Sclerochronology Laboratory

Fisheries & Oceans Canada
Pacific Biological Station

Sclerochronology Laboratory

- is the study of physical and chemical variations in the accretionary hard tissues of invertebrates and fish
- focusing primarily upon growth patterns reflecting daily, seasonal and annual increments of time

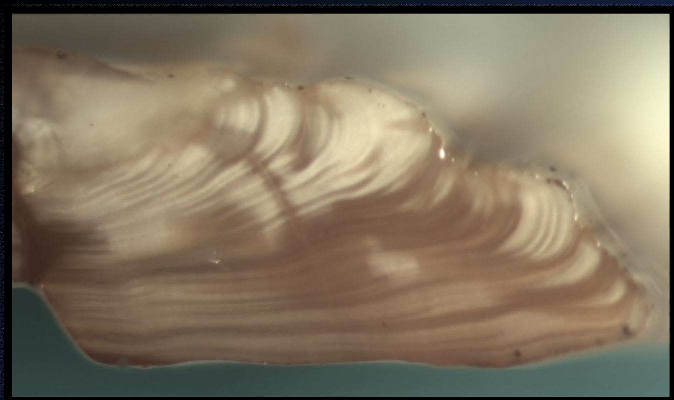
Getting the Biggest Otolith Bang For Your Buck

- Pattern recognition; its how we age fish
- Bias in fish age estimation
- How do we know we aged them correctly?
- Climate change; the effect on fish ageing
- Climate change; new species to Canada's EEZ
- The SCL meeting the challenge of Climate Change
  - Computerizing workstations for Direct Data Entry
  - Otolith Weight
  - Otolith Images
  - FT-NIR Spectroscopy

Pattern recognition; its how we age fish

- Sclerochronologists do not simply count stuff, they interpret patterns.
- Each specimen’s growth pattern is evaluated based on the presence and location of the first year, juvenile stage, maturation transition, mature stage, and edge growth in the structure being examined.
- The presence of annuli in each of these growth regions can be vastly different in context.
- This requires reviewing written documentation, pulling previous years’ samples and reviewing the patterns associated with that species, the area of capture, and any unique characteristics associated with the parameters of that particular species’ growth.
- On average 5yrs. of training is required before new staff are contributing at an intermediate level with multiple species.
- Sclerochronologists are above all pattern recognizers; the by-product of this is an age estimate.

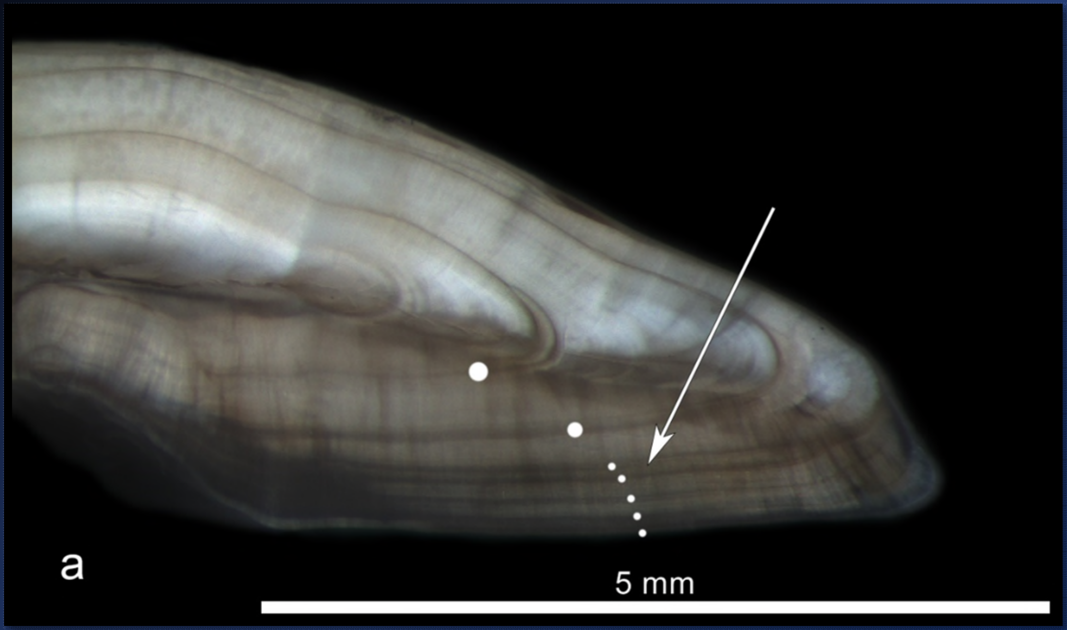
Pattern recognition; its how we age fish



Sablefish – how old?

Rougheye rockfish – how old?

Pattern recognition; its how we age fish



The 1982-83 El Niño Southern Oscillation (ENSO) event produced a natural tag on otoliths of the strong 1980-year class of the offshore stock in the form of a relatively small fourth annual growth zone. The tag established known-age fish and validated the burnt otolith section method for up to 12 years of age. The SCL subsequently extended the validation to 21 years by ageing annual samples till the 1980-year class aged out.

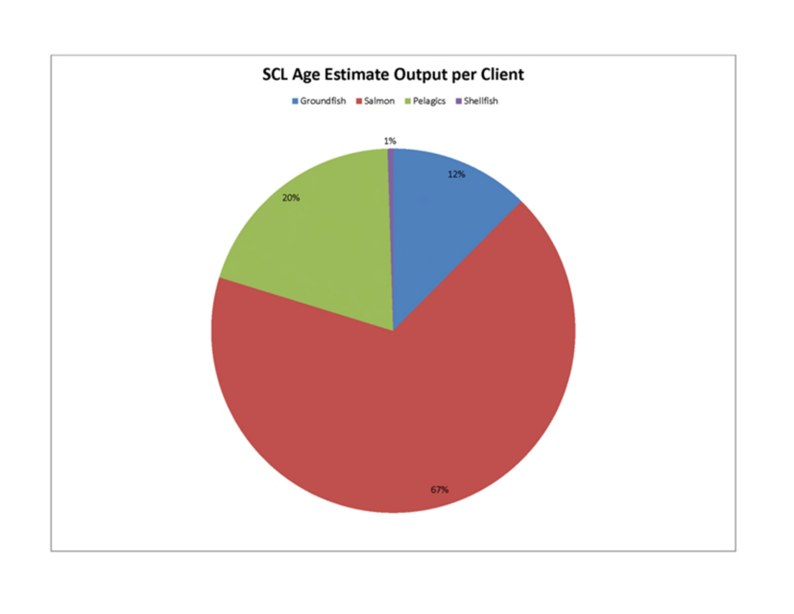
Maximum age of the most commonly aged non-research species

