376**	**13.4**
CI-02-05	**M**	8/2/2002	T(B)	**386**	**14.9**
CI-02-01	**M**	7/29/2002	T(B)	**411**	**20.1**
BM-BLKA-07-01	M	10/7/2007	N	423	*≥21*
DL061307	M	6/13/2007	N	430	*≥21*
692-BLKA-080	M	7/24/2005	N	439	*≥21*
CI-01-07A	M	8/20/2001	T(B)	442	*≥21*
BLKA-076	M	7/25/2002	N	457	*≥21*

a) T(B) = Trocar(Biopsy) samples of live capture and release whales; N = Necropsies of deceased/stranded whales.

b) Ages estimated from allometric body length vs teeth growth layer groups desribed in Vos (2003); assumes 1 GLG/yr
Ages shown in italics are minimum age estimates for females ≥340 cm and males ≥415 cm

Relationship between the single FA ratio that exhibited the best correlation with age for physically immature CI belugas



FA ratio - Age Model

AGE(yrs) = 6.64 * [C16:1n9/iso-C16:0] - 25.96

Predicting the ages of physically mature (adult) belugas from the FA(ratio)-age Model

Animal ID	Sex	Body Length (cm)	C16:1n9/iso-16	**Age(yrs)**[a] (est. from length)	**Age(yrs)**[b] (est. from FA ratio)
CI-01-01	F	257	3.06	**2.5**	**-5.7**
CI-01-03	F	312	6.03	**5.1**	**14.0**
CI-01-04	F	340	7.89	*≥8*	*26.4*
CI-02-02	F	340	7.32	*≥8*	*22.6*
BLKA-073	F	345	5.16	*≥8*	*8.3*
CI-01-05A	F	362	8.52	*≥8*	*30.6*
CI-02-03	F	366	10.27	*≥8*	*42.2*
692-BLKA-081	F	370	7.21	*≥8*	*21.9*
CI-02-07	F	373	7.22	*≥8*	*22.0*
CI-02-04	F	378	15.39	*≥8*	*76.2*
CI-01-06	F	401	6.49	*≥8*	*17.1*
Birchwood K Arm	M	310	4.71	**7.0**	**5.2**
CI-01-02	M	323	4.90	**8.0**	**6.5**
CI-02-06	M	353	6.78	**10.7**	**19.0**
CI-02-08	M	376	5.41	**13.4**	**9.9**
CI-02-05	M	386	6.06	**14.9**	**14.2**
CI-02-01	M	411	6.70	**20.1**	**18.5**
BM-BLKA-07-01	M	423	11.24	*≥21*	*48.6*
DL061307	M	430	6.64	*≥21*	*18.1*
692-BLKA-080	M	439	11.73	*≥21*	*51.9*
CI-01-07A	M	442	8.91	*≥21*	*33.1*
BLKA-076	M	457	7.74	*≥21*	*25.4*

a) Ages estimated from allometric body length vs teeth growth layer groups desribed in Vos (2003); assumes 1 GLG/yr

b) Ages predicted from FA ratio model; Age = 6.64 * [C16:1n9 / iso-C16:0] - 25.96

Conclusions – beluga whales

- Preliminary empirical age/FA model developed for beluga whales
  - Derived from allometric body length vs age relationships described by Vos (2003)
  - Based on the ratio of a single pair of blubber fatty acids; C16:1n9/iso-C16:0
  - Allows ages to be predicted to within ($\sigma = \pm$ 5.8 yrs) for juvenile/sub-adult belugas and appears to be independent of sex
  - Appears to be contiguous thus enabling the ages of physically mature adult belugas to also be estimated

- We anticipate that age prediction uncertainties will be substantially reduced when biopsy samples from animals of exact known age are acquired and their blubber fatty acid compositions fit to a linear combination of two FA ratios, similar to the KW and HW models

- Again, there is no clear understanding of the underlying biological mechanisms responsible for the beluga age/FA relationship

- This technique likely can be extended to many other cetacean species (e.g., we also have some preliminary FA data to suggest that this technique may allow the ages of sperm whales (Bahamas) to be similarly determined (*data not shown*)

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- Beluga samples
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