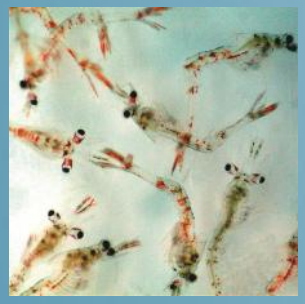
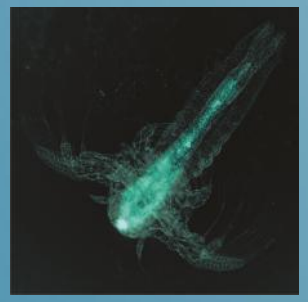
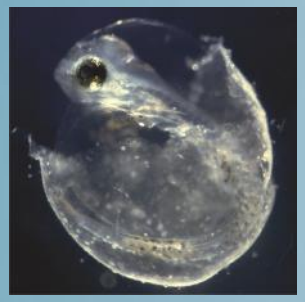
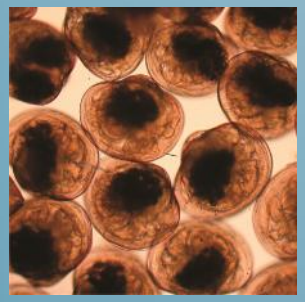




larvi 2013

6th fish & shellfish larviculture symposium

Digital image analysis to aid broodstock management and egg and larval quality assessment

Andrew Davie

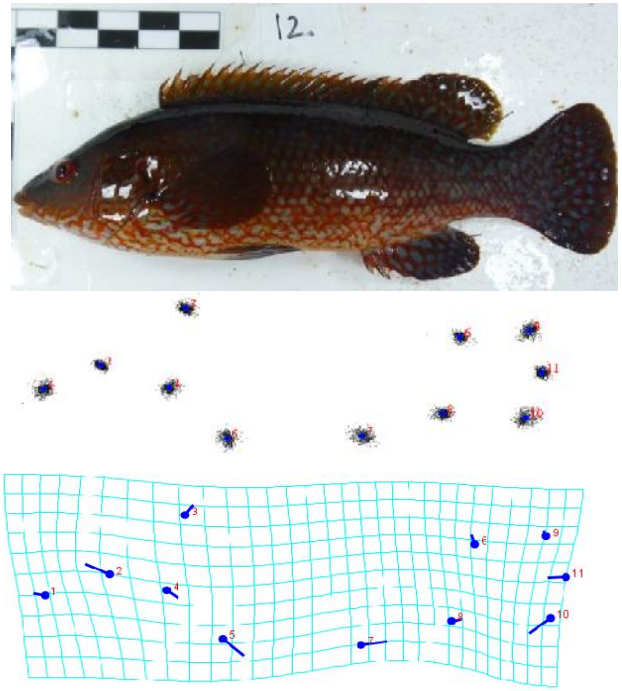
ghent university, belgium, 2-5 september 2013

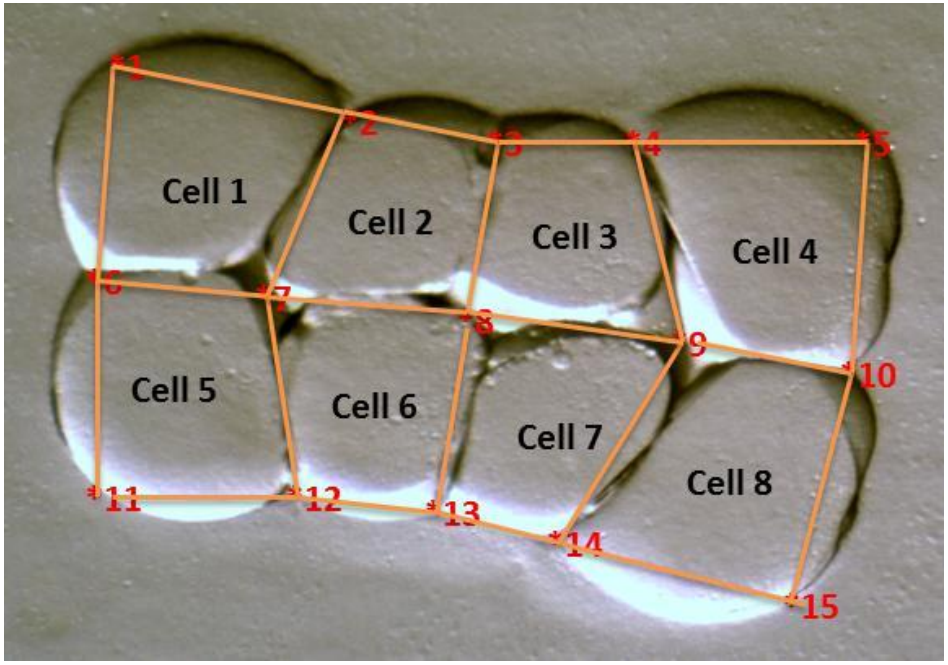
UNIVERSITY OF STIRLING

DIGITAL IMAGE ANALYSIS TO AID BROODSTOCK MANAGEMENT AND EGG QUALITY ASSESSMENT.