ROUNDFISH		
Lingcod	*Ophiodon elongatus*	21
Pacific cod	*Gadus macrocephalus*	8
Pacific hake	*Merluccius productus*	22
Sablefish	*Anoplopoma fimbria*	92
Walleye Pollock	*Theragra chalcogramma*	9
FLATFISH		
Arrowtooth flounder	*Astheresthes stomias*	26
Dover sole	*Microstomus pacifica*	57
English sole	*Parophrys vetulus*	22
Flathead sole	*Hippoglossoides elassodon*	15
Petrale sole	*Eopsetta jordani*	35
Rex sole	*Glyptocephalus zacharus*	15
Rock sole	*Lepidopsetta bilineata*	25
SALMON		
Chinook	*Oncorhynchus tshawytscha*	7
Sockeye	*Oncorhynchus nerka*	8
Coho	*Oncorhynchus kisutch*	4
Chum	*Oncorhynchus keta*	6
Pink	Oncorhynchus gorbuscha	3
HERRING		
Pacific Herring	*Clupea pallasii*	15

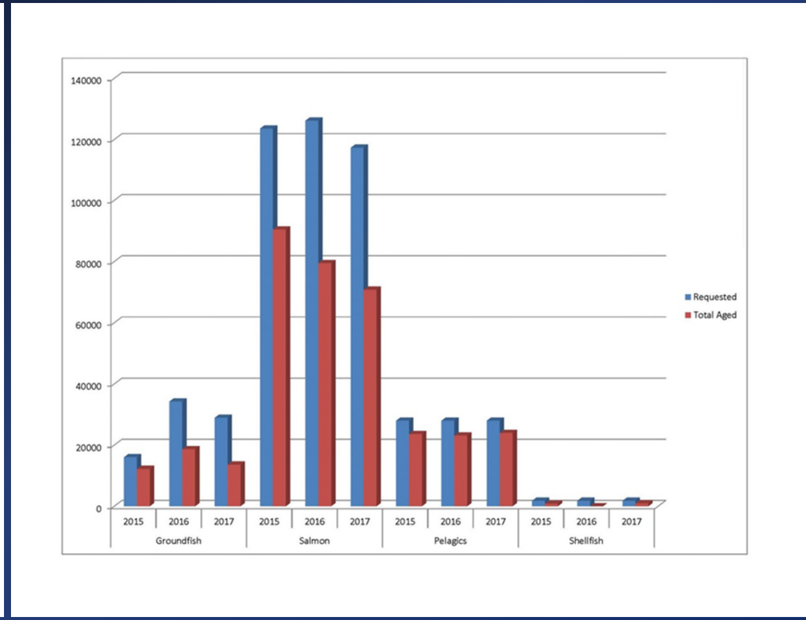
ROCKFISH		
Blackgill Rockfish	*Sebastes melanostomu*	64
Canary rockfish	*Sebastes pinniger*	84
China rockfish	*Sebastes nebulosus*	79
Copper rockfish	*Sebastes caurinus*	51
Darkblotched rockfish	*Sebastes crameri*	48
Harlequin rockfish	*Sebastes variegatus*	48
Pacific ocean perch	*Sebastes alutus*	100
Pygmy rockfish	*Sebastes wilsoni*	24
Quillback	*Sebastes maliger*	95
Redbanded rockfish	*Sebastes babcocki*	93
Redstripe rockfish	*Sebastes proriger*	55
Rougheye rockfish	*Sebastes aleutianus*	147 (205)
Sharpchin rockfish	*Sebastes zacentrus*	58
Shortraker rockfish	*Sebastes borealis*	120
Silvergrey rockfish	*Sebastes brevispinis*	81
Tiger rockfish	*Sebastes nigrocinctus*	69
Widow rockfish	*Sebastes entomelas*	60
Yelloweye rockfish	*Sebastes ruberrimus*	121
Yellowmouth rockfish	*Sebastes reedi*	99
Yellowtail rockfish	*Sebastes flavidus*	59
Vermillion rockfish	*Sebastes miniatus*	60
Shortspine thornyhead	*Sebastolobus alascanus*	50
Longspine thornyhead	*Sebastolobus altivelis*	65

SHELLFISH		
Geoduck	*Panopea generosa*	160 (190)
Manila Clam	*Venerupis philippinarum*	12
Red Abalone	*Haliotis rufescens*	9
Rocky Mountain Ridged Mussel	*Gonidea angulata*	50

SCL Output per Client



Requested vs Total Aged



Groups	Total
Groundfish	13,700
Salmon	73,000
Pelagics	28,400
Shellfish	8,000
	123,100

Biases in Fish Age Estimation

- It is widely accepted that age estimation of hard-part structures requires some degree of subjectivity.
- Two age readers examining the same structure following the same protocol can produces different age estimates.
- Quality control measures are employed to assure high levels of precision are maintained. Typically, ten to 15% of structures are re-aged for the process of precision testing. The goal is zero net bias, however that is nearly impossible to attain.
- There are two aspects to ageing error: precision and accuracy.
- Precision is an aspect of the ageing error that will always occur, as it is inherent to the nature of the structure, the preparatory technique employed, and species being assessed.
- Precision is expressed as percent agreement (PA), average percent error (APE), or coefficient of variation (CV) between independent readers.

Biases in Fish Age Estimation

- Accuracy is the relationship between the postulated age and the true age of the specimen.

