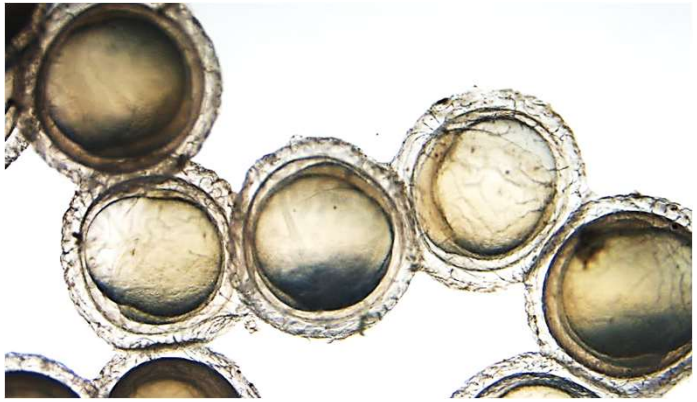
11.03.2021

Bruk av lipidomics for å vurdere eggkvalitet hos berggylt

FHF webinar om produksjon av berggylt

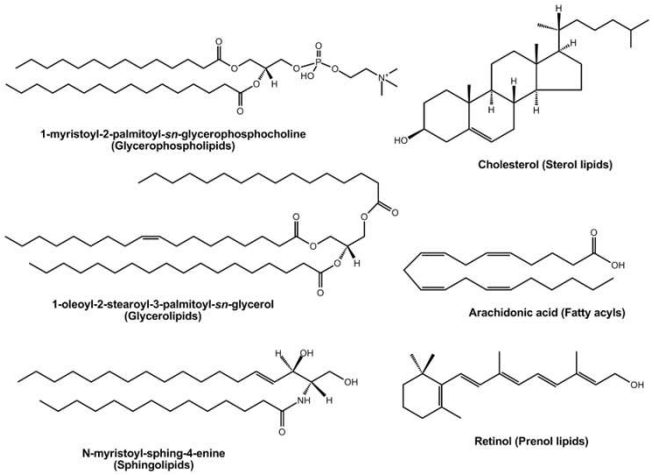


Bjørn Henrik Hansen & Antonio Sarno

Arne Malzahn, Julia Farkas, Tjalling Jager, Trond Nordtug, Deni Ribicic, Luciana Musialak, Bjarne Kvæstad, Elin Kjørsvik & Andreas Hagemann

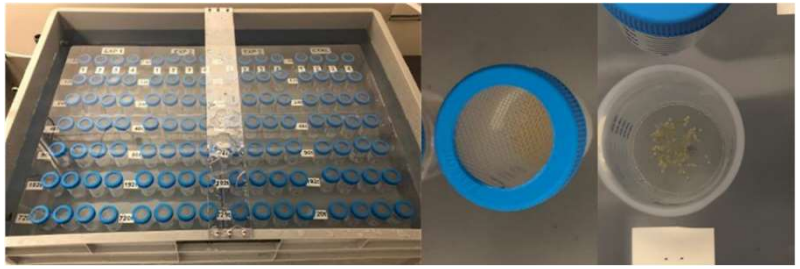
Målsetning

- Bedre forstå tidlig utvikling og fysiologi
- Etablere en bioenergetisk modell for tidlige utviklingsstadier av berggylt
- Etablere metoder for å analysere lipidspecies (lipidomics)
- Vurdere muligheten for å benytte lipidomics-metoder for å identifisere lipidspecies som kan benyttes som et verktøy for å vurdere kvaliteten på egg-batcher