Andrew Davie, Eric Leclerq, & Herve Migaud

Institute of Aquaculture, University of Stirling, Scotland





UNIVERSITY OF STIRLING

Digital Image Analysis

Digital image analysis is deeply ingrained in modern society

Security

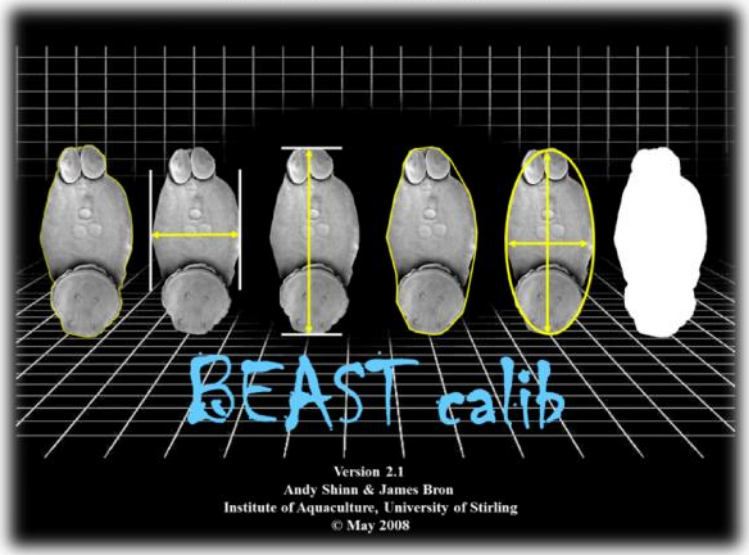
Health and Safety

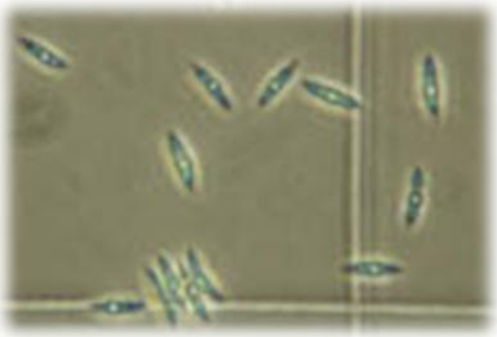
Social Media

However…Digital image analysis appears to be utilised sporadically across the aquaculture industry and research community.

Biomass estimation

Parasite identification





Live feed quantification

UNIVERSITY OF STIRLING

Wrasse Aquaculture

Ballan wrasse (Labrus Bergylta)

Biological control of sea lice in salmon farming

3 hatcheries in the UK

Challenging species to farm

- **Long lived and late maturing (> 6years)**
- **Protogynous hermaphrodites**
- **Benthic substrate spawners**
- **Live in social harems**
- **Larval/juvenile performance problematic**

“Production and implementation of farmed wrasse in the Scottish Salmon industry”

Technology Strategy Board
Driving Innovation

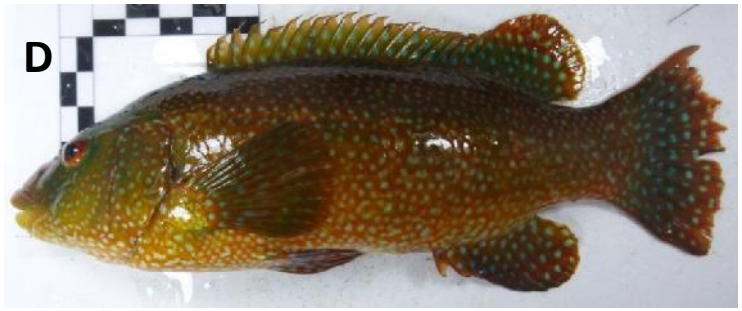
marineharvest
excellence in seafood

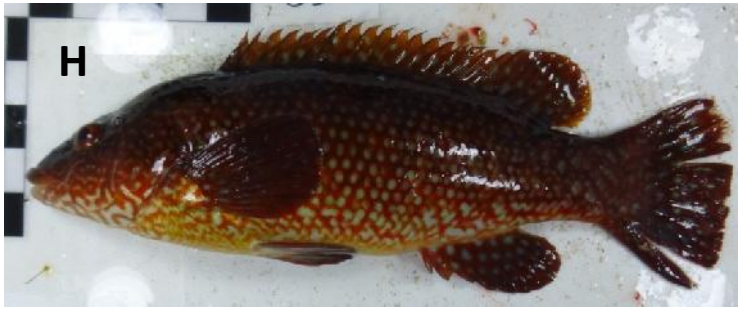
Scottish Sea Farms

January 2012 – December 2014

UNIVERSITY OF STIRLING

Who is Who?