Precision,
or repeatability

Accuracy, or the closeness
to the real age of the fish

It is possible to have
neither accuracy
or precision

- The true age is determined by age validation studies
- Age validation studies are a costly but required element to ensure we are ageing species correctly.

How do we know we aged them correctly?

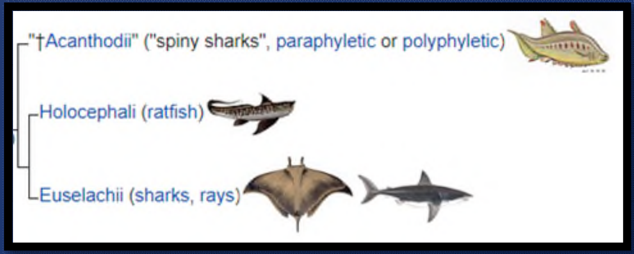
Not all species can be age validated, this is highly dependent on the natural history of the species and the technology of the day.

Actinopterygii (ray-finned fishes)

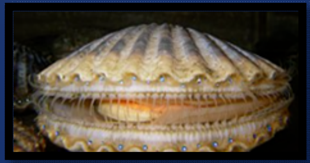
- Many deep-water species including many rockfishes are extremely difficult to validate with traditional means

Chondrichthyes (cartilaginous fishes)

- Only a few species of cartilaginous fishes that display hardening of vertebra have been age validated



Mollusca (shellfish and Cnidaria)



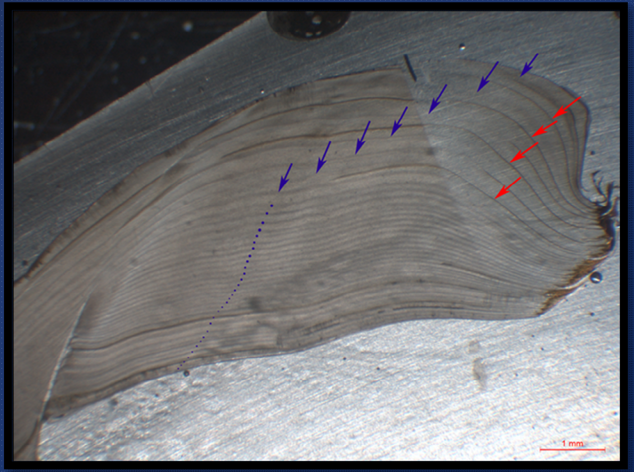
Most common form of <u>direct</u> age validations are; mark-tag-recapture, OTC, release of known age fish, isotopic (^{14}C, ^{18}O), radiochemical dating (Pb-210; Ra-226), natural date specific markers.

How do we know we aged them correctly?

Secondary Ion Mass Spectroscopy (SIMS) (^{18}O, ^{15}N, ^{13}C)

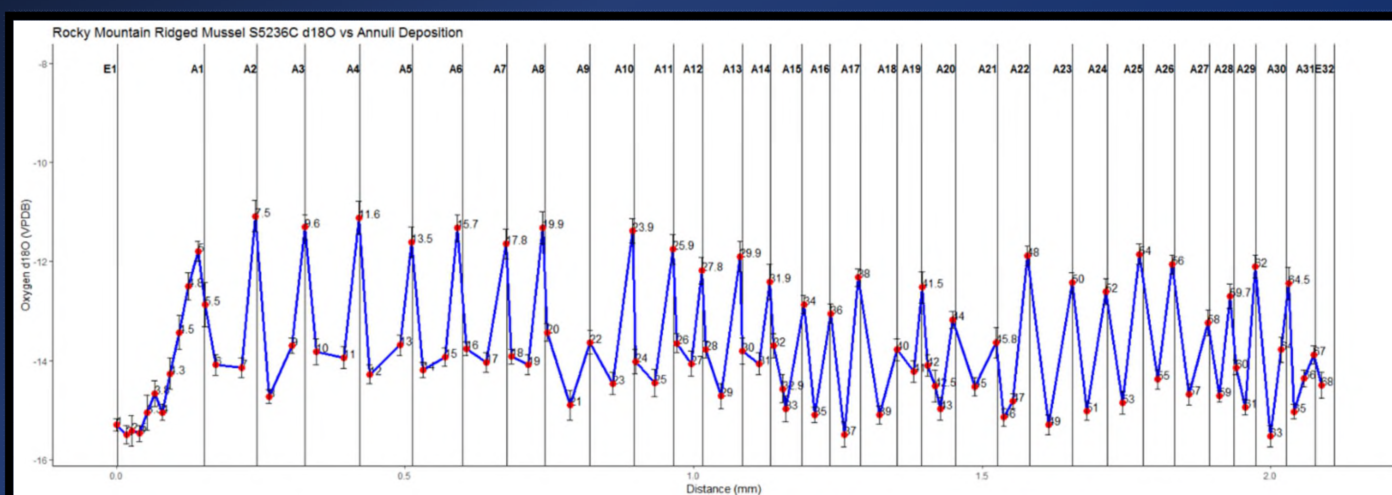
Is a technique used to analyze the composition of solid surfaces by sputtering the surface of the specimen with a focused primary ion beam and collecting and analyzing ejected secondary ions. The mass/charge ratios of these secondary ions are measured with a mass spectrometer to determine the isotopic composition.

- the ability to sample $^{18}0$ (temperature), ^{15}N (trophic level) ^{13}C (metabolic rate)
- the ability to sample down to 20nm
- the ability to sample the exact same location multiple times



How do we know we aged them correctly?