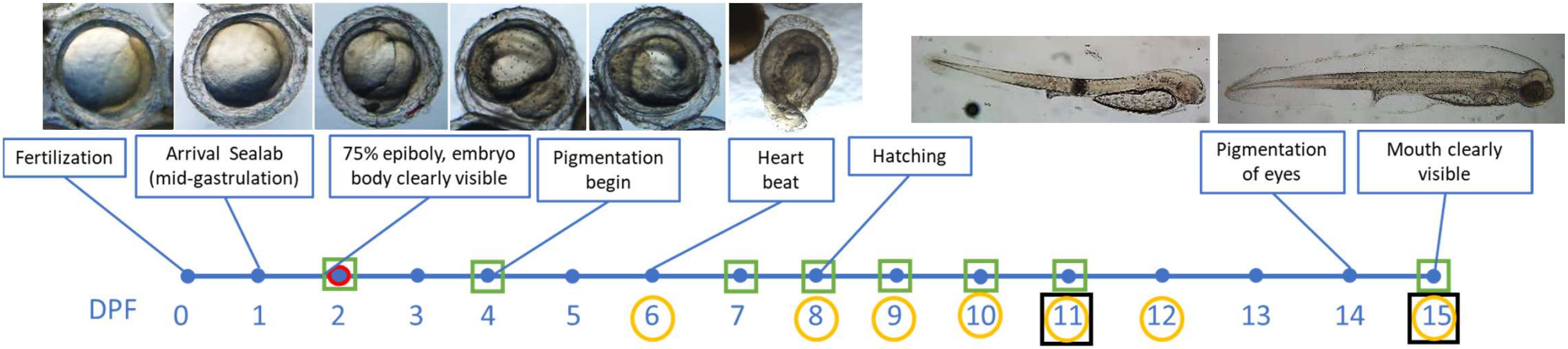


Egginkubering, utvikling og prøvetaking

Batch ID	Information
BG-1	Villfisk fra 2016/17 spesiell diett
Stord1	Villfisk fra 2017 (MOWI Stord)
Stord2	Villfisk fra 2017 (MOWI Stord)
NOV-A	Villfisk fra 2016 and 2017 (MOWI Øygarden)
NOV-B	Villfisk fra 2018 (MOVI Øygarden)
NOV-C	Rekrutter (2014) og villfisk fra 2018 (MOWI Øygarden)

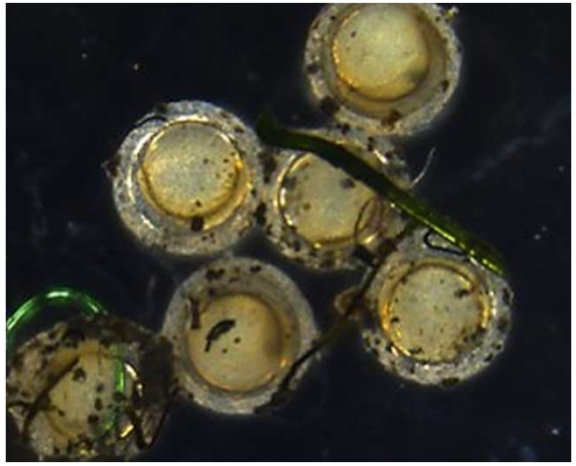


- Microscopy (biometry)
- Lipid sample
- Respiration, DW and CN
- Bone development
- Heart activity



Befruktning og klekking

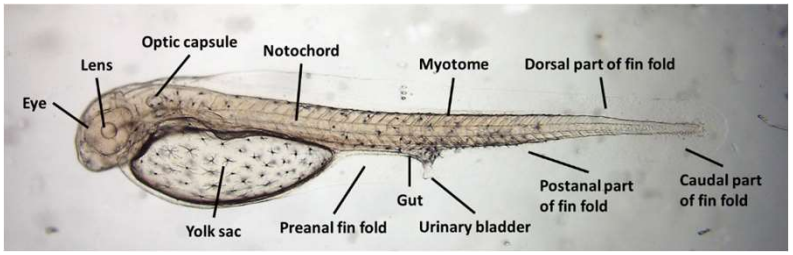
Normale egg med 'limlag'



Egg etter degumming

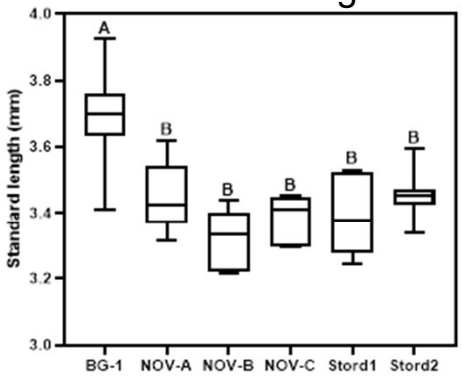
Automatiske biometri-analyser

- Kroppsareal (standard lengde, myotomhøyde)
- Plommesekkareal (max lengde og høyde)
- Øyeareal (max/min diameter)
- Eggdiameter, limlagtykkelse