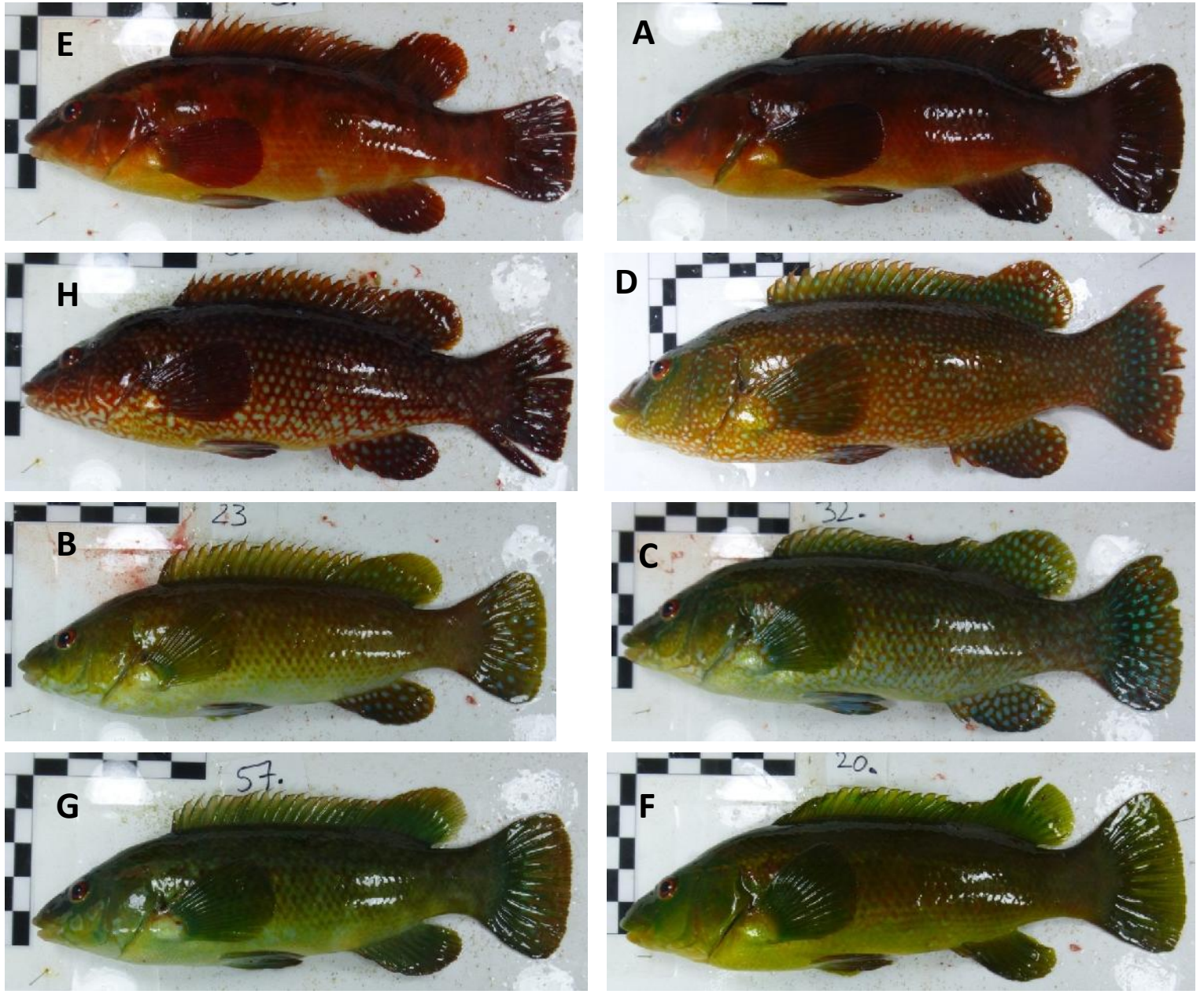




UNIVERSITY OF STIRLING

Highly diverse external coloration

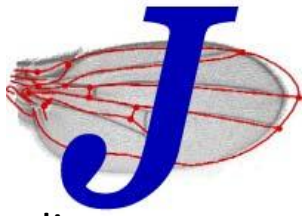
♀



♂

UNIVERSITY OF STIRLING

What is geometric morphometrics

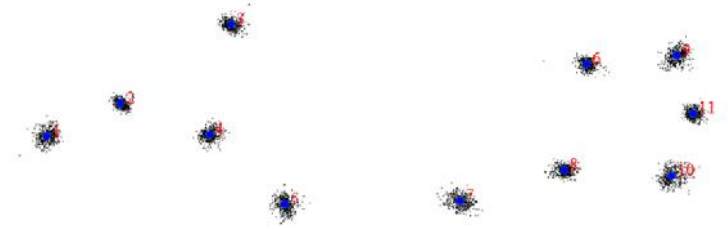


Principal

Analysis of landmark coordinates

Used to separate “size” and “shape”

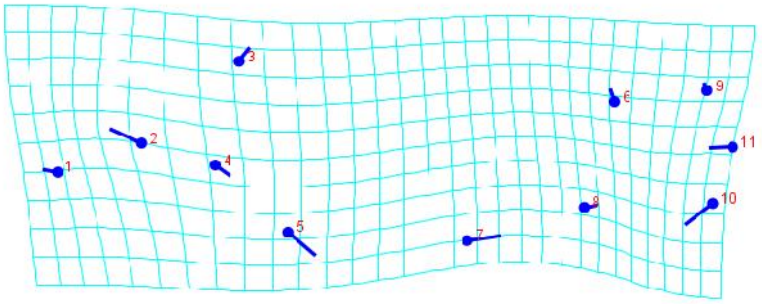
predict sex based on “shape”



“Size” (surface)

Measured from raw coordinates

(Dispersion of landmarks from the centroid)



“Shape”

Raw- transformed into **Procruste-coordinates**

(by translation and rotation)

Used to perform statistical analysis:

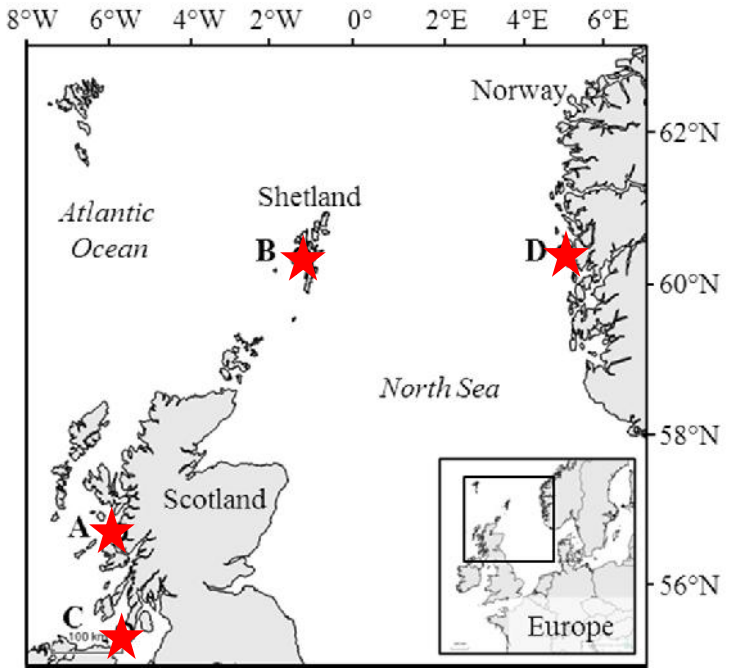
Quantify shape differences between groups

Predict groups based on shape

UNIVERSITY OF STIRLING

Origin of variability in size and shape

- 400 wild ballan wrasse
- 4 locations across Scotland and Norway
  - Weight
  - Length
  - Photograph
  - Age classification by opercula rings
  - Histological confirmation of gender
  - Fin sample for genomics research



	SS	SS (%)	MS	P
(a) Procrustes ANOVA: Centroid size				
Location	2312268	78.99	770756.1	<0.0001
Sex	112942	3.86	112941.7	<0.0001
Individual	501973	17.15	1328.0	<0.0001
Sum	2927183	100.00		
(b) Procrustes ANOVA: Shape				
Location	0.11806	29.03	0.00219	<0.0001
Sex	0.00384	0.94	0.00021	<0.0001
Individual	0.28479	70.03	0.00004	<0.0001
Sum	0.40668	100.00		