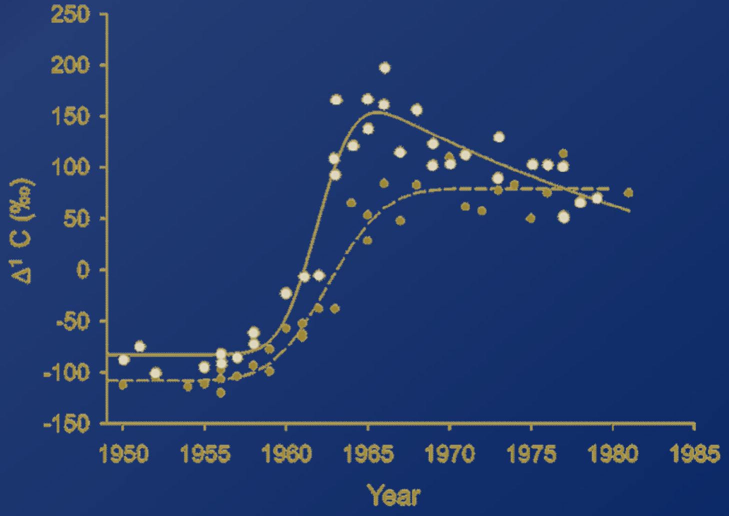
SIMS used to validate Rocky Mountain Ridged Mussel (*Gonidea angulata*)



How do we know we aged them correctly?

Bomb Radiocarbon Validation (^{14}C)

Refers to the use of a unique temporal signal of rapid increase in oceanic radiocarbon levels due to atmospheric testing of nuclear weapons in the 1950s and 1960s. This signature was incorporated into the otoliths of fish. The pattern of changes in radiocarbon levels across years has been used to verify the accuracy of the age assignment of many species of rock and flatfish in the northwest Pacific.

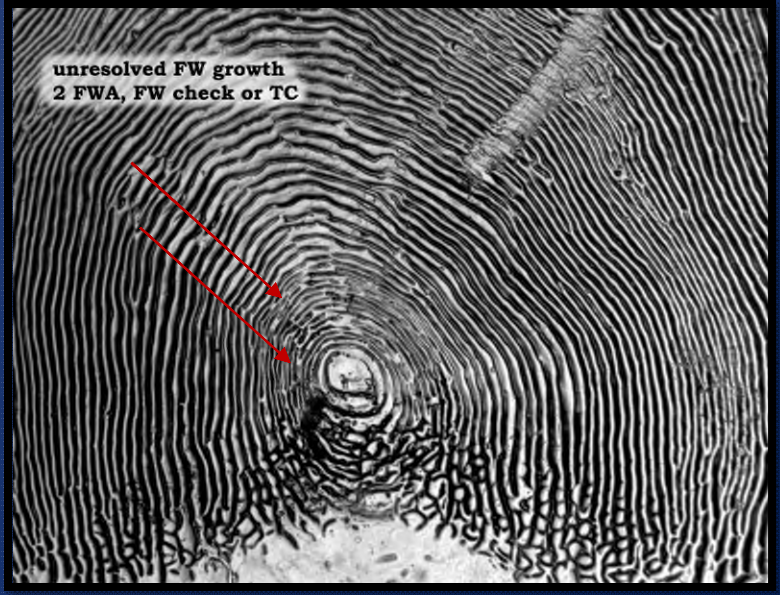


Climate change; the effect on ageing fish

- Climate Change, in the form of increasing water temperatures and acidification will diversely affect fin/shellfish species causing variations in growth and metabolic condition.
- The interpretation of growth patterns for some rockfish and salmon species have become more difficult to interpret because of “noise” in the form of increased false annuli/checks and variable growth patterns.
- False annuli are indicators of asymmetric growth due to stressors that cause enough “hardship” that the event is perpetually recorded within the skeletal hard parts of the individual.
- The last two decades has, for some species, indicated a slow transition away from stable growth patterns to more inconsistent and “noisier” patterns, indicating increasing stress on those species/populations.
- Quality-erosion in the form of increased ageing error creates age estimation ambiguity, the “smearing” of year classes increases the uncertainty in the analytics of determining fishing mortality and population numbers for catch-at-age data.

Climate change; the effect on ageing fish

Salmon (Chinook, Coho and Sockeye) are of particular concern as the SCL has begun to see increasing disagreement between age estimates and CWT data for the same fish. These differences are localized to the freshwater component (for now) of the scale indicating increasing difficulties estimating the number of freshwater winter events, therefore affecting absolute age. This is concerning as the SCL provides the only means to determine freshwater age. CWT, Thermal Marking and PBT methods provide no freshwater residency times only absolute age.



Climate change; the effect on ageing fish

Physical Attributes

- Warmer conditions will result in faster growth but produce smaller adult body size in fish (https://doi.org/10.1111/gcb.12514)
- Rapid growth early in life has a direct tradeoff between lifespan and growth rate. https://doi.org/10.1098/rspb.2012.2370
- Adults that don't grow as large due to warmer temperatures are likely to produce smaller offspring. These juveniles may in turn grow more rapidly to compensate for their undersized start to life, and this rapid growth may lead to shorter lifespans. (https://doi.org/10.1016/S0169-5347(01)02124-3)
- Climate change has already caused changes in metabolic rate, heart rate, and enzymatic activity up to 20% in the last two decades (https://doi.org/10.1038/nclimate2457)
- Life history strategies, and even physiologies can provide a buffer to increasing temperatures, but it may not be enough for long term survival or fittness. (https://doi.org/10.1111/1365-2435.12874)

Climate change; new species to Canada's Exclusive Economic Zone

The world's fish are on the move

- Climate change is driving the world's fish toward Earth's poles as species seek preferred habitat. https://doi.org/10.1111/j.1365-2486.2009.01995.x
- Species from the U.S. and Canadian west coast including the Gulf of Alaska had the highest projected magnitude shifts in distribution, and many species shifted more than 1000 km under the high greenhouse gas emissions scenario. https://doi.org/10.1371/journal.pone.0196127

What does this mean for the SCL?

- Some commonly aged species will potentially move out of Canada's EEZ and new unfamiliar species will move in.
- It is expected that if the commercial fishing industry targets these new species the SCL will be required to provide age estimates.
- Depending on the rate of immigration the SCL may not be able to address the numbers of new species requiring age estimation especially if validations studies are required.

The SCL meeting the challenge of Climate Change

Climate change is certain to increase the difficulty of estimating fish age. To provide more age certainty the SCL is working to provide more access to new validation technologies, the otoliths' other age contributing characteristics, and more ages with fewer sclerochronologists.