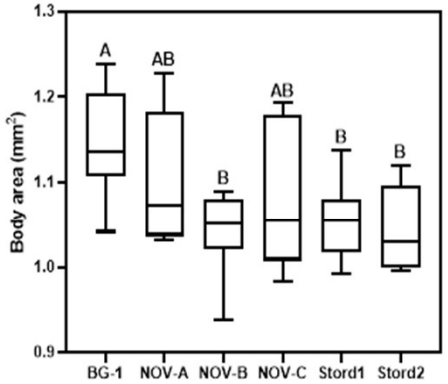


Utvalgte parametere

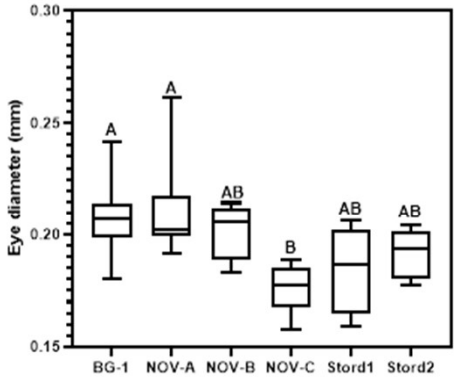
Standard lengde



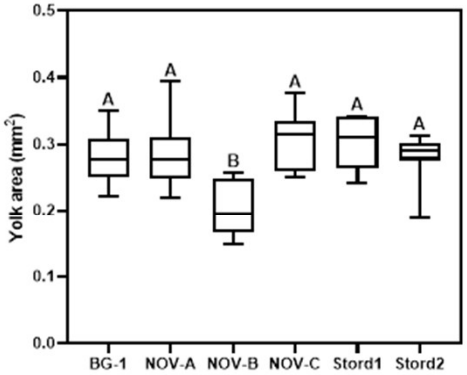
Kroppsareal



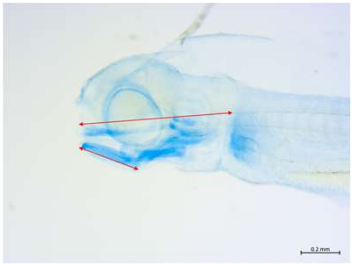
Øyediameter



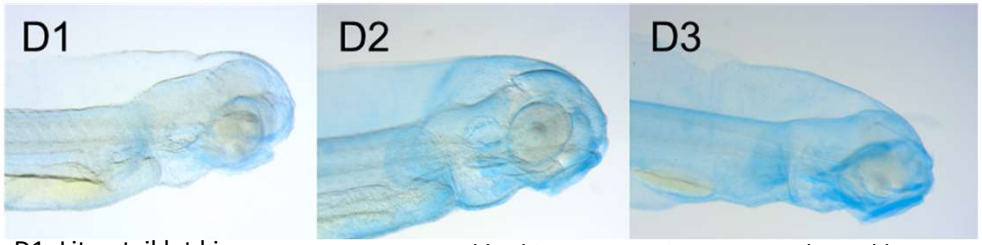
Plommesekkareal



Kjeve- og hodelengde



Kjeveutvikling

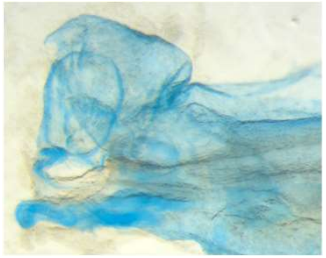


D1: Lite utviklet kjeve

D2: Noe utviklet kjeve

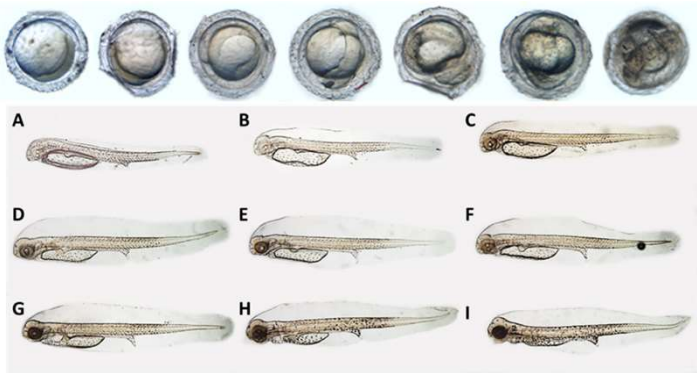
D3: Kjever godt utviklet

Deformiteter



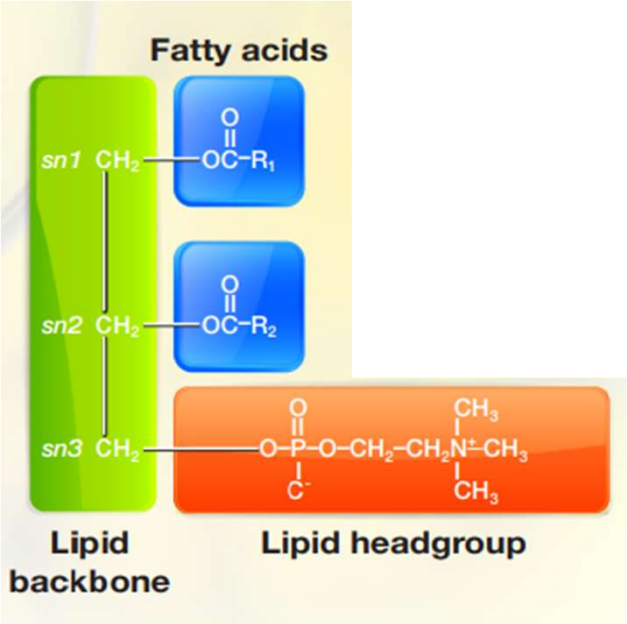
Kvalitetsdata – input til statistikk

- Mangler pålitelige data på befruktnings- og klekkesuksess
- Ingen replikasjon (en batch pr gruppe)
- 22 utvalgte parametere
- Relevans som eggkvalitetsmål?



