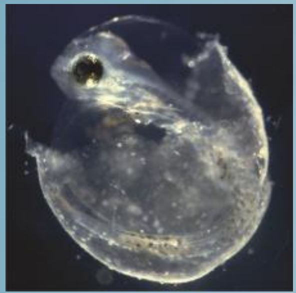
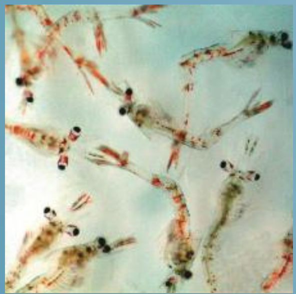
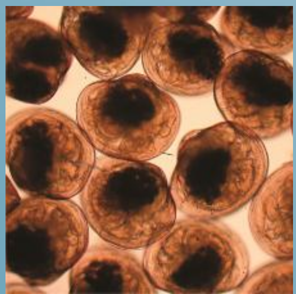
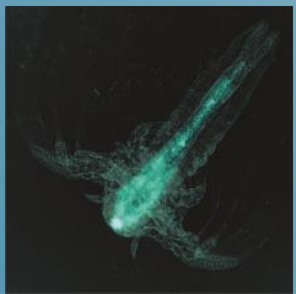



larvi 2013

6th fish & shellfish larviculture symposium

The function of wax esters in larval fish transition from endogenous to exogenous nutrition freshwater fish, exception or the rule?

Konrad Dabrowski

ghent university, belgium, 2-5 september 2013

The function of wax esters in larval fish transition from endogenous to exogenous nutrition. Are freshwater fish the exception or the rule?

Konrad Dabrowski & **Malgorzata Korzeniowska**

School of Environment and Natural Resources , Ohio State University, U.S.A.

Troy M. Farmer, **Stuart A. Ludsin** & **Elizabeth A. Marschall**

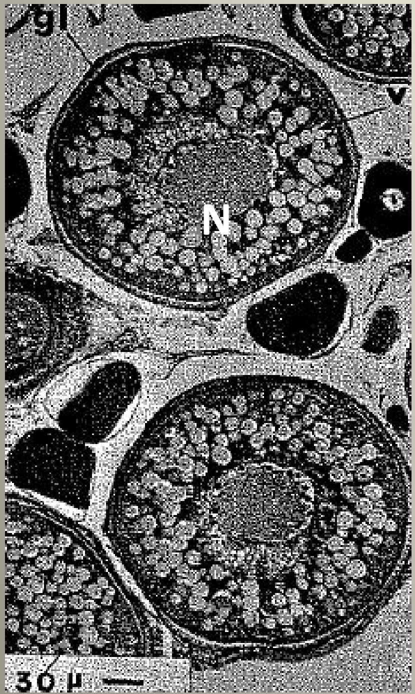
Department of Evolution, Ecology and Organismal Biology, OSU

Plan of presentation

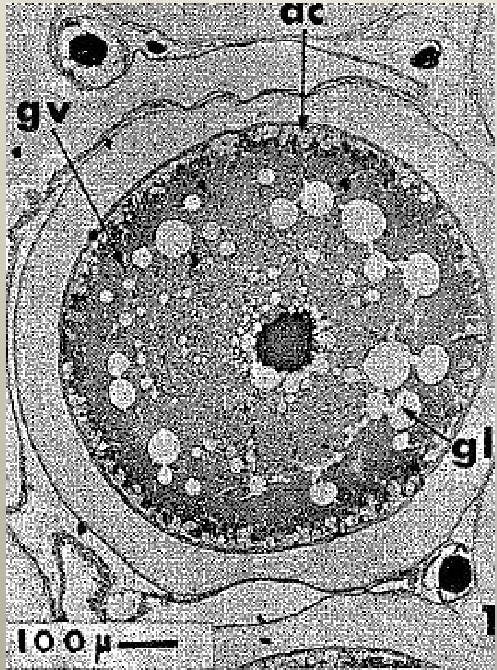
1. Oogenesis and early life history of yellow perch
2. Yolk utilization and critical points in early life history
3. Function of lipids and fatty acids in larval fish viability
4. Materials and Methods
5. Results
6. Lipids classes and fatty acid in yellow perch
7. Why waxes?
8. New research directions to link perch waxes to physical environment during early life and recruitment

Yellow perch oogenesis in North America

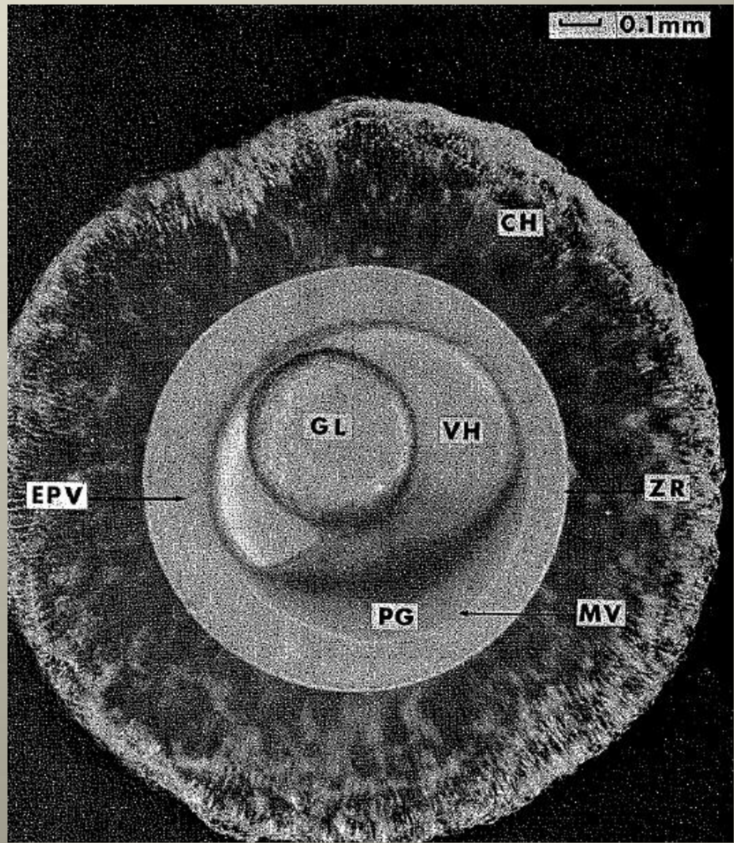
(Malservisi and Magnin 1968. Nat. Canad.)



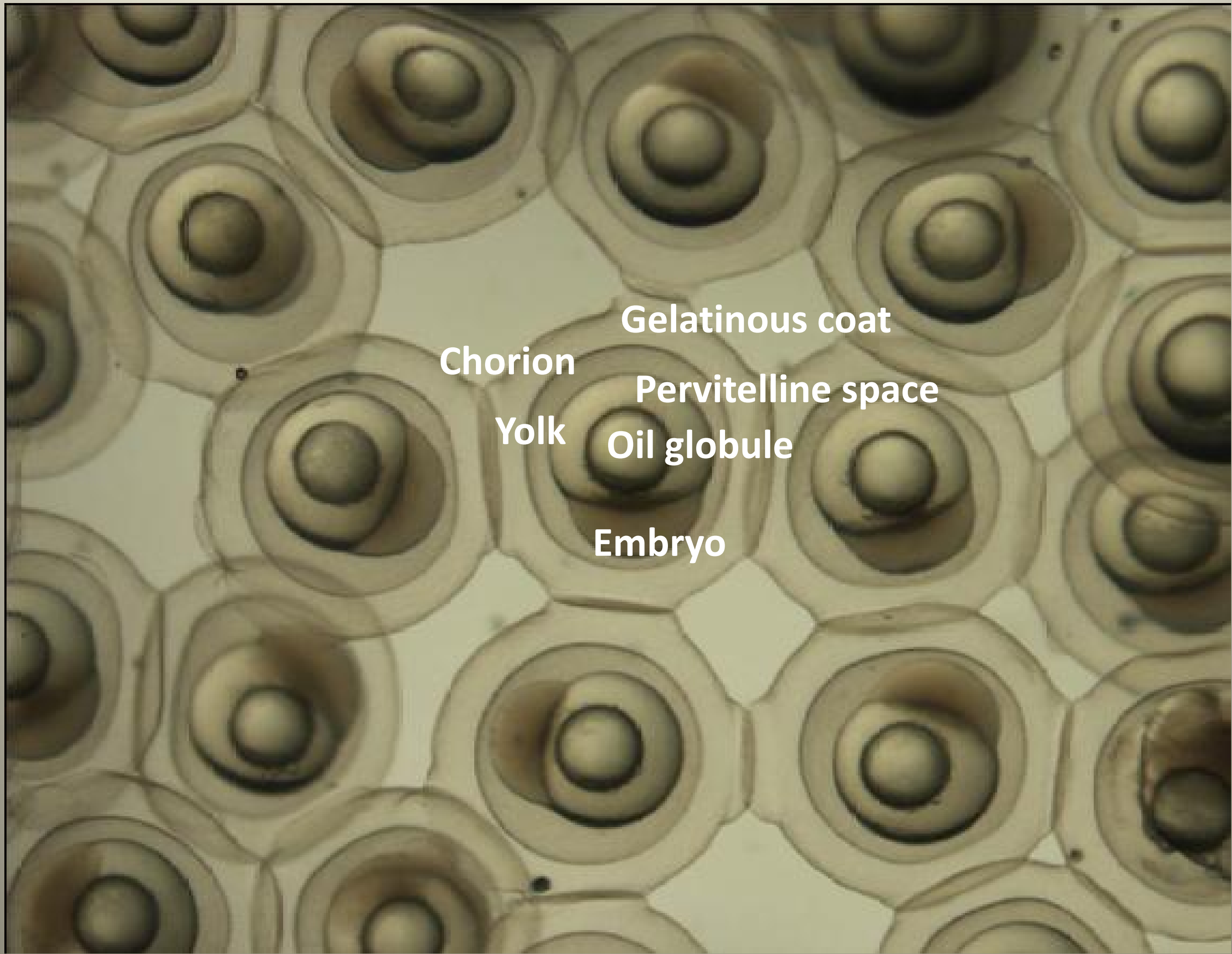
Lipid dropletes (gl), yolk
Vesicles (v), nucleus (N).



Oocyte in the process of hydration,
Oil globule (GL), pervitelline space
(EPV), germonal cell (PG).