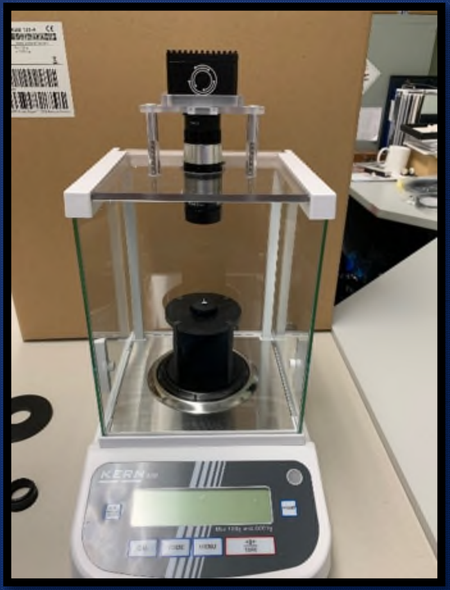
- More support for validations studies are needed to deal with changing patterns or new commercial harvestable species that require assessments.
- Otolith weight and otolith shape can be loosely considered *analogues* to age, and when used collectively with otolith age data in a quality control manner can provide age parameters.
- Are more ages statically better? Typically not, however, with more ambiguity with the age data produced it might be needed. Fournier Transfer Near Infrared (FT-NIR) spectroscopy is a rapid form of spectral age estimation that reduces the time to produce an age estimate and can provide more ages than traditional agers.

The SCL meeting the challenge of Climate Change

Direct Data Entry (DDE) has been a historic priority for the SCL. The vision of DDE has changed since its original conception. Then, the main objective was to;

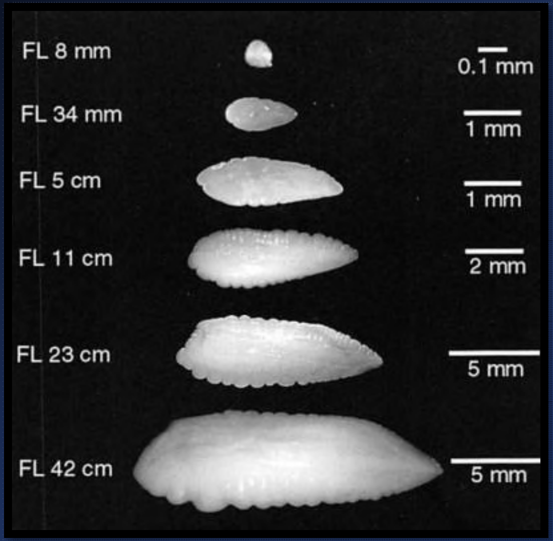
- increase work throughput
- eliminate paper usage
- eliminate/reduce transcriptional errors
- add real time age-determination statistical analysis for increased QA/QC
- eliminate client key punching

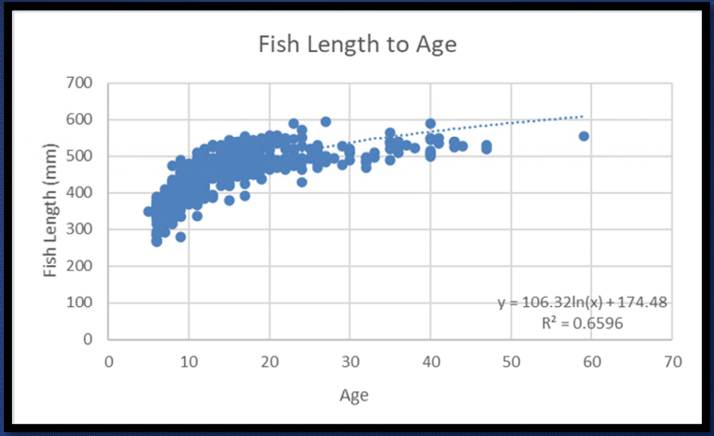
However, the hidden advantage of computerized workstations is the capacity to capture otolith images and measures of otolith weight that were in the past to time consuming or cumbersome to record.

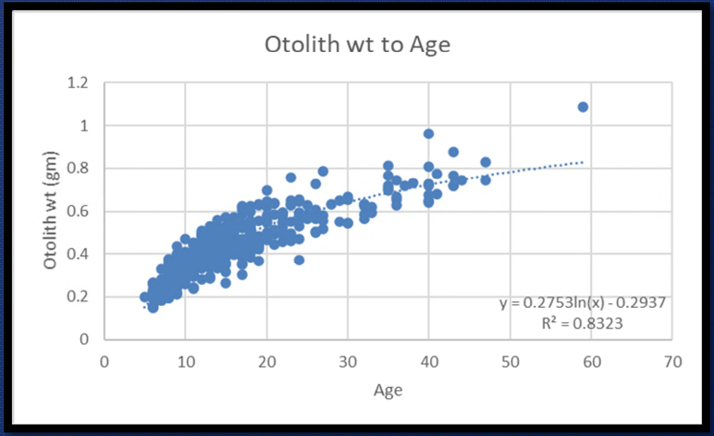


The SCL meeting the challenge of Climate Change

Otolith weight – during the biomineralization process the otolith continues to increase in weight until the death of the individual.