Parameter	Time point	BG-1	NOV-A	NOV-B	NOV-C	Stord1	Stord2
Egg resp	1 dpf	1.39	1.74	3.00	1.99	1.74	1.33
Dry weight	1 dpf	83.46	91.46	95.52	87.94	90.64	98.57
C egg	1 dpf	43.48	45.43	45.89	42.04	44.87	44.78
N egg	1 dpf	9.85	9.88	9.93	8.94	9.67	9.89
CN ratio egg	1 dpf	4.42	4.60	4.62	4.70	4.64	4.53
Total egg diameter	1 dpf	1.098	1.07	1.084	1.05	1.11	1.1
Chorion diameter	1 dpf	0.899	0.843	0.881	0.855	0.902	0.859
Gumlayer diameter	1 dpf	0.100	0.113	0.102	0.098	0.104	0.121
Egg yolk volume	1 dpf	0.246	0.094	0.124	0.145	0.087	0.098
Egg volume	1 dpf	0.697	0.644	0.669	0.606	0.717	0.697
Chorion volume	1 dpf	0.383	0.322	0.359	0.327	0.385	0.332
Gumlayer volume	1 dpf	0.314	0.322	0.310	0.280	0.332	0.365
Δresp	Change between 1-16 dpf	0.4350	0.4980	0.4160	0.8211	0.2734	0.3680
ΔDW/ΔT	Change between 1-16 dpf	-3.0230	-3.0584	-3.5871	-2.9593	-2.6539	-4.0167
ΔC/ΔT	Change between 1-16 dpf	-2.3540	-1.6940	-1.8554	-1.6825	-1.6633	-1.8098
ΔN/ΔT	Change between 1-16 dpf	-0.6222	-0.3525	-0.3846	-0.3459	-0.3447	-0.4022
ΔCN/ΔT	Change between 1-16 dpf	0.0639	-0.0149	-0.0171	-0.0118	-0.0132	0.0021
Eye diameter	8 dpf	0.2068	0.2111	0.2022	0.1763	0.1846	0.1926
Body area	8 dpf	1.1473	1.0985	1.0427	1.0794	1.0541	1.0413
Yolk area	8 dpf	0.2787	0.2854	0.2025	0.3068	0.3028	0.2802
Yolk fraction	8 dpf	0.2431	0.2586	0.1939	0.2838	0.2867	0.2703
Standard length	8 dpf	3.7826	3.5181	3.3905	3.4760	3.4765	3.5254