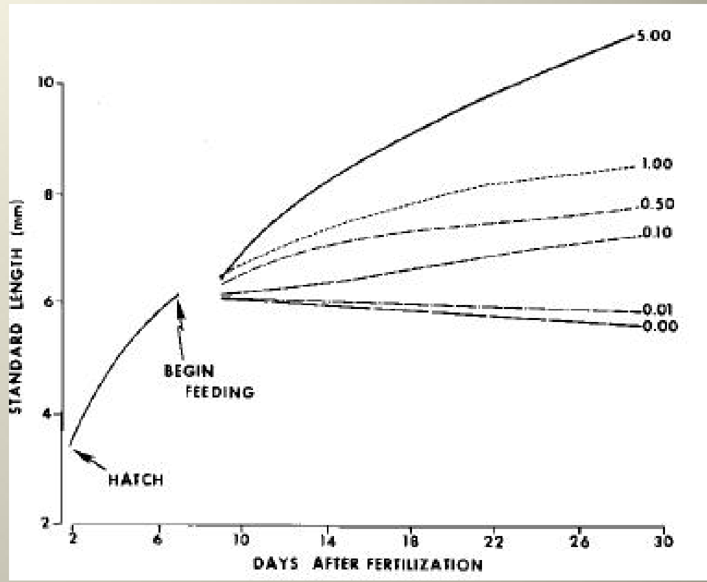


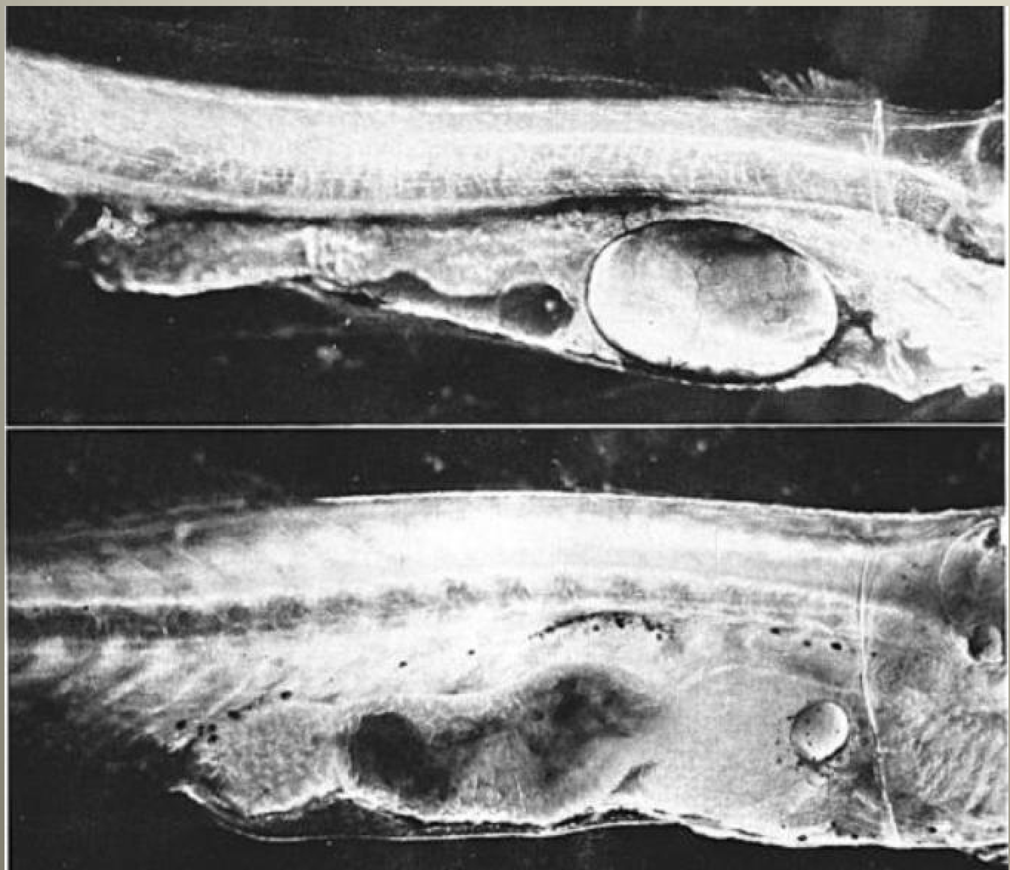
Perch (*Perca flavescens*) blastula…….. 100% fertilization



Oil globule utilization in striped bass larvae

(Eldridge et al. 1981.TAFS 110:111)

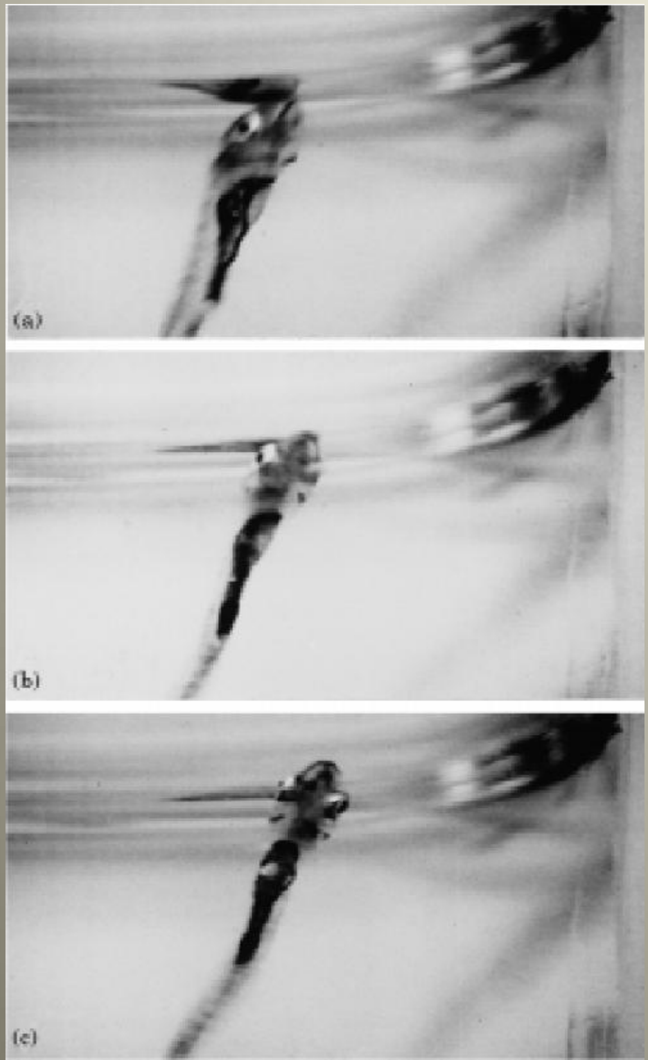




Larva starving for 12 days after hatching

Larva feeding for 12 days after hatching

Swim bladder inflation process and physical conditions accompanying the successful event in walleye (Regier and Summerfelt 2001. J.Fish Biol. 53:93)



1. Water surface tension
2. Duration of pneumatic duct opening
3. Swim bladder lipid content and profiles

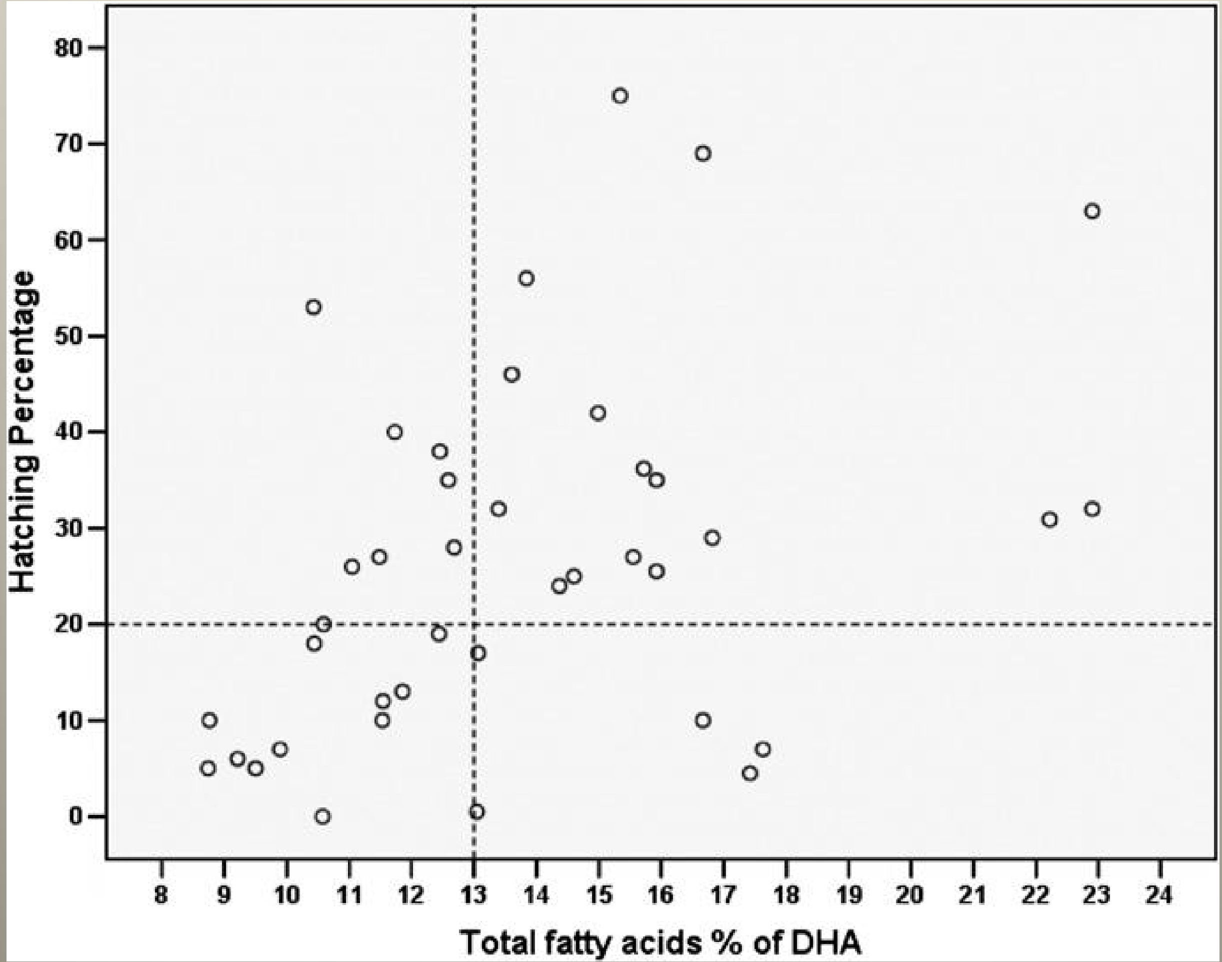
Are there alternative strategies for larvae of different fish species that do not rely on maternal phospholipids?