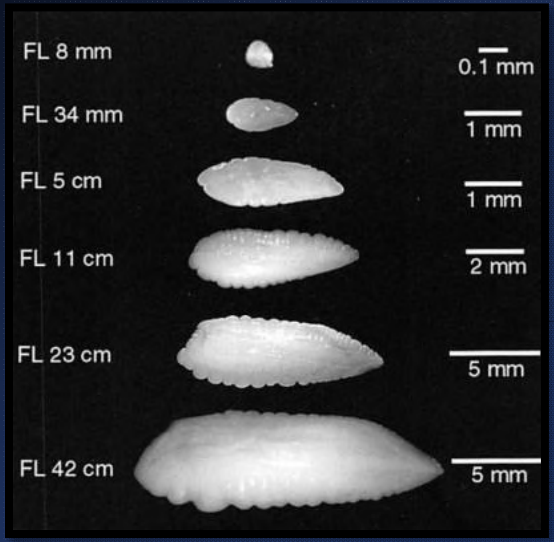




S. pinniger - age to length verses age to otolith wt.

The SCL meeting the challenge of Climate Change

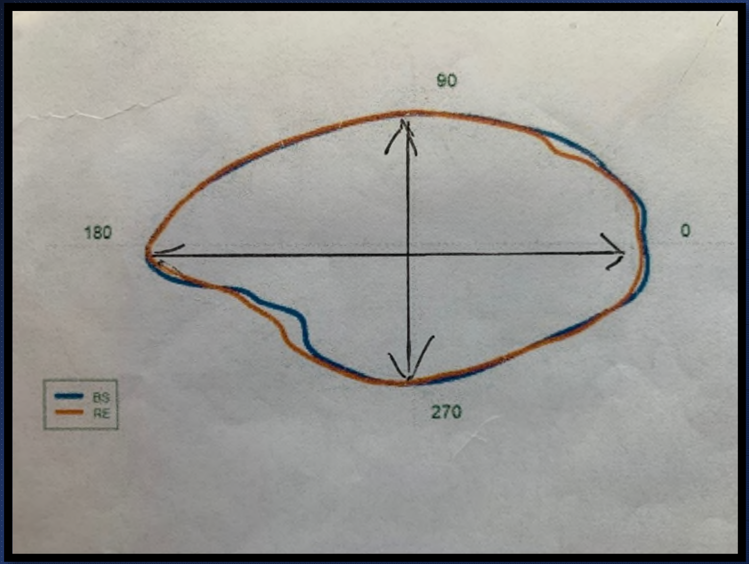
Otolith images – during growth, the shape of the otolith changes and continues to change until the death of the individual.

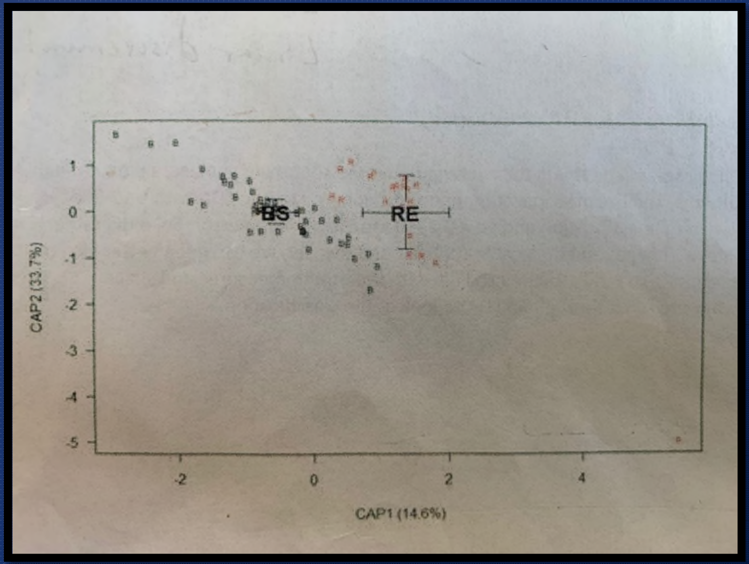


Otolith Shape Analysis (OSA) has in recent years become a powerful tool for the identification of species based on morphometric measure of the otolith. Its utility has been implemented in the identification of populations within species that reveal no genetic disparity by current molecular biology methods.

The SCL meeting the challenge of Climate Change

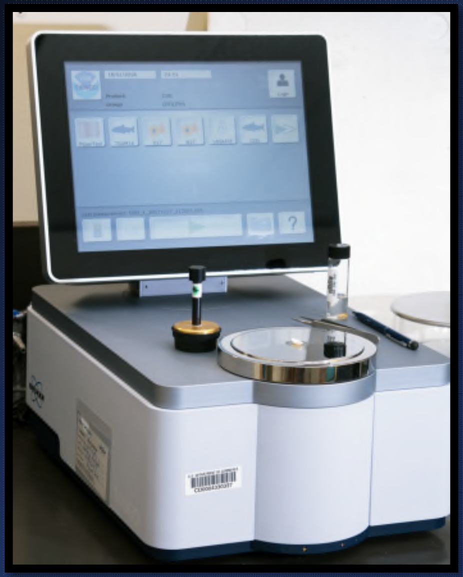
A recent pilot study had good success in identifying the species within the rockfish complex of Rougheye/Blackspotted. A very difficult complex to morphologically identify. Based on OSA, otolith weight, fish length and age from ~700 specimens 85% of specimens were correctly identified to species based on DNA analysis.

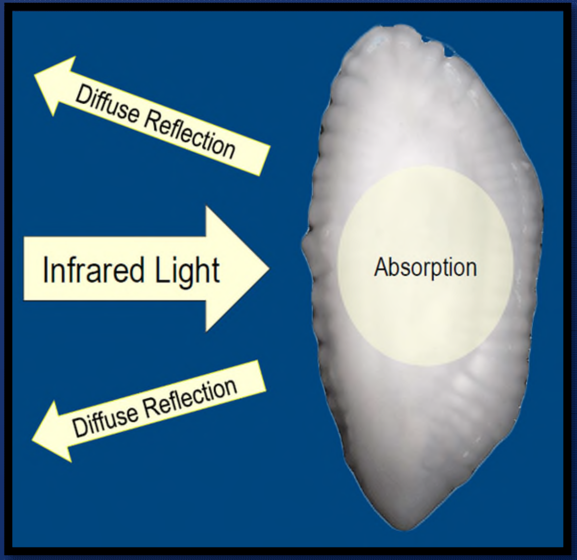




The SCL meeting the challenge of Climate Change

Fournier Transfer-Near Infrared (FT-NIR) spectroscopy is a recent development in automatize fish ageing, a technology widely used in many industries for quality control measures. Investigative work at NMFS has indicated the application on otoliths may provide more efficient, timely and a cost-effective method of estimating fish age.