Leclercq et al (2013) ***In Review***

Variability in Shape/Size due to

1. **Location**
2. **Individual**
3. **Sex**

UNIVERSITY OF STIRLING

Optimisation of size-based analysis

- 5 sets of variables tested

(1) BW, TL and K

(2) BW, TL and K + centroid size

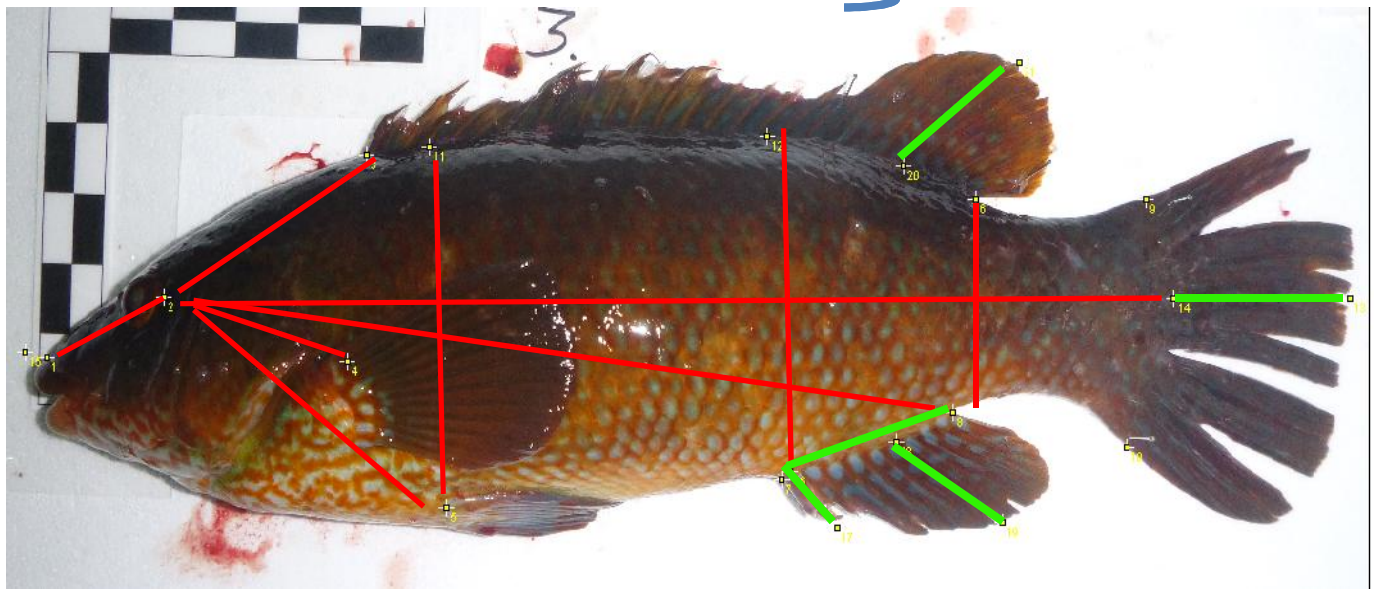
(3) Procruste distances: *from coordinates*
Body
Fins

(4) Body and fin lengths + Centroid-size

(5) ALL

Simplest formula had a 91.4% accuracy

Accuracy of predicting gender was >85% in all cases

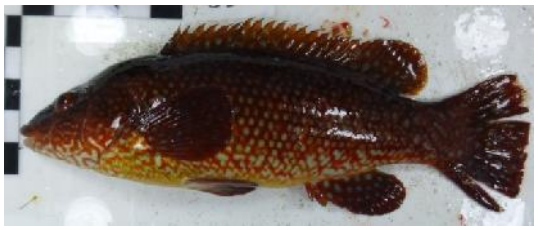


Leclercq et al (2013) *In Review*

Simplified on farm size-based analysis

$$D = (0.010\ BW) + (-0.016\ TL) + (-3.835\ K) + 6.252$$

1.459<<1.504

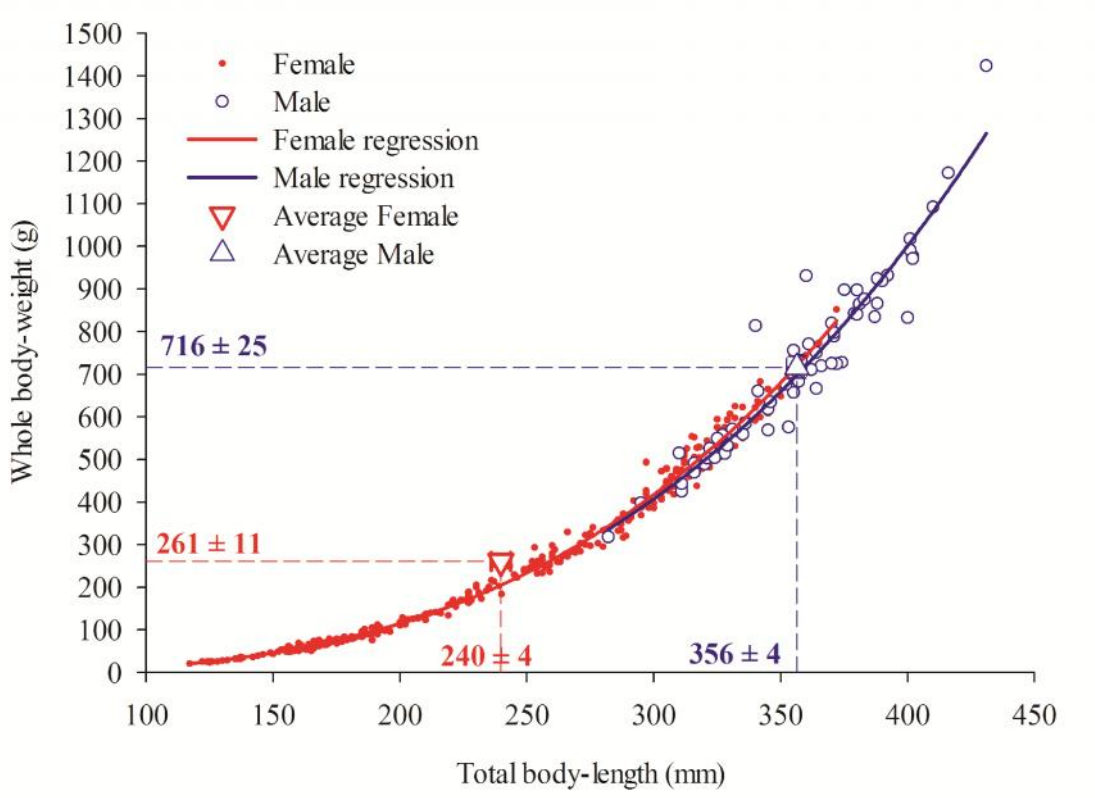
♀   ♂

Simple calculation that can be performed in the field or on farm to predict gender

Size selection of gender in a protogynous hermaphrodite is not necessarily surprising.