Sargent J. et al. 1999. Aquaculture 179: 217

"Marine fish phospholipids: the gold standard in larval fish nutrition"

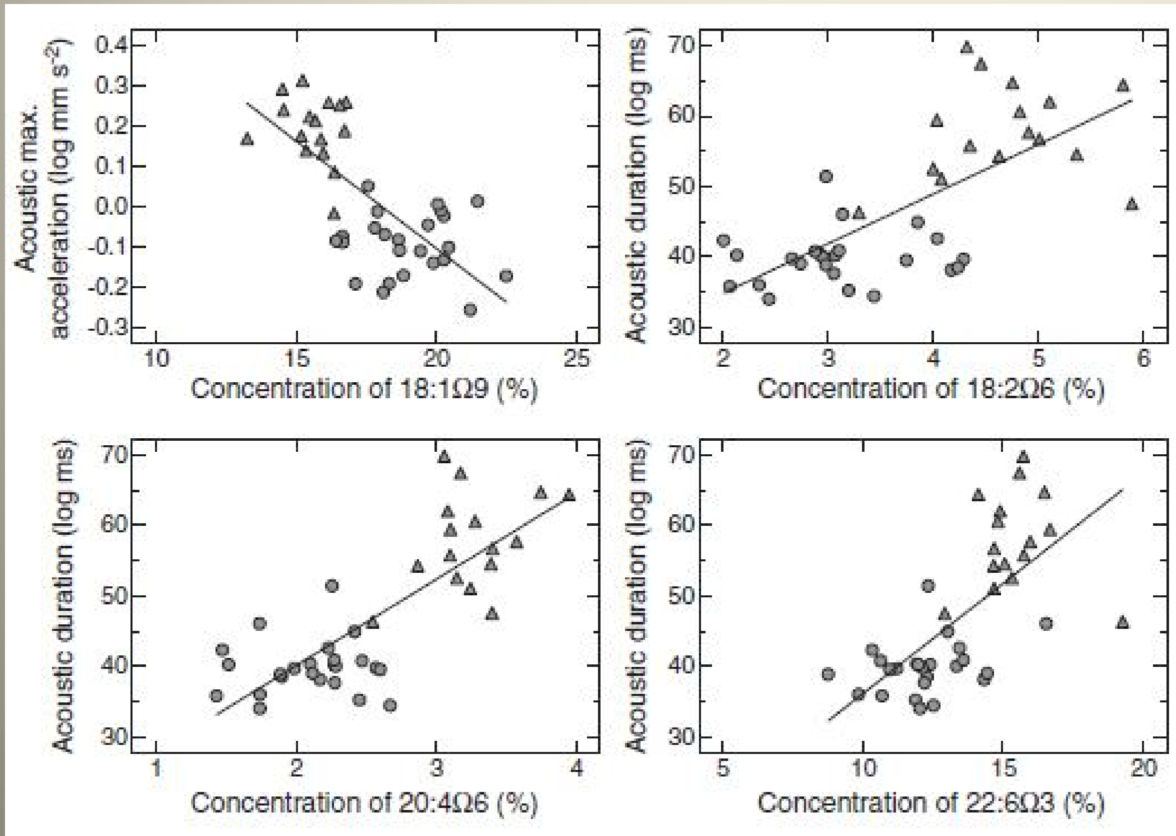
- Fish larvae have a limited ability to biosynthesise phospholipids *de novo* but can exchange fatty acids within and between PL and TG.
- Dietary phospholipids can, in principle, be utilized (assimilated) unchanged.
- Ideal larval diet is the one that matches yolk sac and natural exogenous diet lipid composition.

Effect of fatty acid (specifically DHA, C22:6n3) composition in eggs on embryo viability in common snook (Atlantic, Florida) (Yanes-Roca et al. 2009. Aquaculture 287: 335).

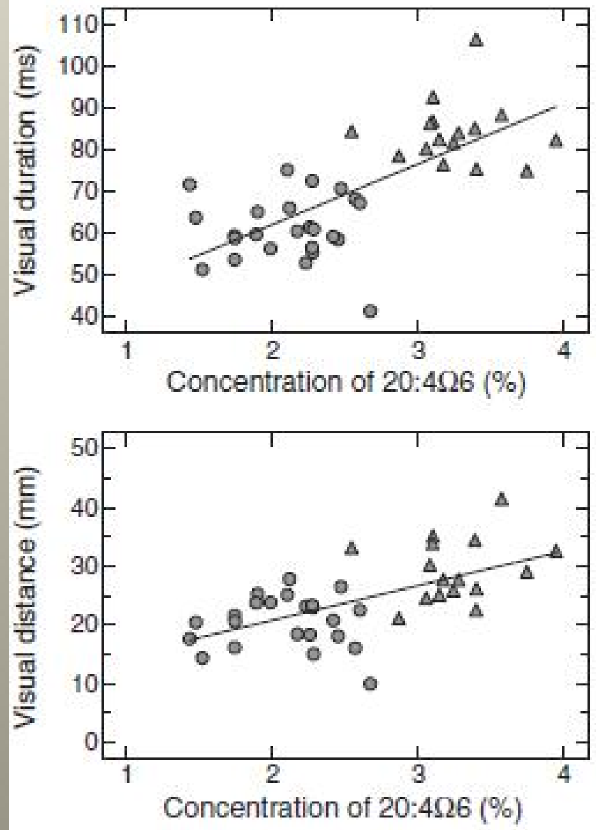


Effect of snook eggs DHA level on hatching rate in 2002 - 2005

Fatty acids affect antipredator performance of red drum larvae (Fuiman, Ojanguren 2011. J.exp.Mar.Biol.Ecol. 407:155



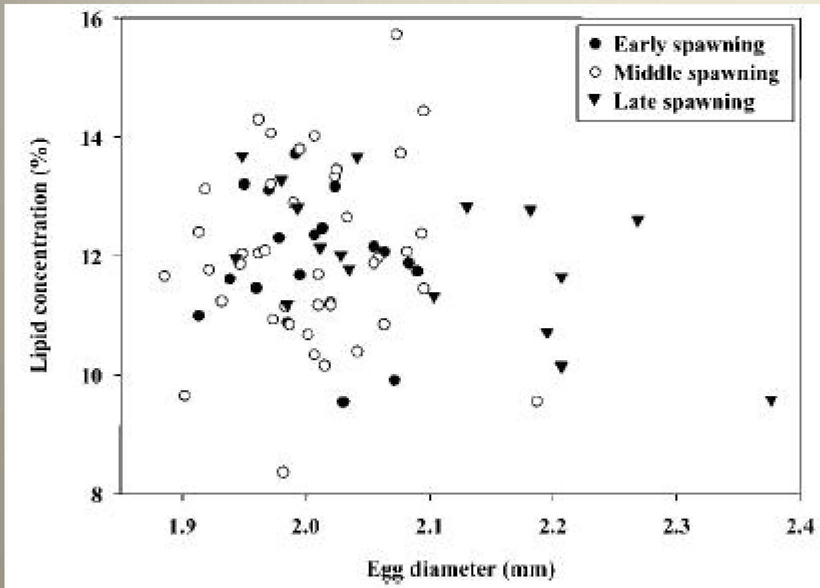
Lighthouse Restaurant (Corpus Christi, TX) - August 1988

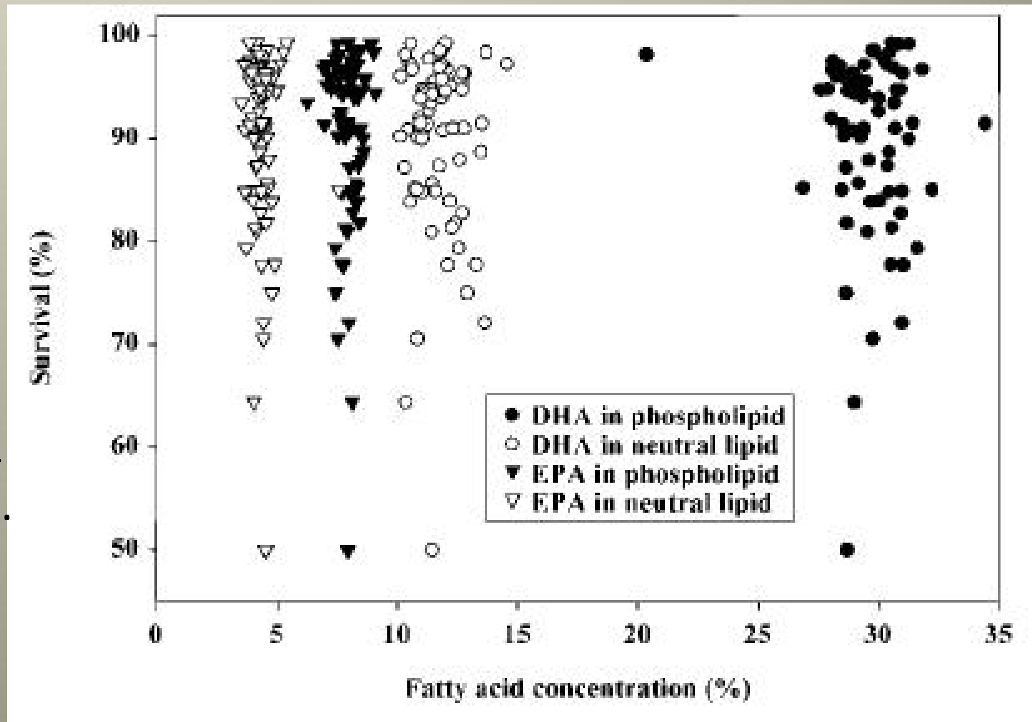


Conclusions:

1. Low ARA and DHA reduced escape behavior in larvae
2. Reduced maternal essential FA was not compensated in initial feeding

Evaluation of walleye egg lipid/fatty acid composition in relation to embryo viability (survival to eyed stage) (Czesny, Dabrowski 2005. North Am.J.Fisheries Manag. 25: 122).



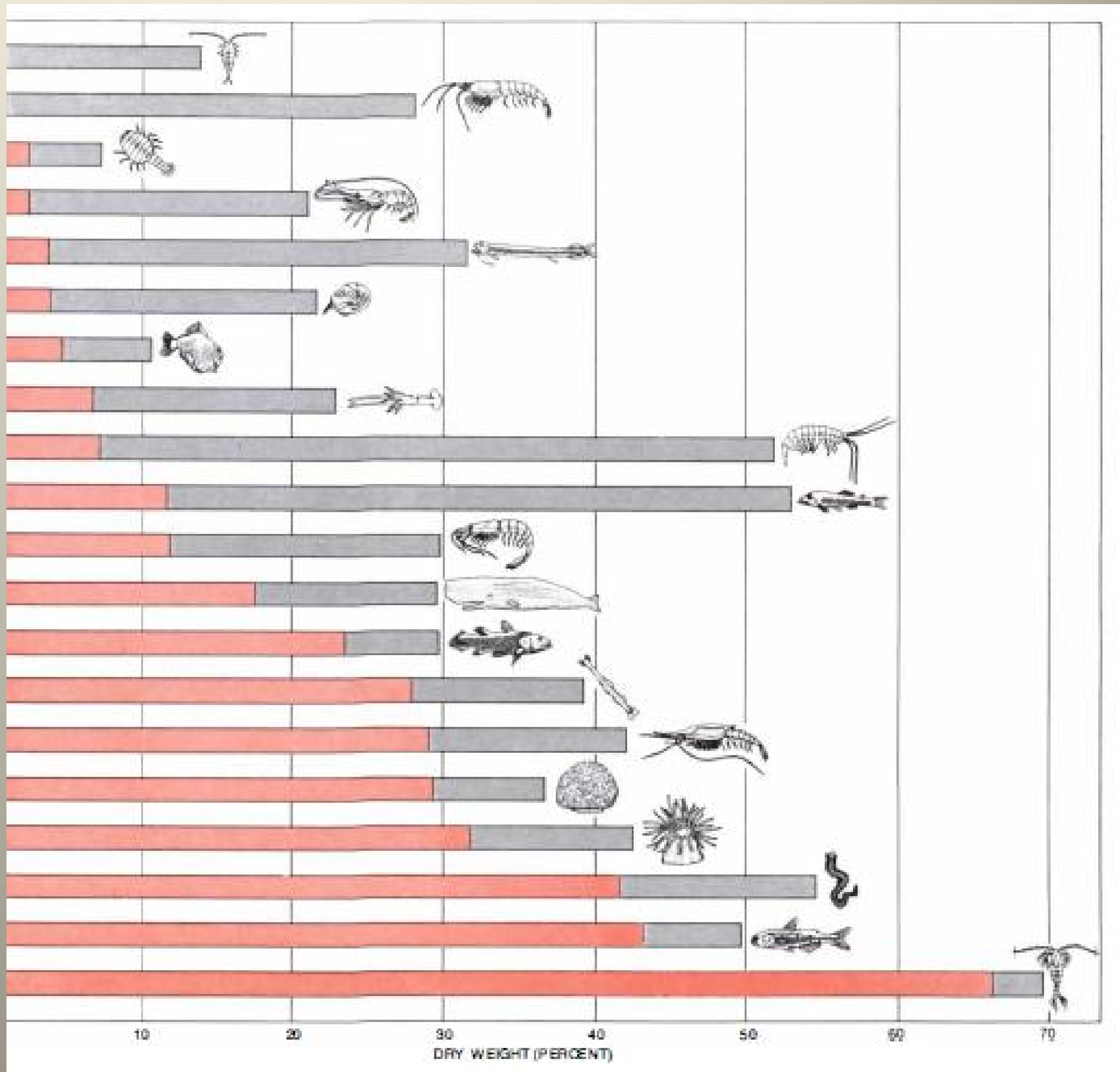


Conclusions:

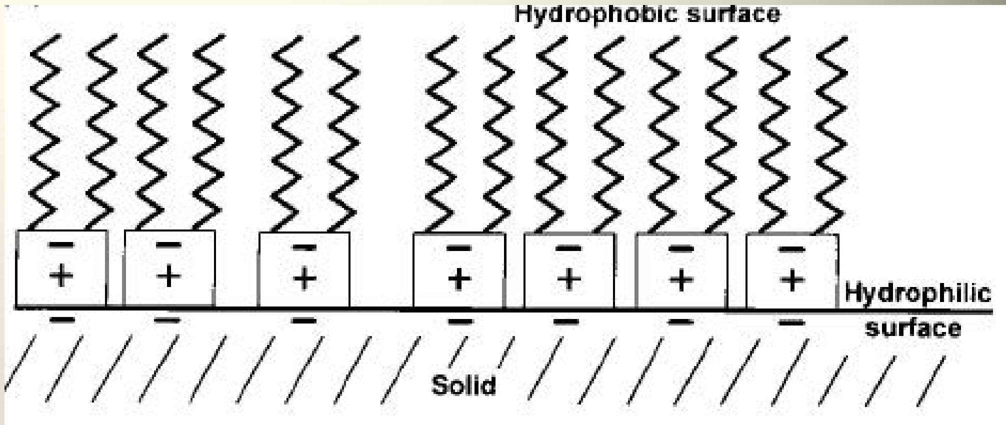
1. Polyunsaturated fatty acid (PUFA) are at higher concentration in PL than in NL
2. Lipid content was unrelated to egg size.
3. PUFA concentration play no role in high viability of eggs (90%).

The role of wax esters in marine food chains, phytoplankton, copepods, fish (Benson 1975. Scientific American

Calanus plumchrus



Wax esters biological insights and advantages over triglycerides and phospholipids!



Hills 2002

1. Hydrophobic lipids in the larval fish (swim bladder and pneumatic duct) will facilitate inflation by acting as surfactants (lubricants) and preventing inner surfaces adhering and collapsing.

2. Osmoregulatory functions of larval fish enhanced (preventing transepidermal water rush)

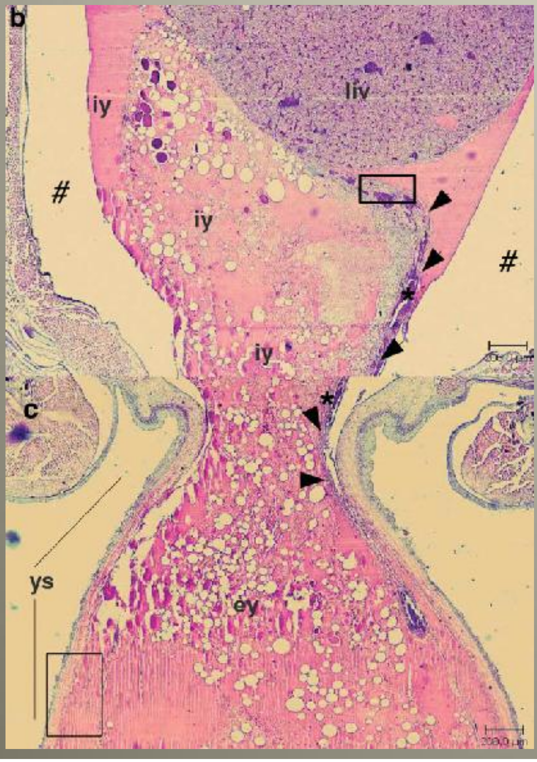
3. Preventive role in bacterial and viral attachments and infections

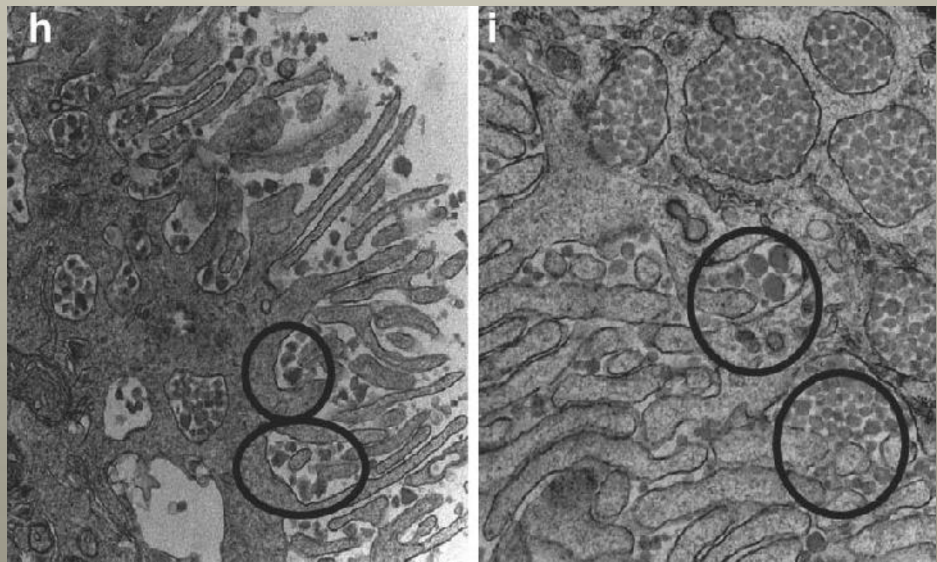
4. Waxes have lower susceptibility to oxidation than other lipids (higher stability in low temperatures).

Comparative aspects of lipid mobilization, transport and utilization (digestion) – does "arowana" model applies to wax esters in "oil globule" possessing larval fish?

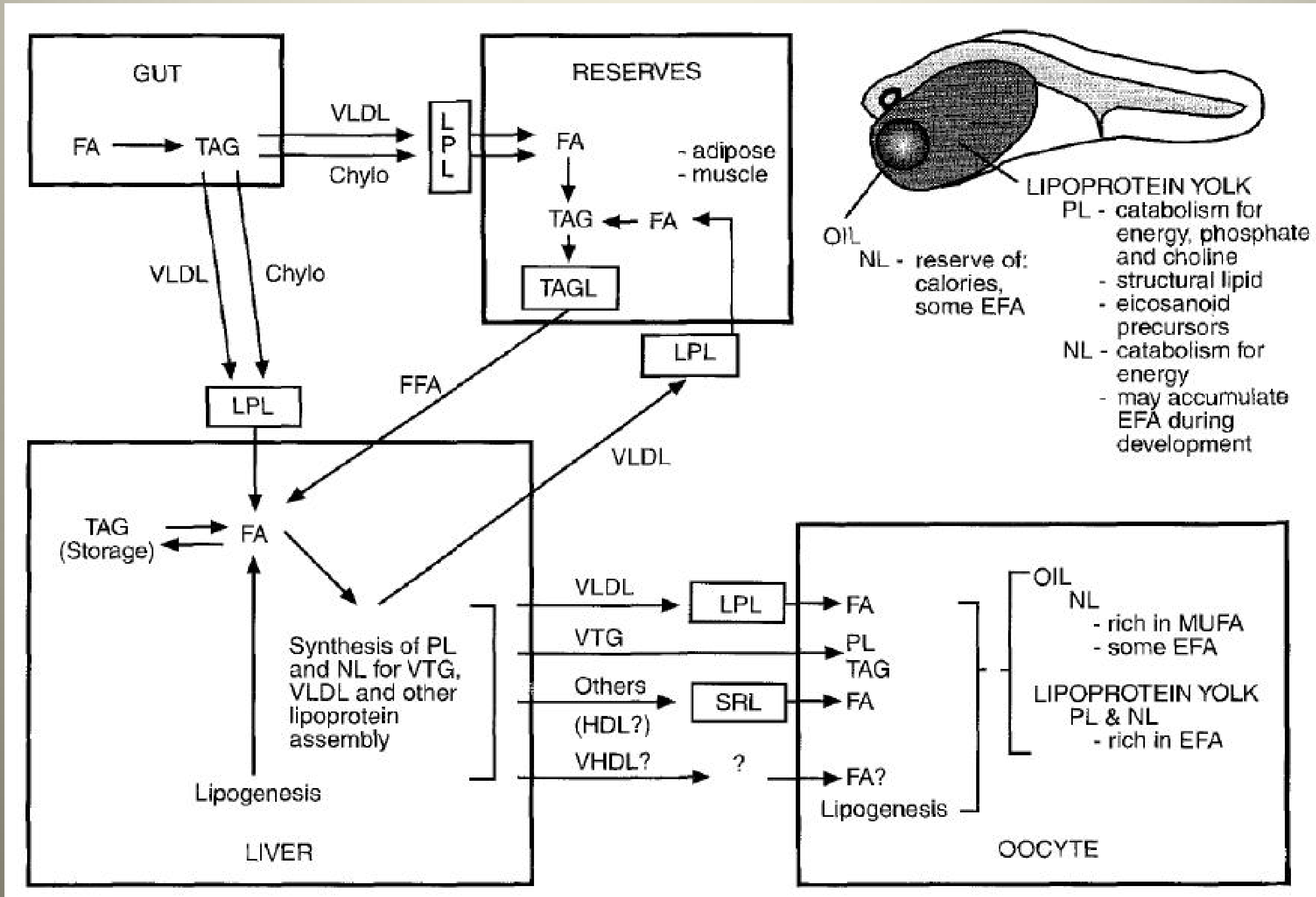
Mobilization of wax esters contained in yolk oil globule may proceed through earlier described "endocytotis" of microparticles (decreased polarity compared to TG) into circulation….

Jaroszewska, Dabrowski 2009. Anatomical Record 292: 1745.