The SCL meeting the challenge of Climate Change

NIR is a vibrational spectroscopy technique where the amount of energy absorbed by specific functional groups (carbon-hydrogen (C-H), oxygen-hydrogen (O-H) and nitrogen-hydrogen (N-H) in a molecule is measured and correlated to a sample's composition. This absorption of energy is detected by NIR instrumentation and produces a spectrograph of the source material.

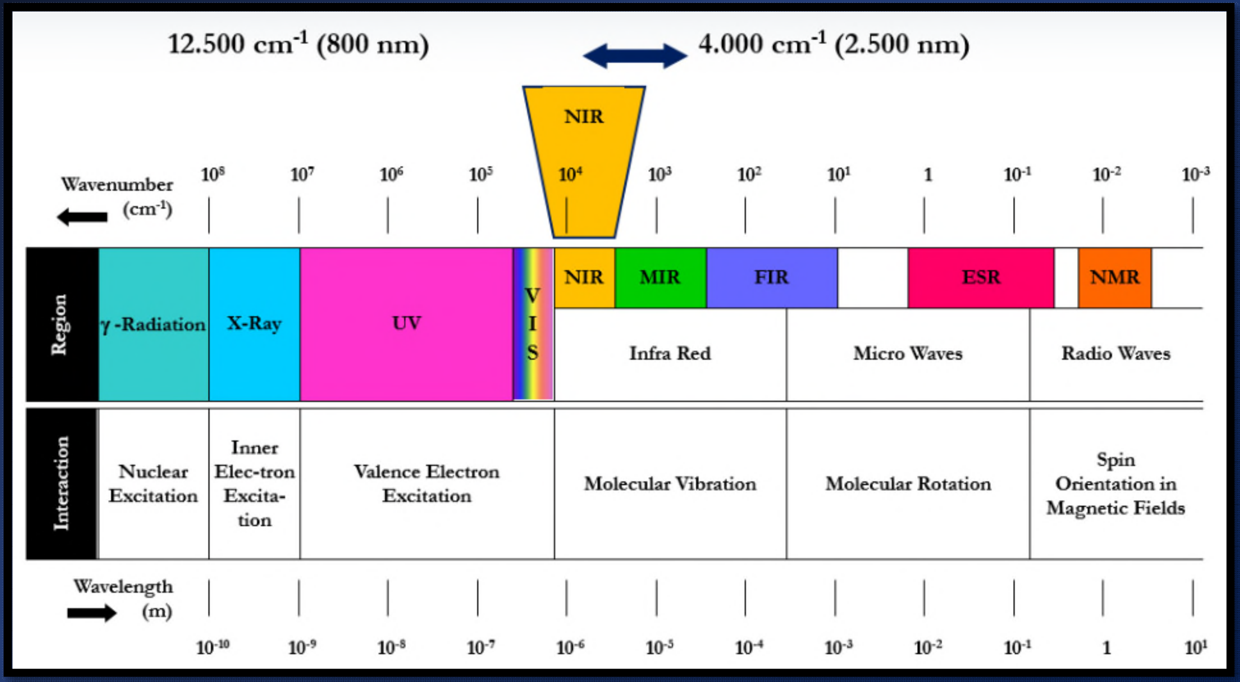
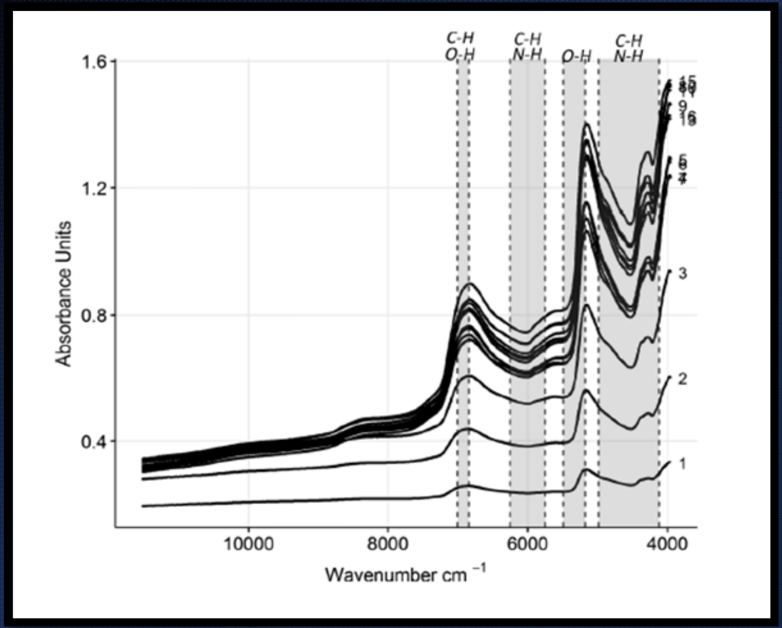
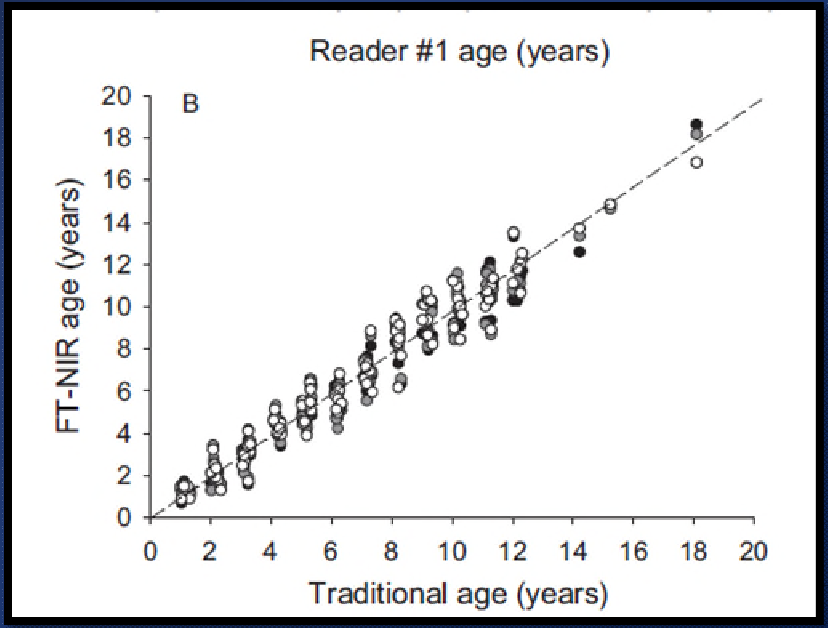