Helps resolve the gender identification in sizes where both genders overlap, 30-40 cm TL.

Digital image analysis was central in deciphering the size/shape interactions



Leclercq et al (2013) *In Review*

UNIVERSITY OF STIRLING

Blastomere Morphology

ELSEVIER

Aquaculture 155 (1997) 1–12

Aquaculture

Blastomere morphology as a predictive measure of fish egg viability

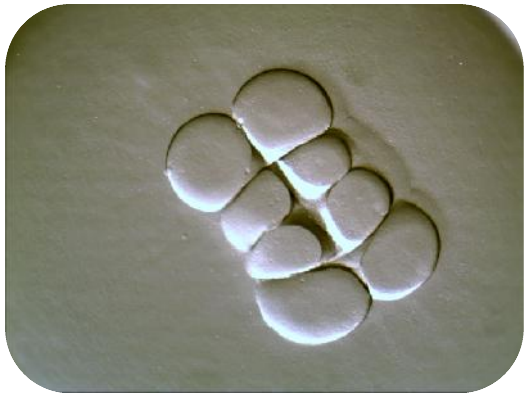
R.J. Shields [a,*], N.P. Brown [b], N.R. Bromage [b]

[a] Sea Fish Industry Authority, Marine Farming Unit, Ardtoe, Acharacle, Argyll PH36 4LD, Scotland, UK
[b] Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK

Subjective scoring methodology of the "shape" of recently fertilised oocyte, blastomeres.

Score (1-5) of the blastomere:

- **Cell symmetry**
- **Cell wall adhesion**
- **Cell size**
- **Cell margin definition**
- **Abundance of inclusion bodies**



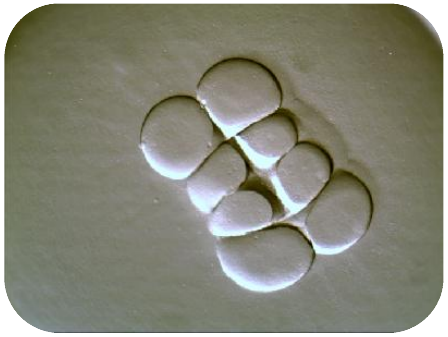
Good

Bad

Ugly

UNIVERSITY OF STIRLING

How subjective is a subjective scoring criteria?

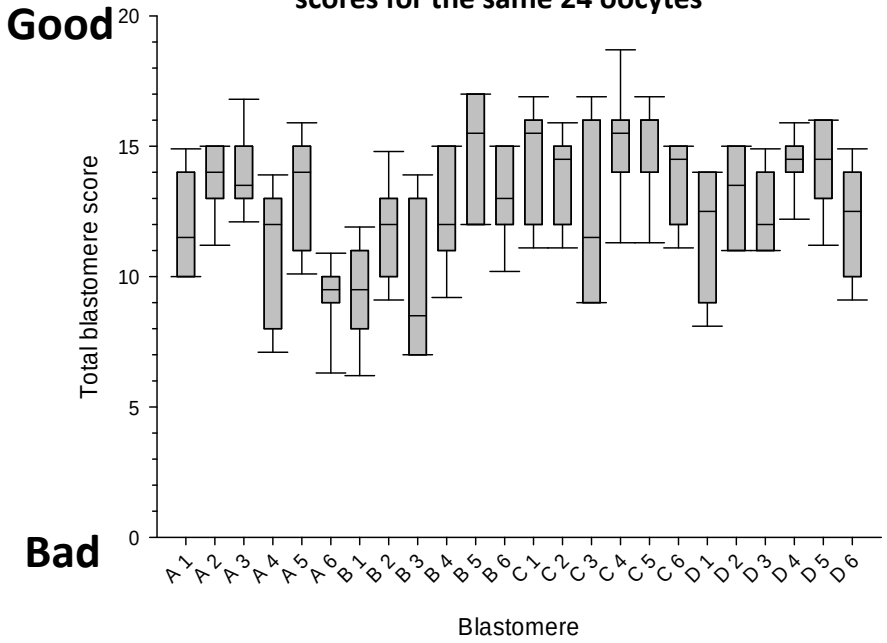


Criteria	Score
Symmetry	4
Cell size	3
Adhesion	2
Margins	4
Inclusions	3
Total Score	**16/20**

24 typical blastomere images were scored by 6 "informed" operators

- **Previous training on the methodology**
- **Pictorial example scale for all criteria**

Box & Whisker plots of 6 individuals blastomere scores for the same 24 oocytes



Conclusion

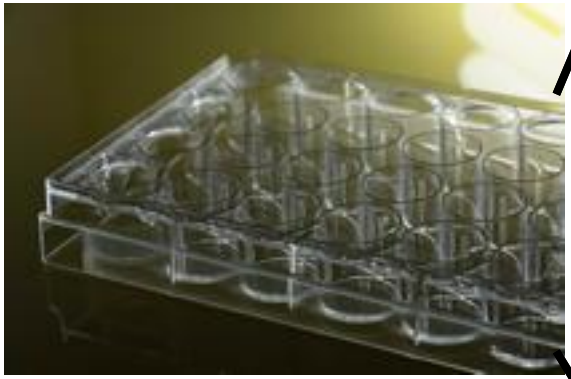
- **"Operator variability" introduced approximately <u>20-30% noise</u> on each blastomere**
- **There is a tendency to balance or compensate the scoring which results in a lack of variability in data points to be associated with the characteristic examined (i.e. quality)**
- **<u>Operator subjectivity may, in part, explain the conflict in the literature</u> as to the efficacy of the methodology.**