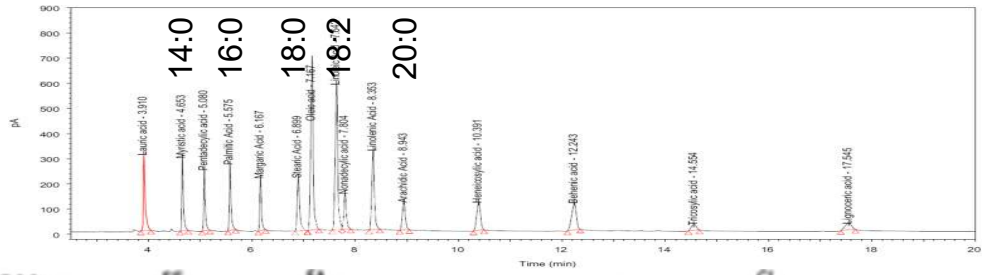
Lipidanalyser

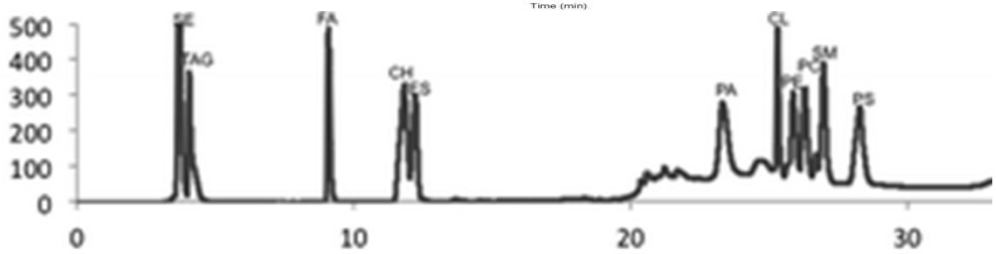


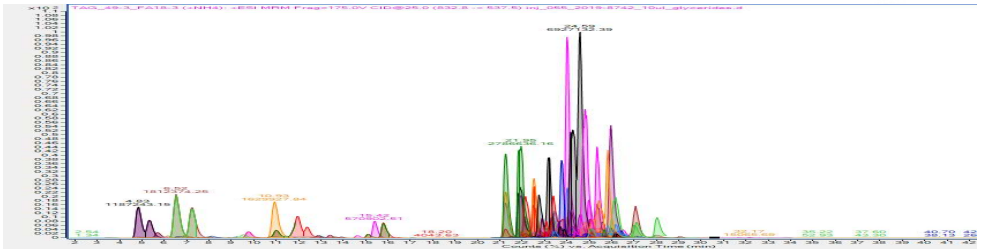
fettsyre-sammensetning

lipidklasse analyse

lipidomics

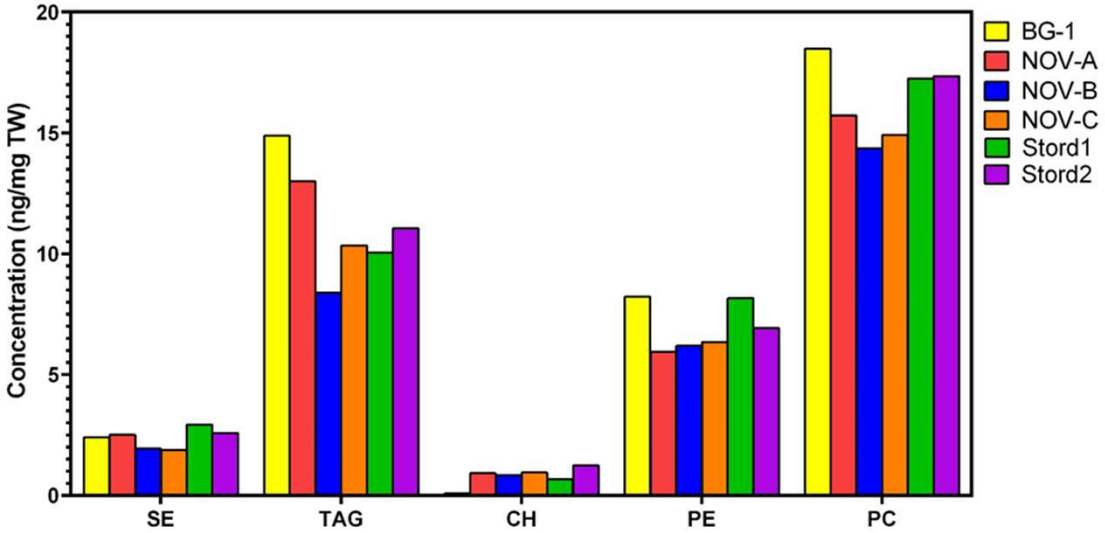






Totallipidinnhold og lipidklasser

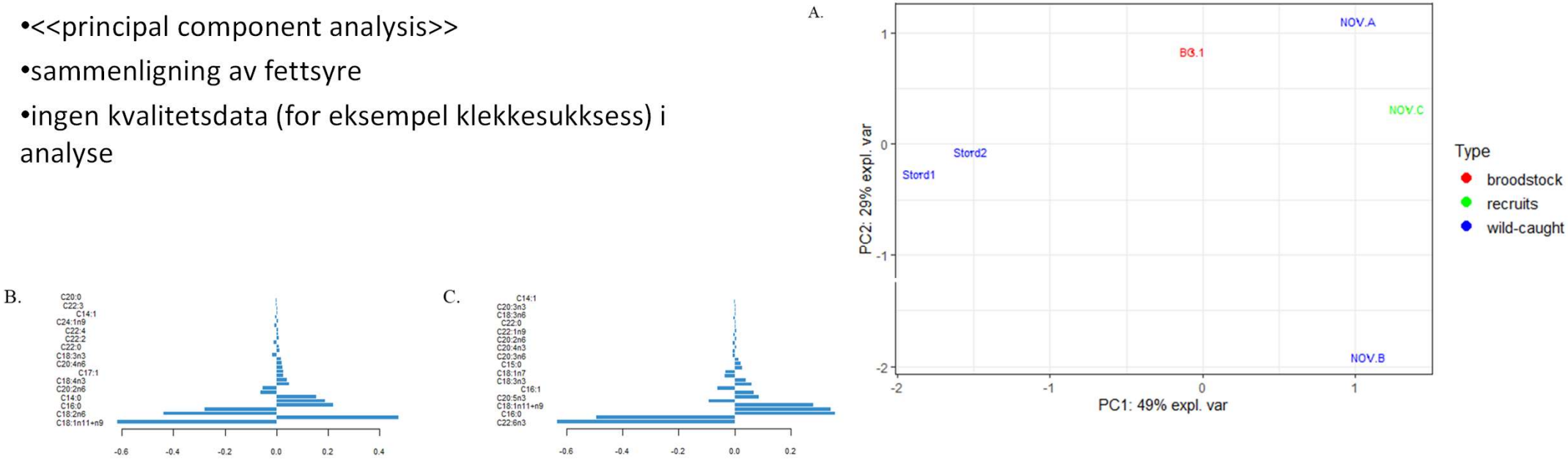
Batch ID	total weight (mg)	total lipid content (mg)	% lipid content
BG-1	37.64	n.d.	NA
Stord1	50.31	0.29	0.58%
Stord2	65.55	0.29	0.44%
NOV-A	72.93	0.59	0.81%
NOV-B	76.71	0.65	0.85%
NOV-C	72.99	0.73	1.00%



Batch ID	
BG-1	broodstock
Stord1	wild-caught
Stord2	wild-caught
NOV-A	wild-caught
NOV-B	wild-caught
NOV-C	recruits