Image borrowed from Irina Benson - Transformative Approach to Ageing Fish Otoliths Using Fourier Transform Near Infrared (FT-NIR) Spectroscopy

The SCL meeting the challenge of Climate Change

Bering Sea walleye pollock (*Gadus chalcogrammus*) otoliths, the shaded regions of absorbance signatures by wavenumber show molecular vibrational group frequencies most relevant to age estimation from FT-NIR.





Preliminary estimates for walleye pollock precisions indicate results are as good or slightly better over traditional ageing and showed little to no bias for samples under 12 years of age. Walleye pollock model age can be reliably predicted within ±1 year of traditional age. https://dx.doi.org/10.1139/cjfas-2018-0112

The SCL meeting the challenge of Climate Change

- FT-NIR is secondary method of age estimation. A primary calibration is required by traditional means to calibrate the predictive model. A reference set (~20% of sample) will be required to predict the age of the unknown samples.
- Potential to produce more age estimates then traditional means
- FT-NIR offers the considerable potential to reduce costs of estimating age
- FT-NIR is non-destructive
- Subjectivity is reduced and repeatability increased due to the quantitative measurement properties of FT-NIR spectral absorbance in otoliths
- Potential other applications; gonad scanning for maturity status, analysis of stomach contents for fish, marine mammal diet analysis and units designed to sample collection at-sea.

The SCL meeting the challenge of Climate Change

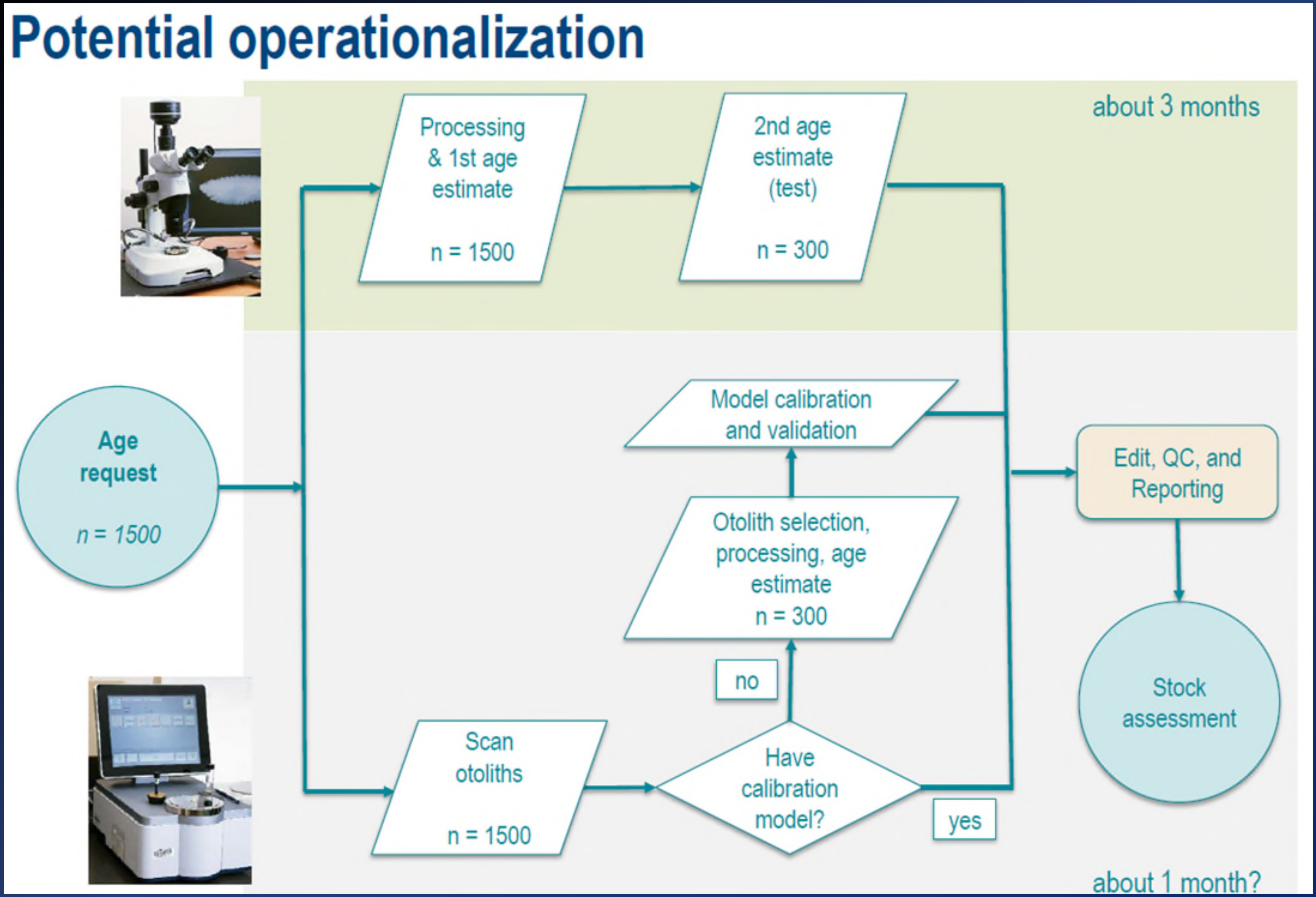


Image borrowed from Irina Benson - Transformative Approach to Ageing Fish Otoliths Using Fourier Transform Near Infrared (FT-NIR) Spectroscopy

The SCL meeting the challenge of Climate Change

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