UNIVERSITY OF STIRLING

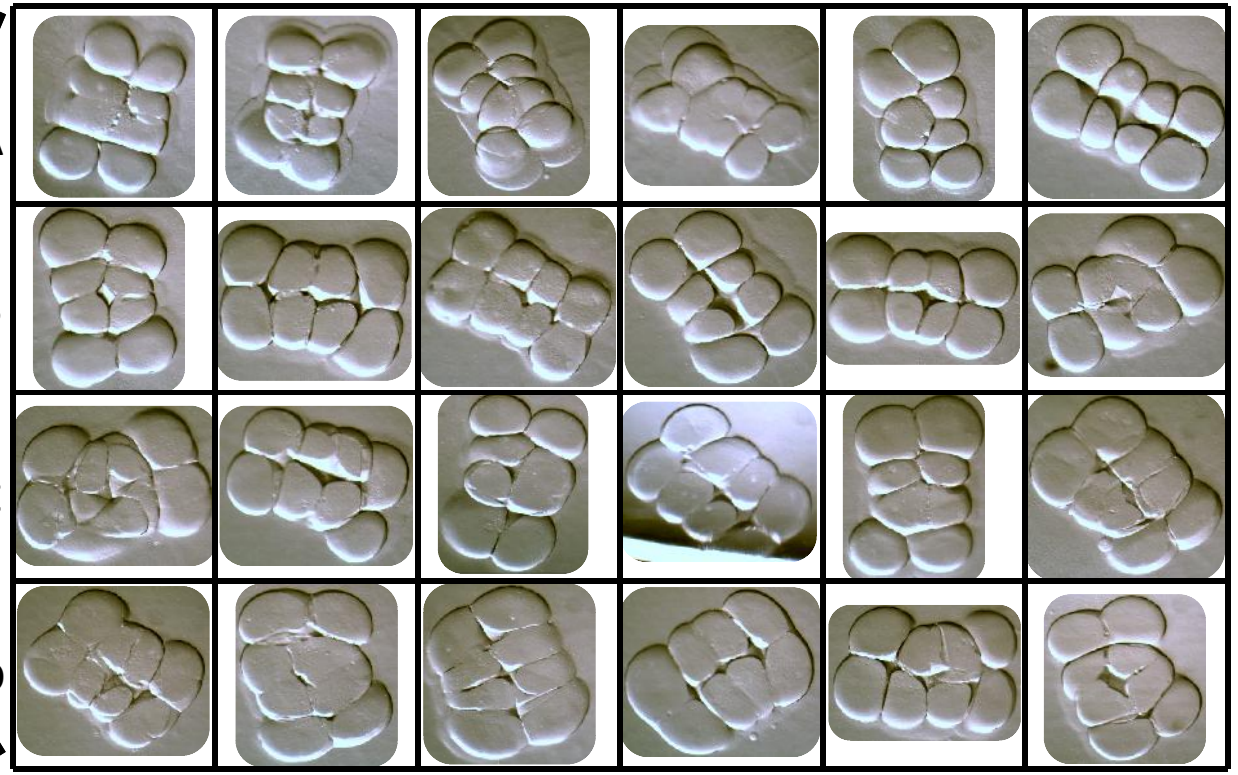
Reverse engineering a DIA measuring method

Samples from a total of 8 Atlantic halibut egg batches were collected from Otterferry seafish Ltd.

Using a routine plate culture method we photographed blastomeres (24 per batch, 192 in total) and then maintained them in constant environmental conditions to hatch.