Fettsyresammensetning

- <<principal component analysis>>
- sammenligning av fettsyre
- ingen kvalitetsdata (for eksempel klekkesukksess) i analyse



Fettsyresammensetning

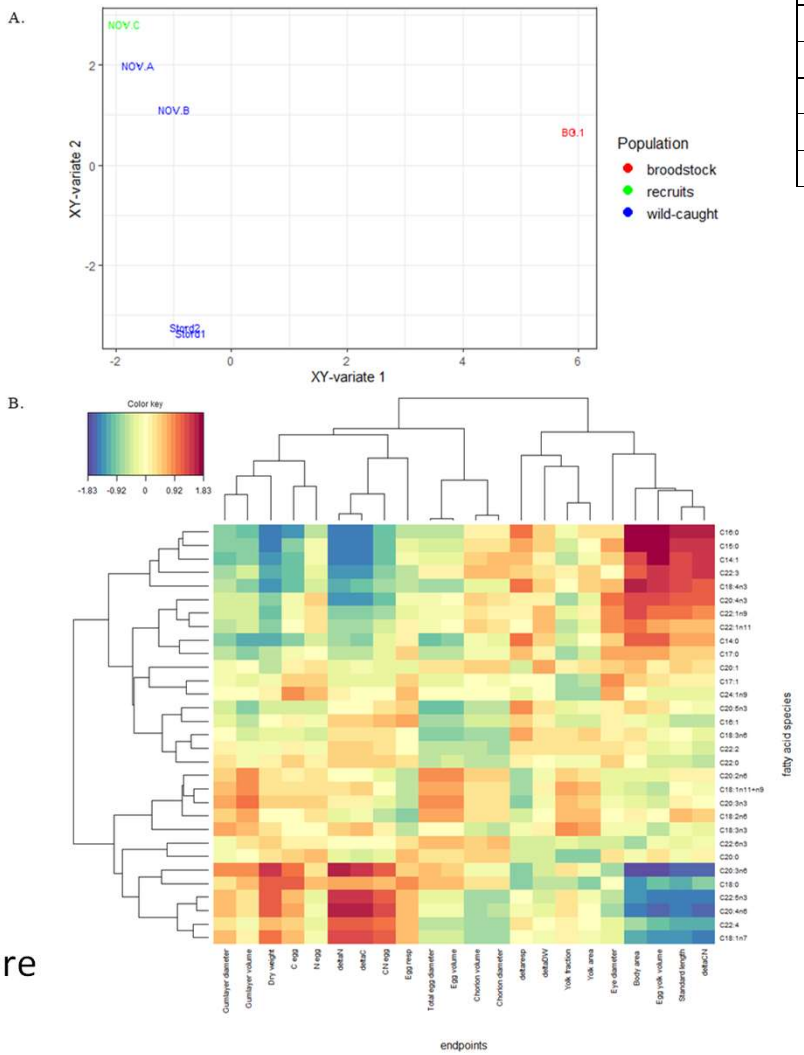
•<<(sparse) partial least squares>> analyse
•sammenligner fettsyrer med kvalitetsparametere

•<<clustered image map>>
•rødt -> positiv korrelasjon
•blått -> negative korrelasjon

•C16:0, C15:0 og C14:1 korreleres med Δ CN, myotomlengde, plommesekkareal og kroppsareal

•C20:4n6 og C20:3n6 negative-korreleres med same parametere

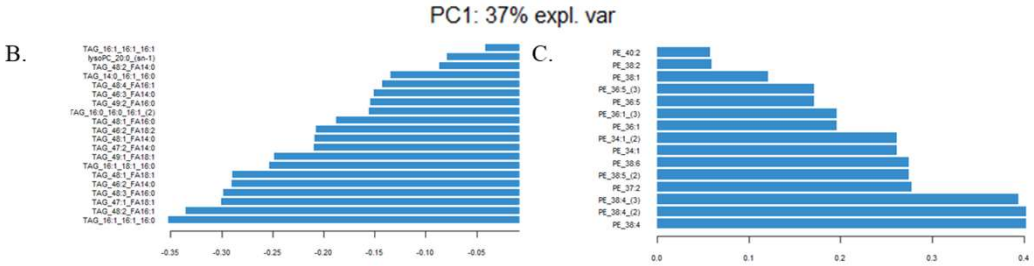
Batch ID	
BG-1	broodstock
Stord1	wild-caught
Stord2	wild-caught
NOV-A	wild-caught
NOV-B	wild-caught
NOV-C	recruits