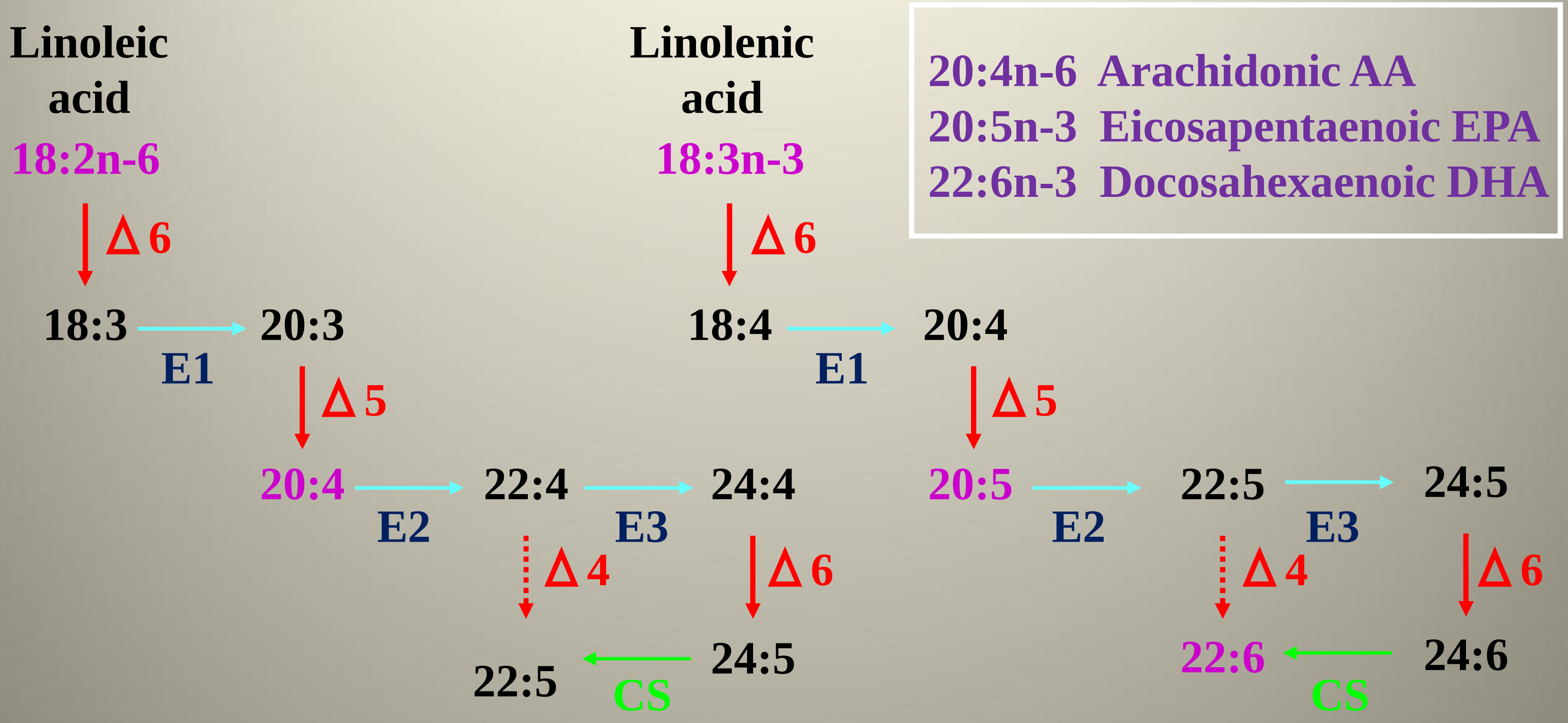




The fate of dietary lipids, synthesis, transfer, accumulation and metabolism in fish (Wiegand 1996. Reviews in Fisheries)



Pathways for the biosynthesis of C20 and C22 HUFA

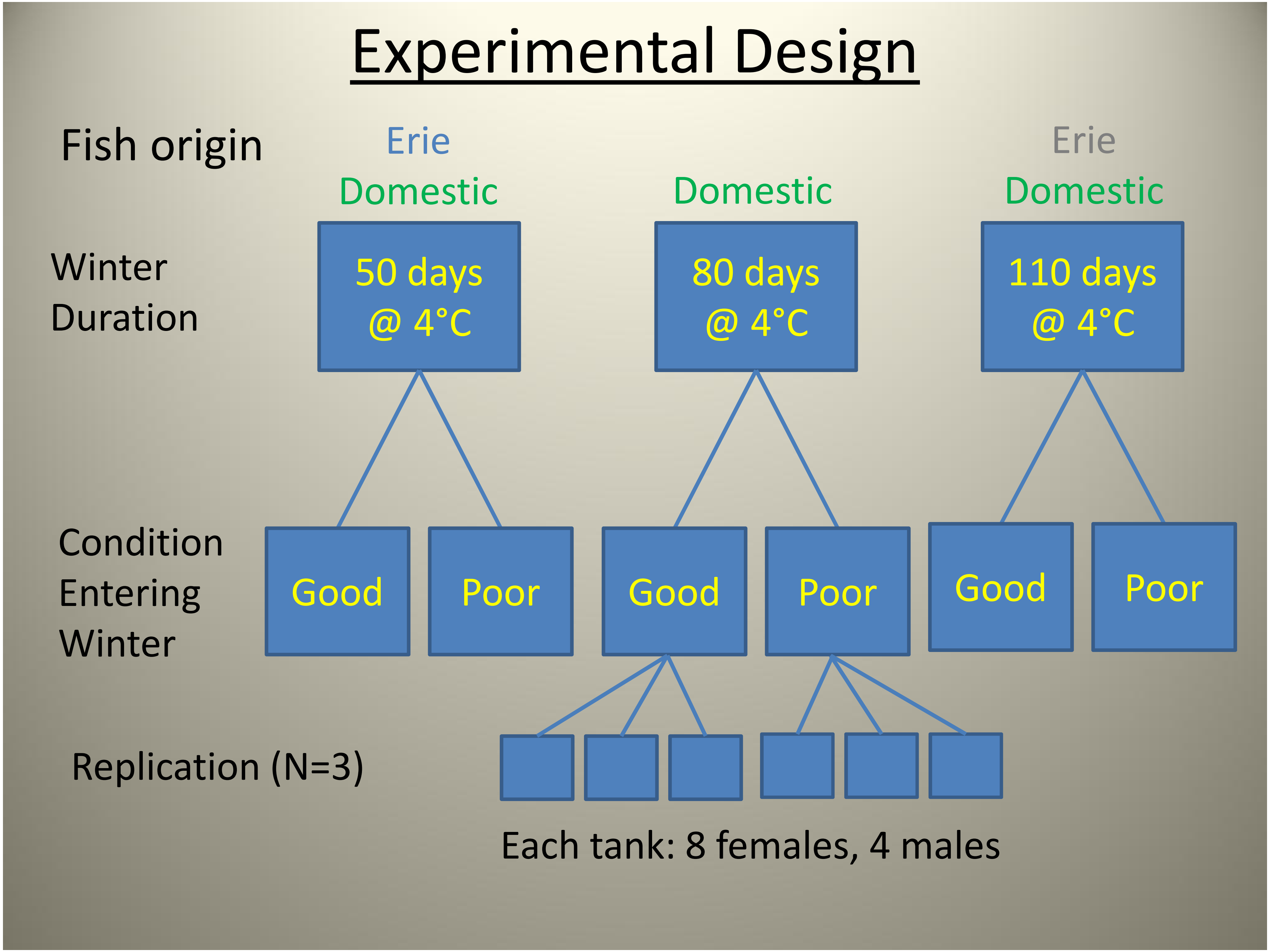


Δ : microsomal fatty acil desaturase

CS : peroxisomal chain shortening

E : microsomal fatty acil elongase

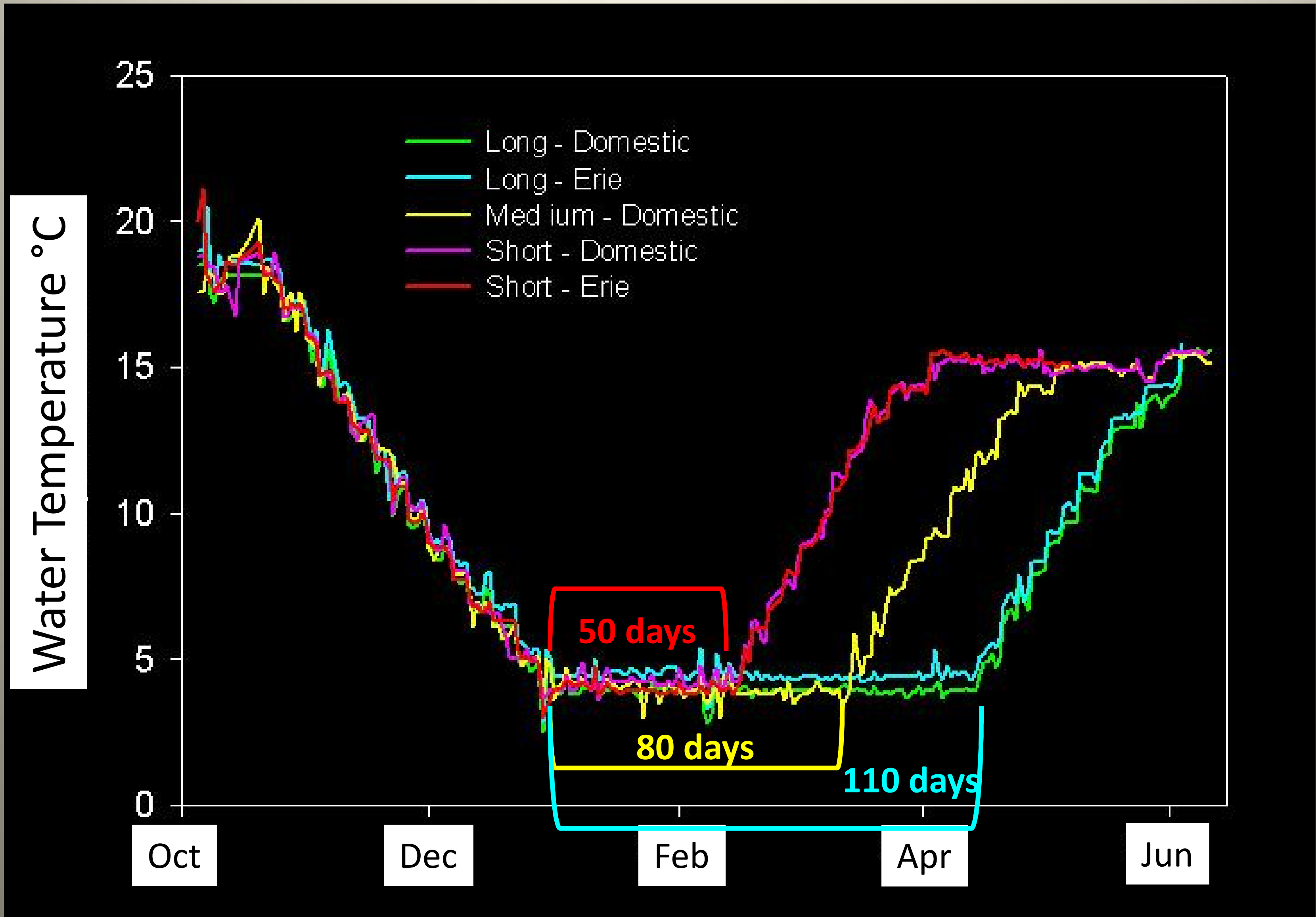
Materials and Methods



Experimental Design
Fish origin
Erie
Domestic
Domestic
Erie
Domestic
Winter Duration
50 days @ 4°C
80 days @ 4°C
110 days @ 4°C
Condition Entering Winter
Good
Poor
Good
Poor
Good
Poor
Replication (N=3)
Each tank: 8 females, 4 males