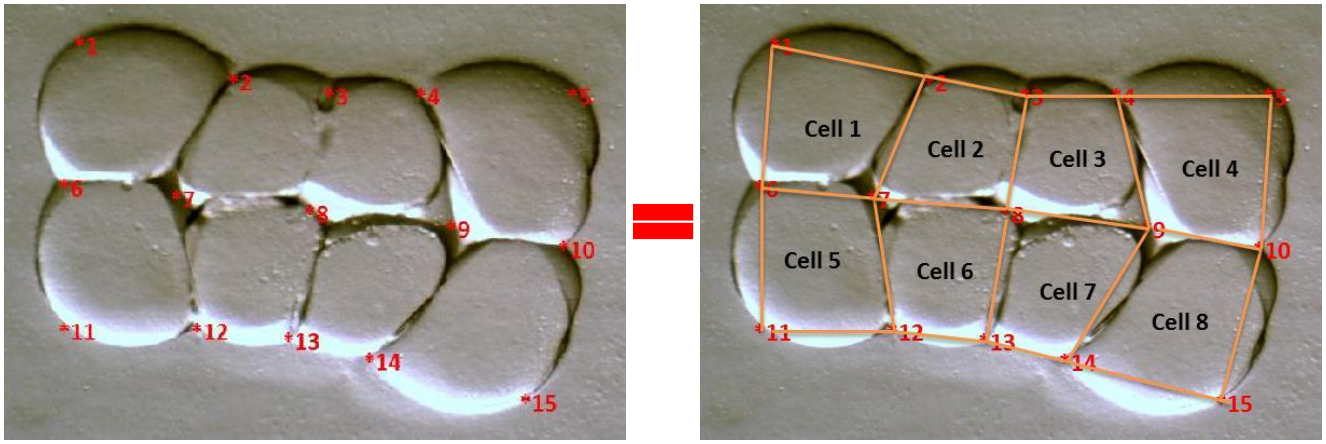
Batch 6: 77.9% fertilisation rate

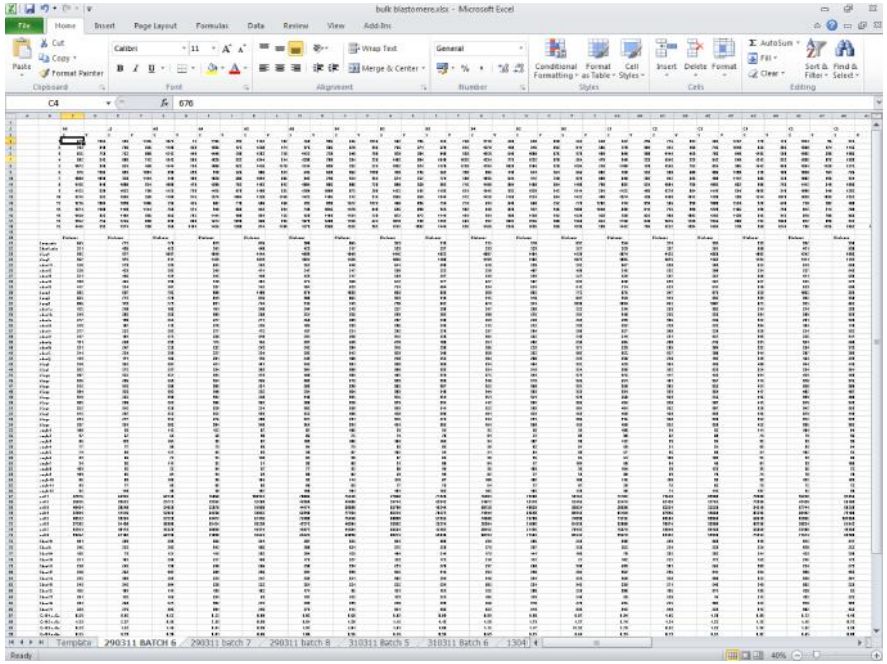


UNIVERSITY OF STIRLING

Reverse engineering a DIA measuring method

Analysis was based on 15 landmark points which in essence converts the blastomere into a crude 4 x 2 cell grid.





The X, Y coordinates of each landmark are imported into excel from which we calculated 74 separate measurements that were then amalgamated into 10 summary values for each blastomere

Long to short axis ratio
Diagonal axis ratio
CV short lengths(%)
CV long lengths (%)
CV angle intersection
CV angle intersection 2
CV angle intersection 3
CV all intersection angles
CV Cell box area
CV Cell width ratio

Davie et al (2013) *In Review*

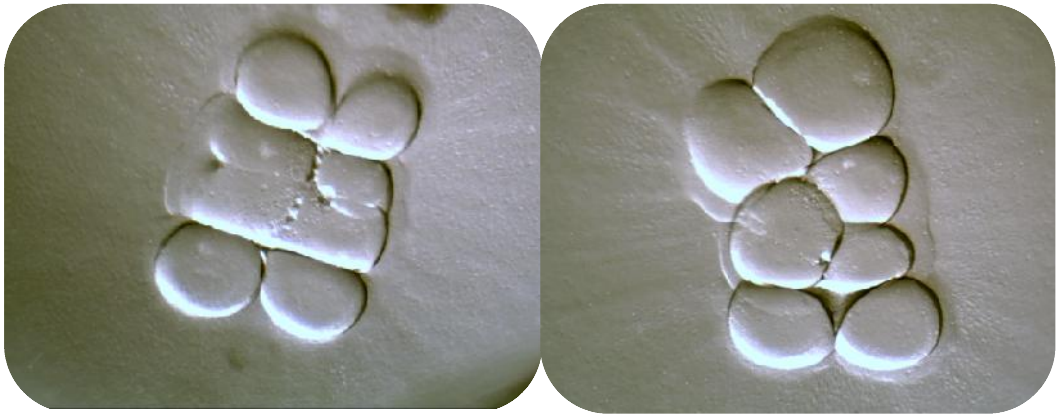
UNIVERSITY OF STIRLING

Reverse engineering a DIA measuring method

Blastomeres with:

- **Abnormal cell counts**
- **Margins poorly defined which prevents landmark point designation**

Did not hatch. i.e. no evidence of "self repair"



Summary values in association with blastomere fate (hatched or failed) were then subjected to discriminant function analysis.

Summary of classification

	True Group	
Put into Group	0	1
0	85	5
1	18	35
Total N	103	40
N correct	85	35
Proportion	0.825	0.875

N = 143 N Correct = 120

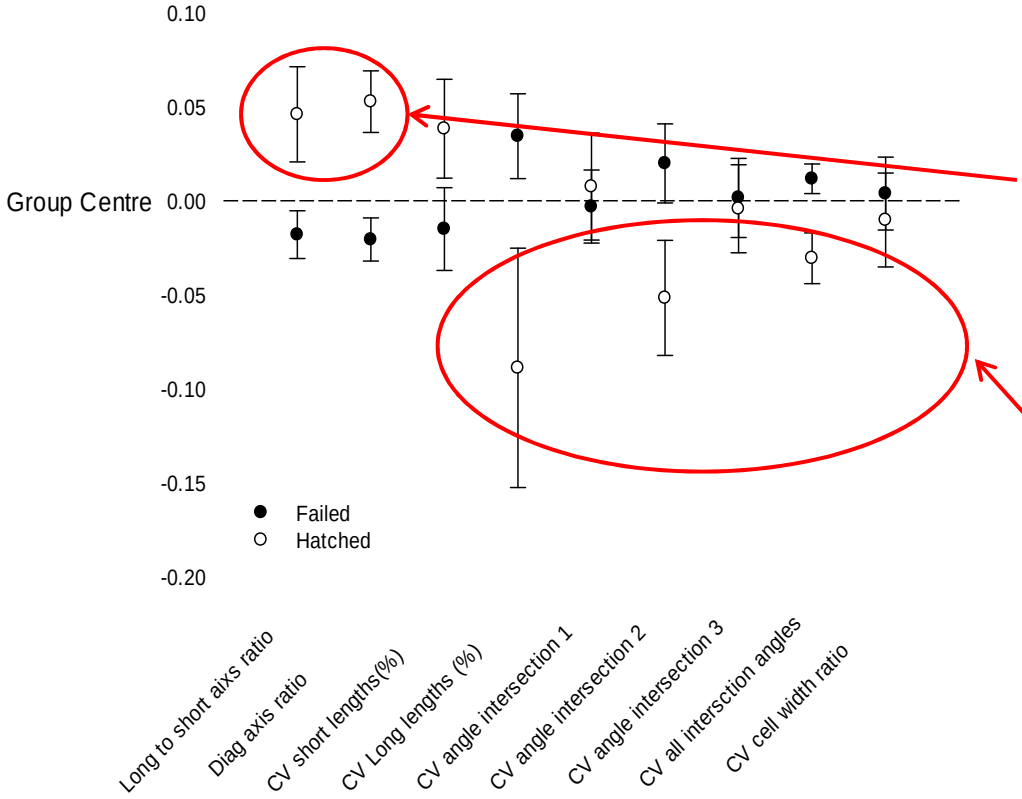
Proportion Correct = 0.839

Based on the summary values discriminant function analysis <u>can correctly assign blastomere fate (hatch or fail) to 83.9% of oocytes</u>

Davie et al (2013) *In Review*

UNIVERSITY OF STIRLING

What shape is a good blastomere?