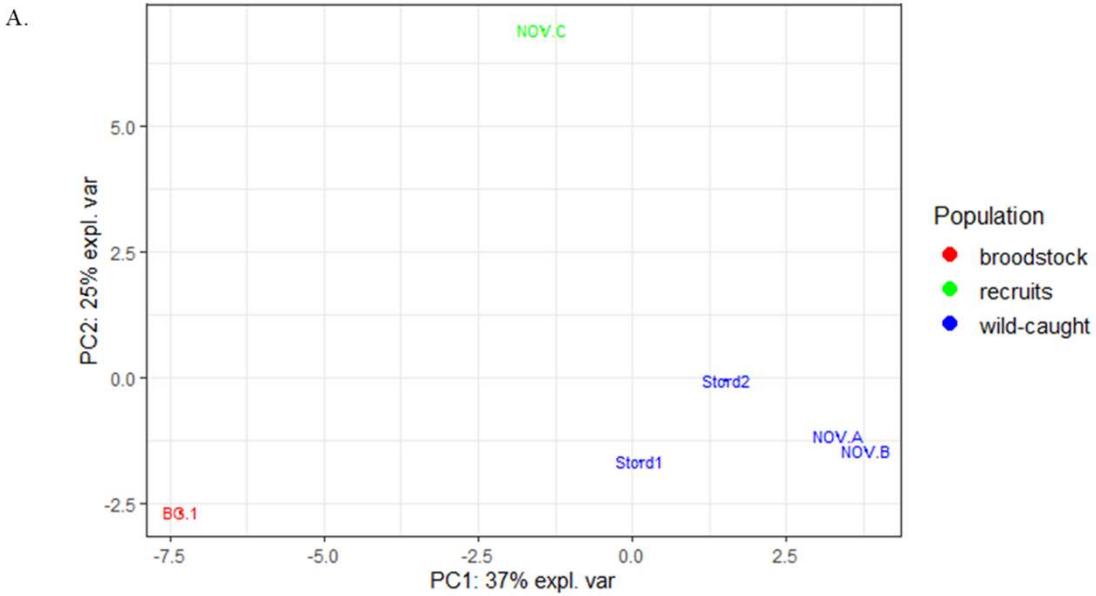


Batch ID	
BG-1	broodstock
Stord1	wild-caught
Stord2	wild-caught
NOV-A	wild-caught
NOV-B	wild-caught
NOV-C	recruits

Lipidomics

- <<(sparse) principal compontent analysis>>
- sammenligning av begrenset antall lipider
- ingen kvalitetsdata (for eksempel klekkesukksess) i analyse