Laboratory Experiments

- Fall 2011 – Winter duration treatments began



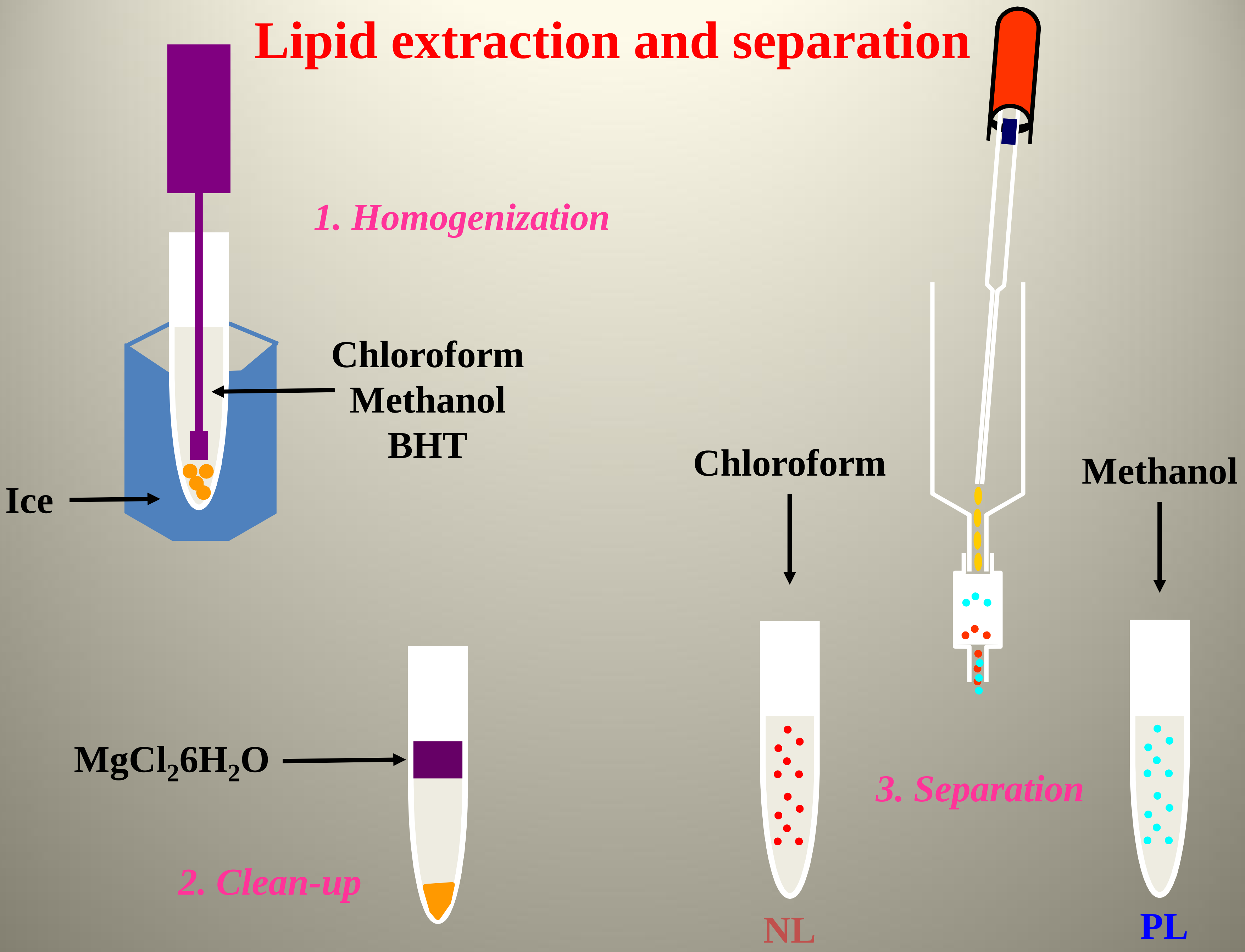
Methods of analysis:

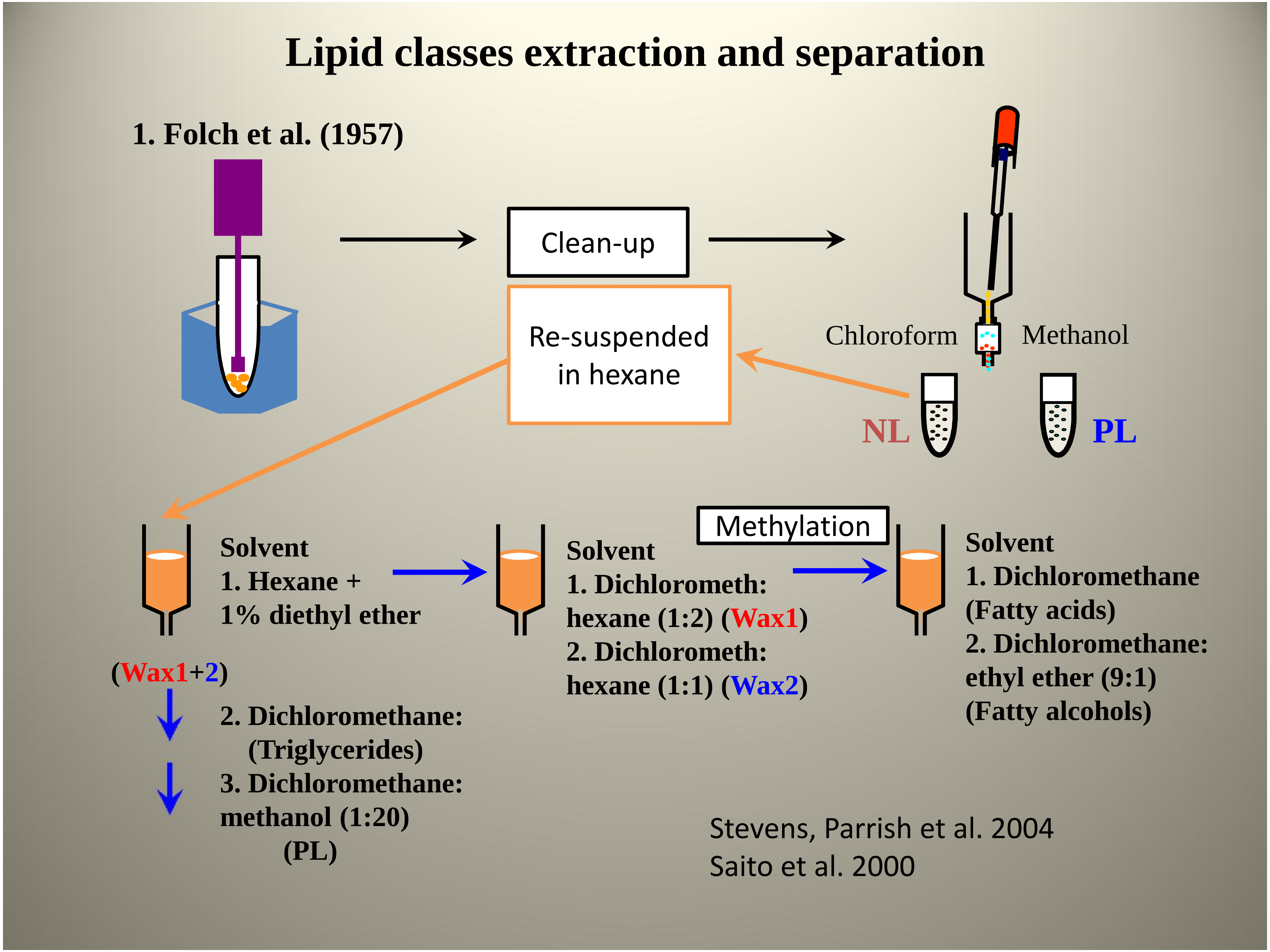
Lipid classes

Fatty acid composition

1. Separation of lipids into phospholipids (PL) and neutral lipids (NL)
2. Separation of the neutral lipid classes, triglycerides (TG) and wax esters (WE)
3. Methylation of fatty acids (FAME) and fatty alcohols (FAL).

Lipid extraction and separation




Lipid classes extraction and separation
1. Folch et al. (1957)
Clean-up
Re-suspended in hexane
Chloroform
Methanol
NL
PL
Solvent
1. Hexane +
1% diethyl ether
(Wax1+2)
2. Dichloromethane:
(Triglycerides)
3. Dichloromethane:
methanol (1:20)
(PL)
Methylation
Solvent
1. Dichlorometh:
hexane (1:2) (Wax1)
2. Dichlorometh:
hexane (1:1) (Wax2)
Solvent
1. Dichloromethane
(Fatty acids)
2. Dichloromethane:
ethyl ether (9:1)
(Fatty alcohols)
Stevens, Parrish et al. 2004
Saito et al. 2000

Lipid classes and fatty acid composition of perch food (fathead minnows) offered in the course of gametogenesis 2011-2012

Category	Mean %			
	Oct. 13*	Nov. 11*	Jan. 20†	Feb. 2‡
Total Lipids	4.1	3.8	2.7	3.4
Neutral Lipids	69	67	58	64
Phospholipids	31	33	42	36
Linoleic C18:2 (n6)	0.05	0.02	0.02	0.07
Linolenic C18:3 (n3)	0.3	0.5	0.3	0.6
Arachidonic C20:5 (n3)	7.7	7.6	2.4	3.7
EPA C20:4 (n6)	0.01	0.03	0.03	0.02
DHA C22:6 (n3)	7.3	9.6	2.2	3.1
n3 / n6	9.4	13.2	6.5	4.3

* St. Mary's State Fish Hatchery (ODOW)

† Jones Fish Hatchery (Cincinnati, OH)

‡ R&R Sports Headquarters (Columbus, OH)

Proportion of neutral lipids in oocytes (eggs) of fish (% total lipids)

(after Wiegand 1996 unless reference given)