Scatter plot of mean ± SEM of the divergence from population mean dimension for hatched (n=40) and failed (n=103) halibut blastomeres.

Hatched blastomeres have a :

Greater
- **Long to short axis ratio**
- **Diagonal axis ratio**

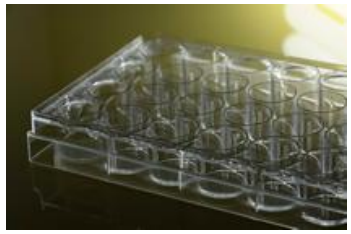
Lower
- **Variation in long axis lengths**
- **Variation in the angles of intersection between cells**

Conclusion: <u>Blastomeres which hatch are more rectangular and symmetrical</u>

Davie et al (2013) ***In Review***

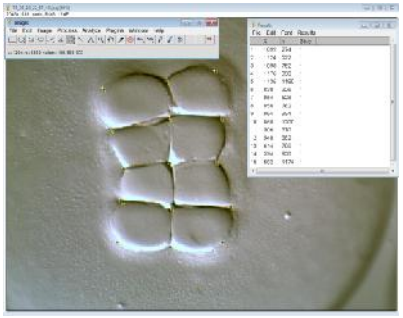
UNIVERSITY OF STIRLING

Development and validation of a digital blastomere morphology measurement tool.

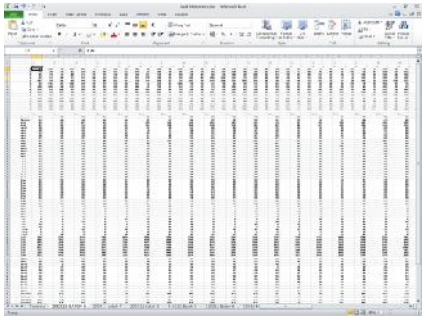


Plate

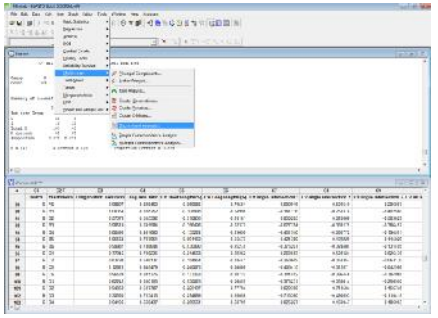
Image



Analyse in ImageJ



Process in Excel



Determine likelihood of hatch in Minitab

Analysis takes under 1 hour for a batch of 24 blastomeres
You could have hatch predictions within 24 hours of fertilisation

This protocol has been designed in Halibut, which other species could it be applicable to…

EU FP7 project: 232305 "Prospawn"

SEVENTH FRAMEWORK PROGRAMME

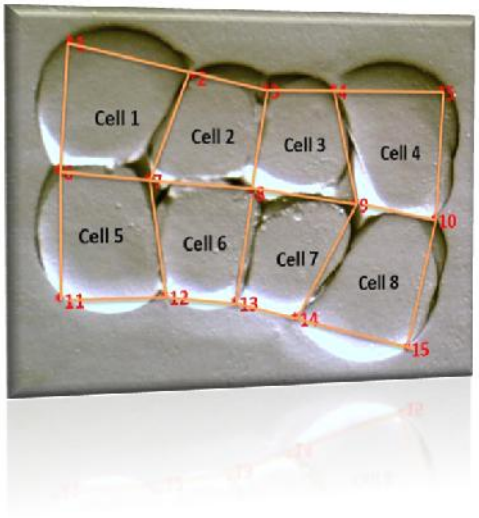
Davie et al (2013) *In Review*

UNIVERSITY OF STIRLING

Image analysis in hatchery management

Digital image analysis allows us to:

- **Capture and describe small differences accurately**
- **Removes operator subjectivity**



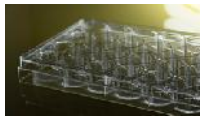
+ Open source software is accessible to all users be they academia or industry based

+ Provides real-time results

+ Capable of rapid large dataset set computation

+ Precision improves with use as reference databases expand.

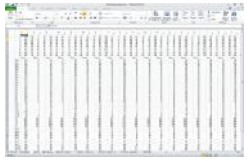
- Not always the most practical technique on farm



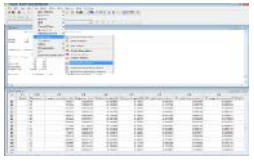
Plate

Image

Analyse in ImageJ

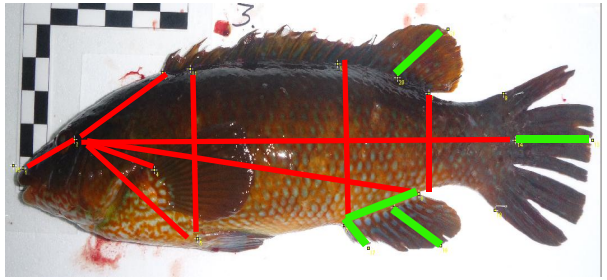


Process in Excel



Determine likelihood of hatch in Minitab

Analysis takes under 1 hour for a batch of 24 blastomeres
You could have hatch predictions within 24 hours of fertilisation



UNIVERSITY OF STIRLING

Acknowledgments

www.aqua.stir.ac.uk

andrew.davie@stir.ac.uk

Reproduction and Genetics Group
Institute of Aquaculture

Machrihanish Wrasse Farms
- Paul Featherstone
- Dave Cockerill
- Chris Hempleman

- Prof Herve Migaud
- Dr Eric Leclercq
- Bridie Grant

Otter Ferry Seafish Ltd
- Alasdair Barge
- David Patterson

Prospawn Consortium

Technology Strategy Board
Driving Innovation

marineharvest
excellence in seafood

Scottish Sea Farms

SEVENTH FRAMEWORK PROGRAMME

EU FP7 project: 232305 "Prospawn"