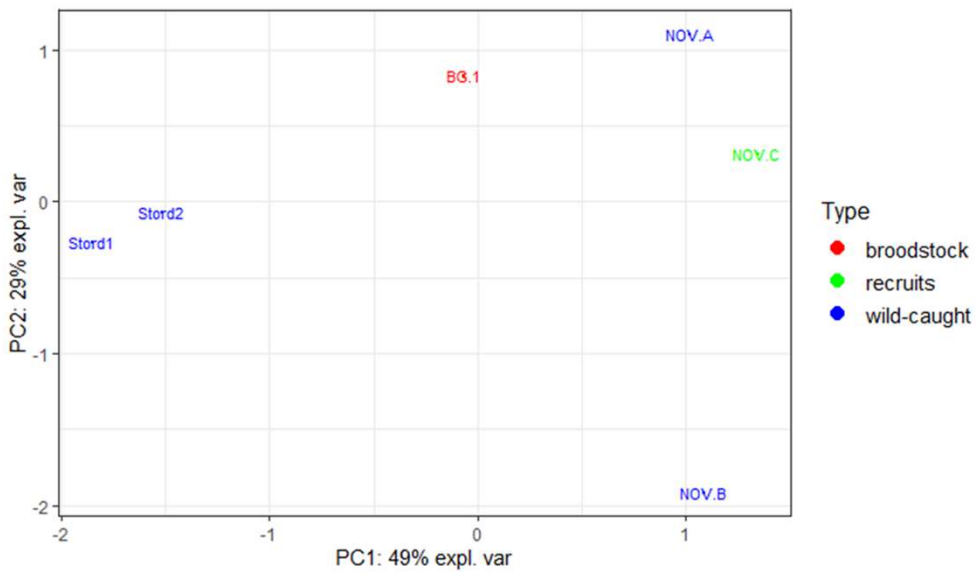




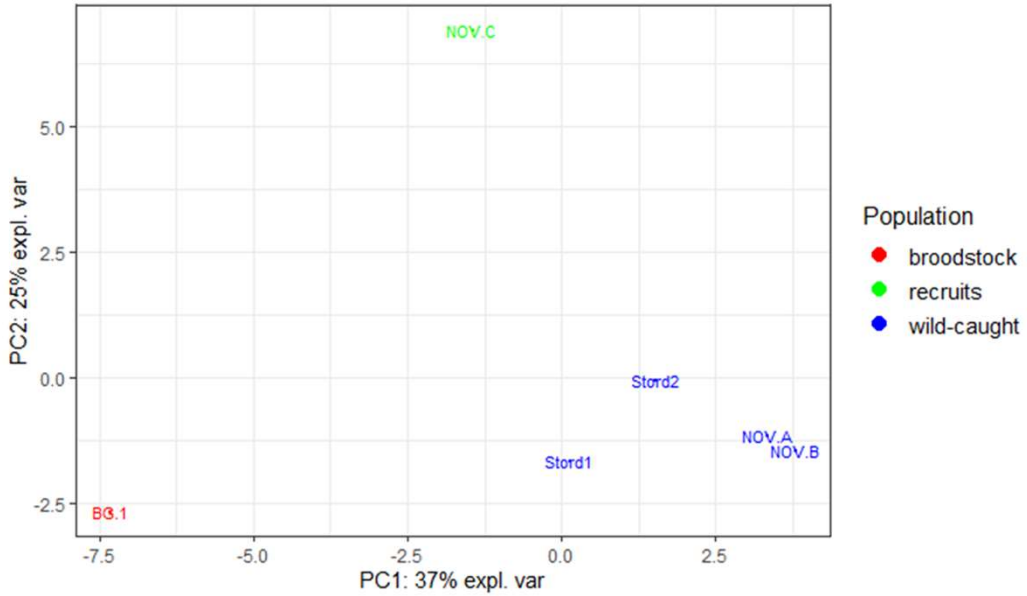
Batch ID	
BG-1	broodstock
Stord1	wild-caught
Stord2	wild-caught
NOV-A	wild-caught
NOV-B	wild-caught
NOV-C	recruits

Fettsyresammensetning vs lipidomics

FAA



lipidomics

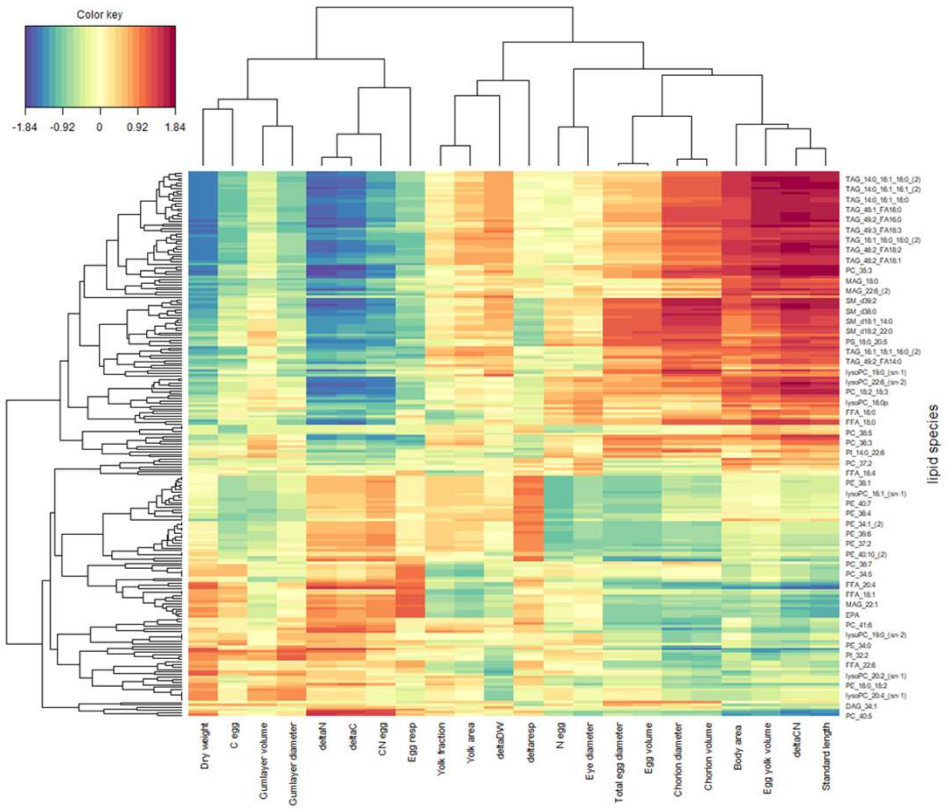


Lipidomics

•<<clustered image map>>
•sammenligner lipidomics med kvalitetsparametere
•rødt -> positiv korrelasjon
•blått -> negative korrelasjon

•<u>Noen</u> triglyserider (TAG) og fosfotidylkoliner (PC) positiv-korreleres med vekst-parametere

•<u>Noen</u> fosfotidyletanolaminer (PE) negativ-korreles med vekst-parametere



Diskusjon

Konklusjoner

•automatiske biometrianalyser kan brukes til å monitorere larveutvikling

•både fettsyresammensetning og lipidomics kan korreleres med kvalitetsparametere

Begrensinger

•biologiske replikater kreves for en ordentlig statistikkanalyse

•data bør bekreftes

•omics data er degenerative: flere løsninger for samme dataset

Veien videre

•lipidomics som biomarkøranalyse: kan vi predikere eggkvalitet?

•kan fôrregime forandres basert på lipidomics data?

Internasjonalt fremragende sammen

SINTEF

NTNU