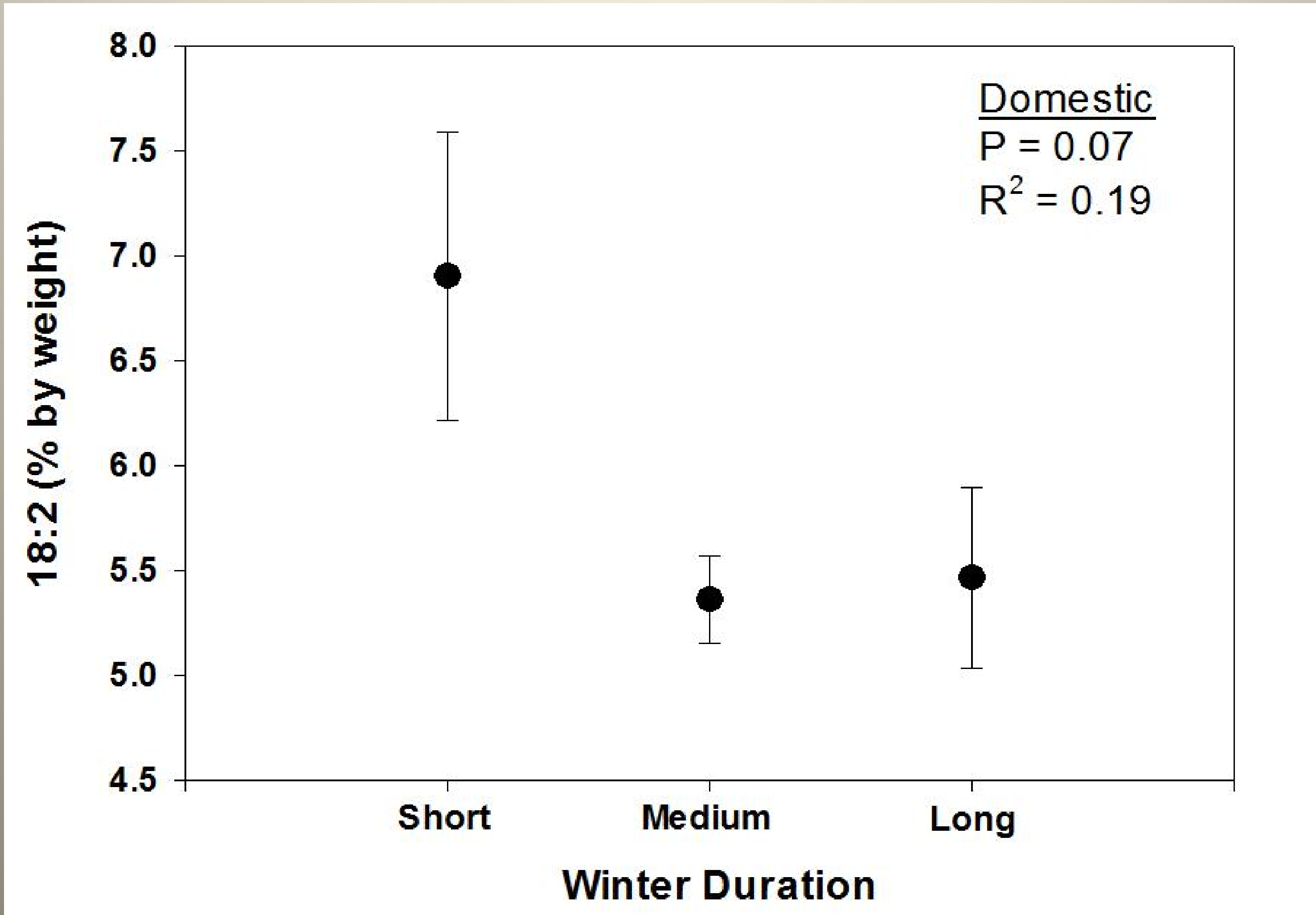
Lipid fraction	PL	NL	TG	WE
Eggs with no oil globule				
Cod	71.7		12.5	3.7
Atlantic halibut	70.8		12.9	4.3
Plaice	65.8		14.2	2.7
Eggs with oil globule				
Turbot	40.0		29.0	23.0
Rainbow trout [1,2]	49.7		46.8	0.2
	37.3	62.8	56.5	0
Japanese eel [3]	19.4	80.6		
Walleye [4]	21.8	78.2		0
Burbot	12.6		4.1	81.8
Striped bass [5]	11.0		11.0	79.0
Whitefish (*Coregonus sp.)* [6]	31.7		64.9	1.8
Perca fluviatilis [7]	13.5 – 17.3	82.4 - 86.5	8.1 - 12.4	68.0 – 68.9
(Eurasian perch)	14.3		1.1	83.7
P. flavescens **[8]**	**23.1**		**2.8**	**77.8**
(yellow perch)				

1 Watanabe et al. 1984; 2Vassalo-Agius, Watanabe 2001; 3 Furuita et al. 2006; 4 Wiegand et al. 2004; Czesny, Dabrowski 2005; 5 Sullivan; 6 Henrotte et al. 2010; 7 Kaitaranta, Ackman 1981; 8 present work

Linoleic acid 18:2n6

<u>Domestic females:</u>

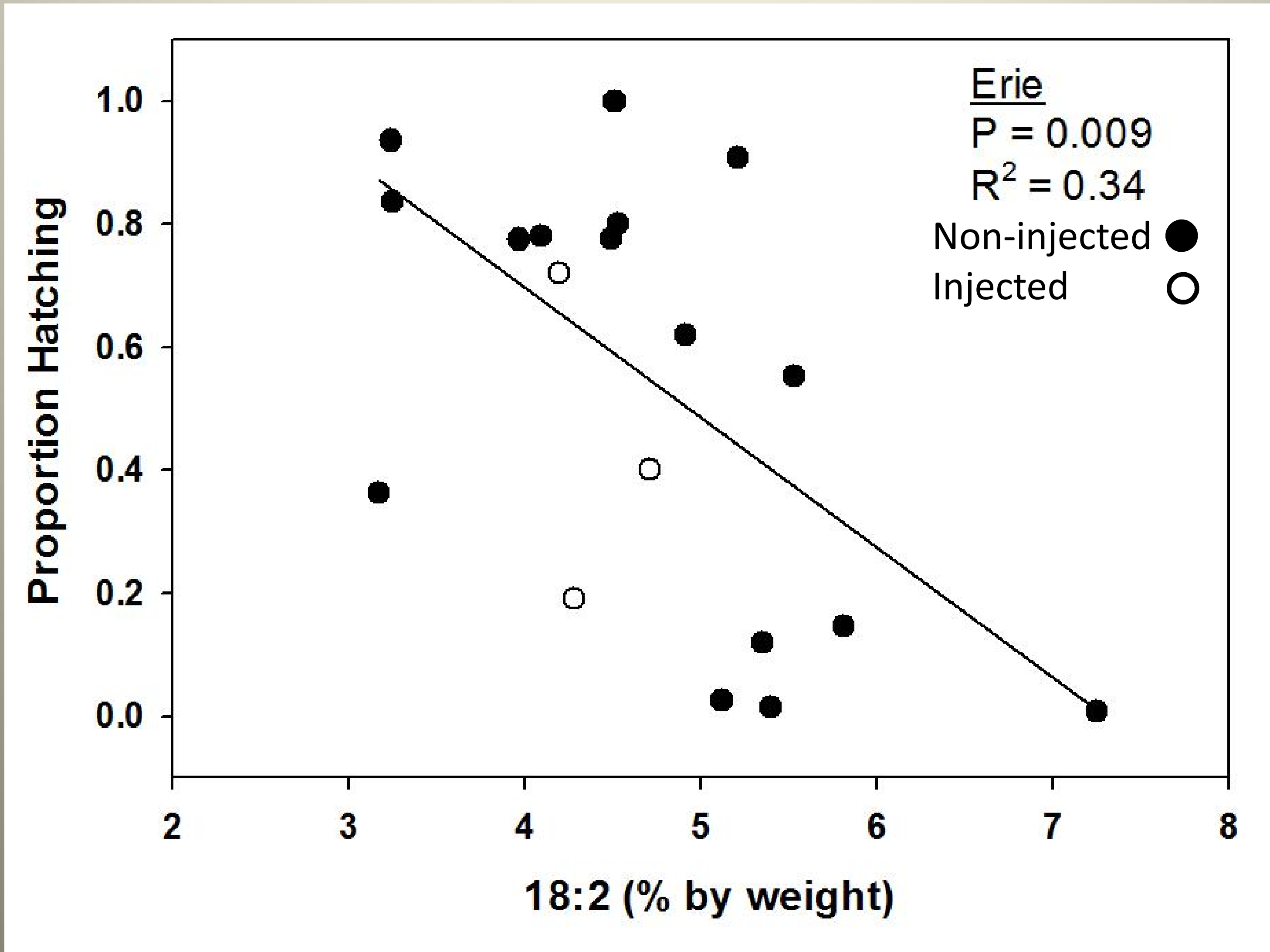
Declining fatty acid as winter duration increases



Linoleic acid 18:2n6

Erie females:

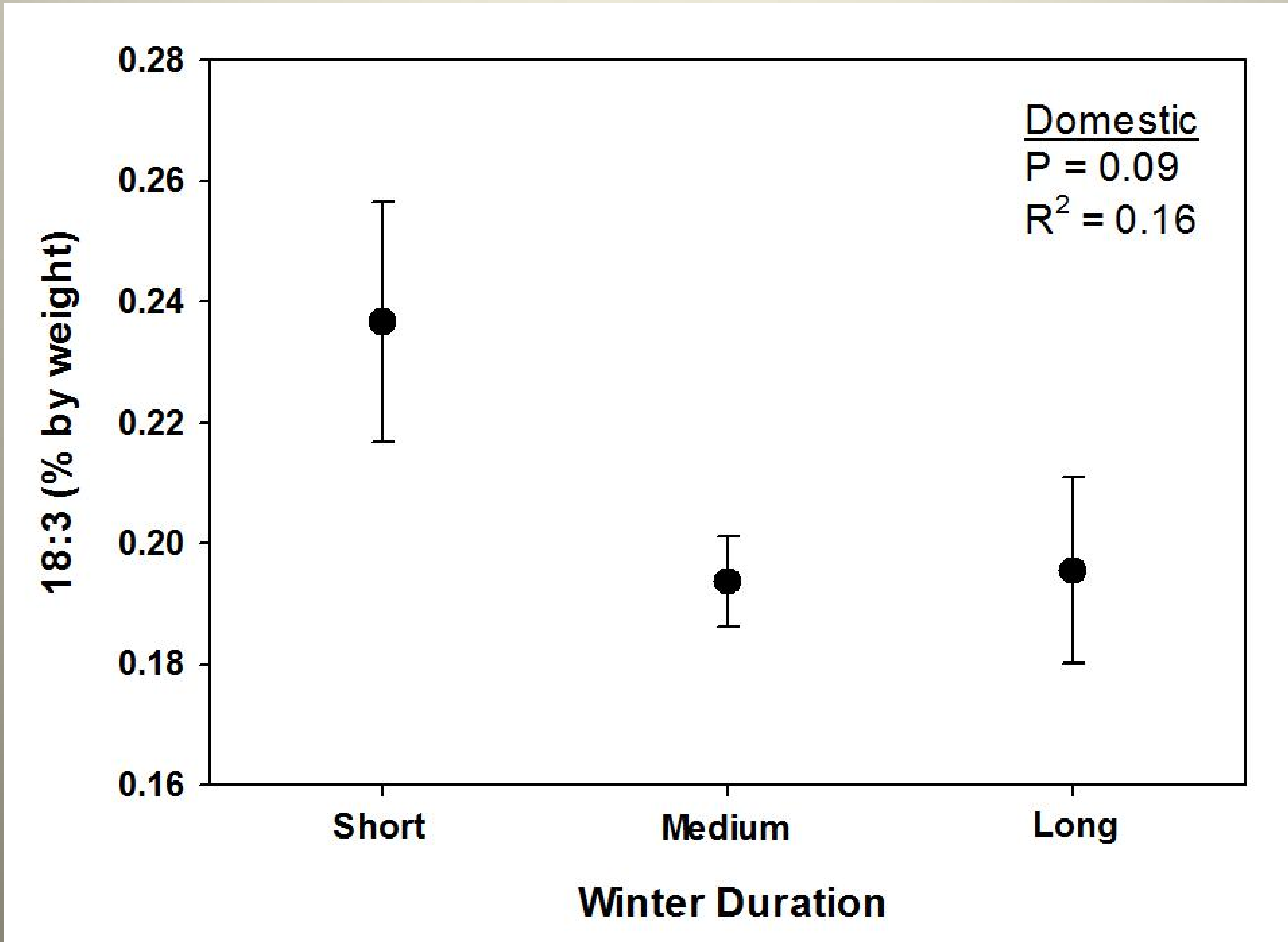
Declining hatching success as fatty acid increases



Linolenic acid 18:3n3

Domestic females:

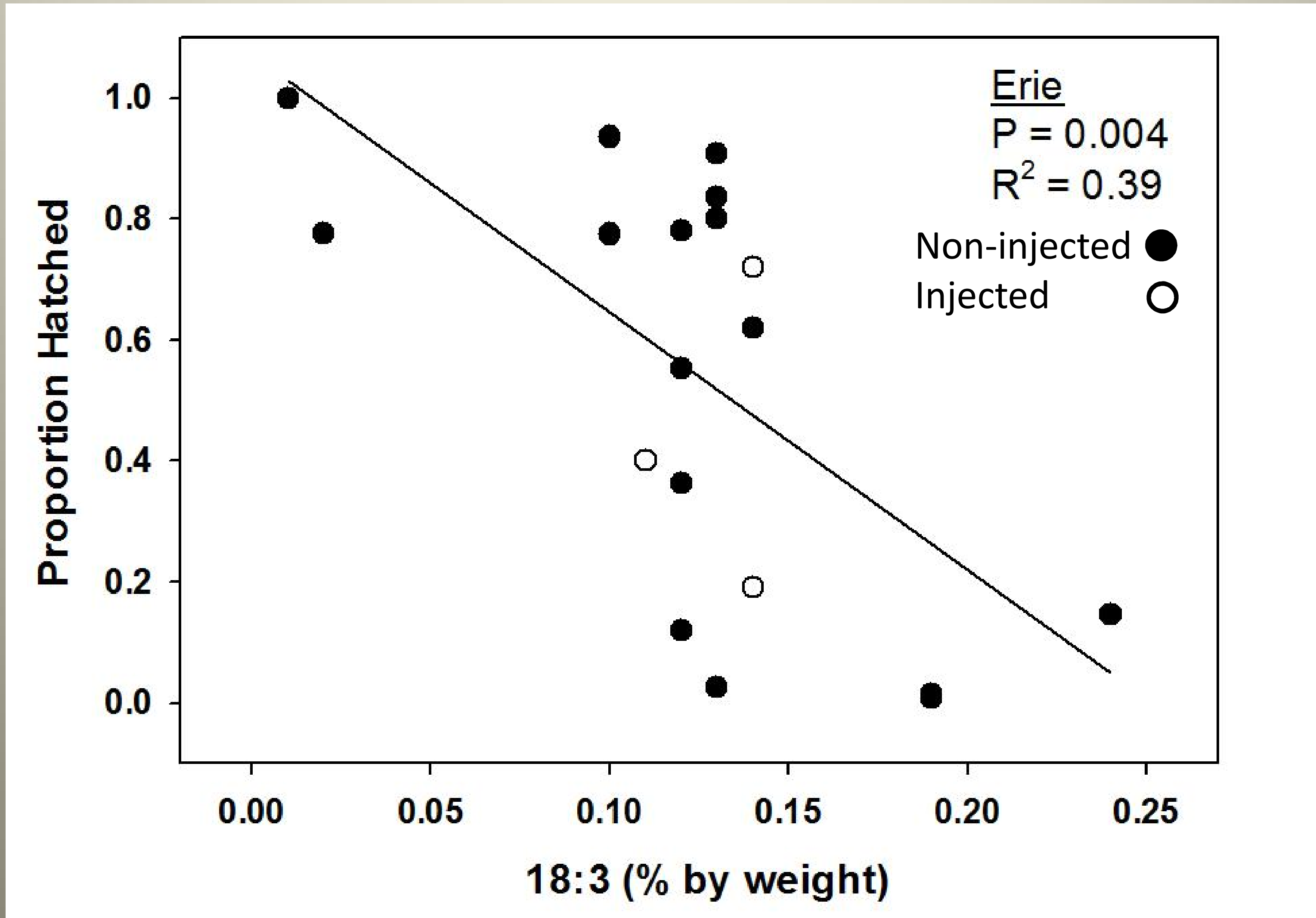
Declining fatty acid as winter duration increases



Linolenic 18:3n3

Erie females:

Declining hatching success as fatty acid increases



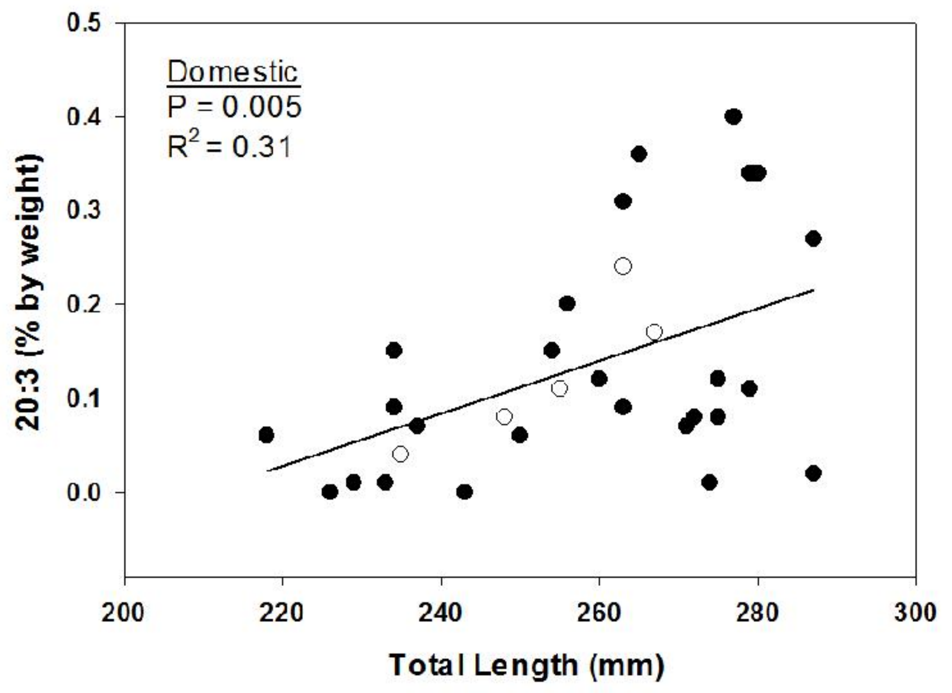
“Mead” acid 20:3n9 ?

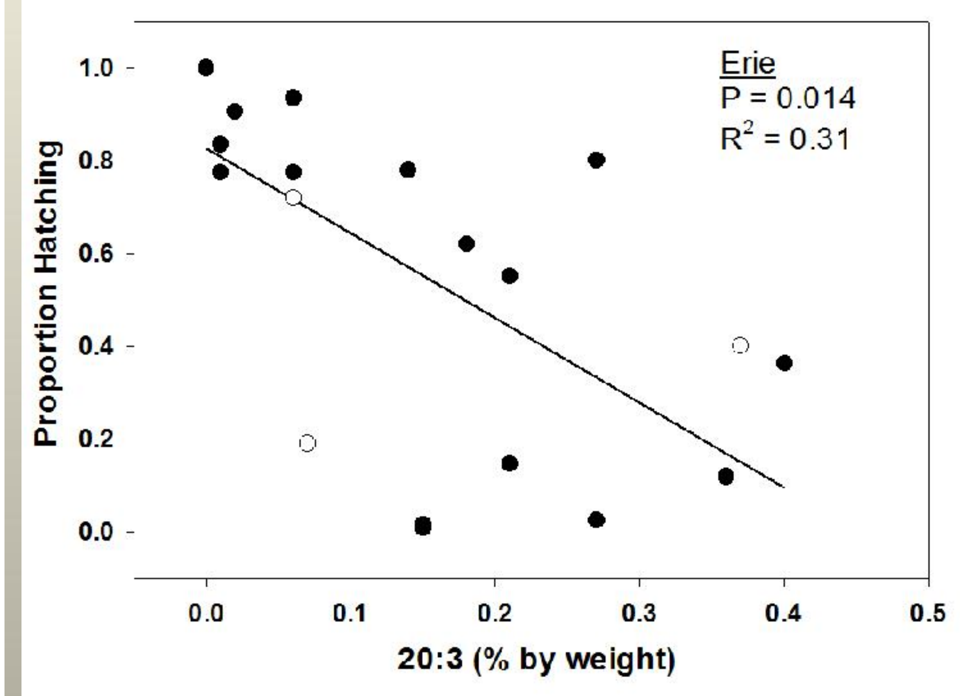
Domestic females:

↑Length = ↑Fatty acid

Erie females:

↑Fatty acid = ↓Hatching success





Non-injected ●
Hormone Injected ○

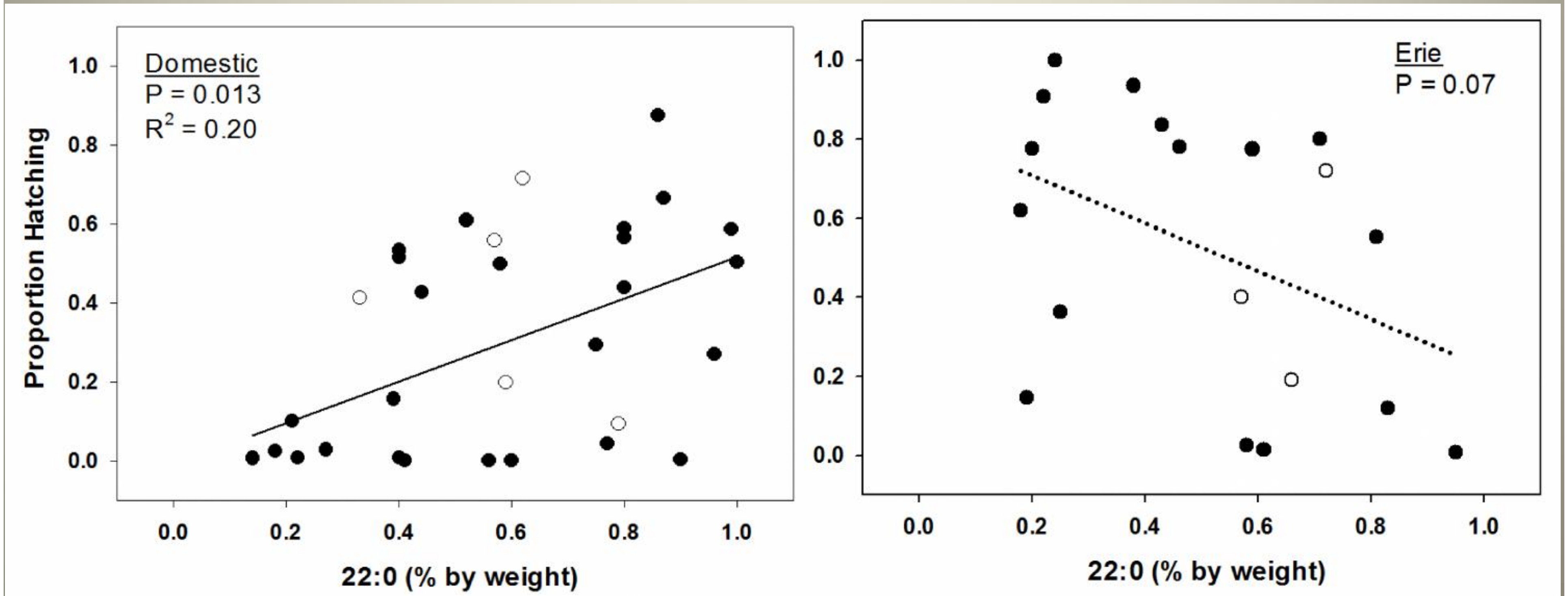
C 22:0 (saturated)

Domestic females:

↑Fatty acid = ↑Hatching success

Erie females:

↑Fatty acid = ↓Hatching success

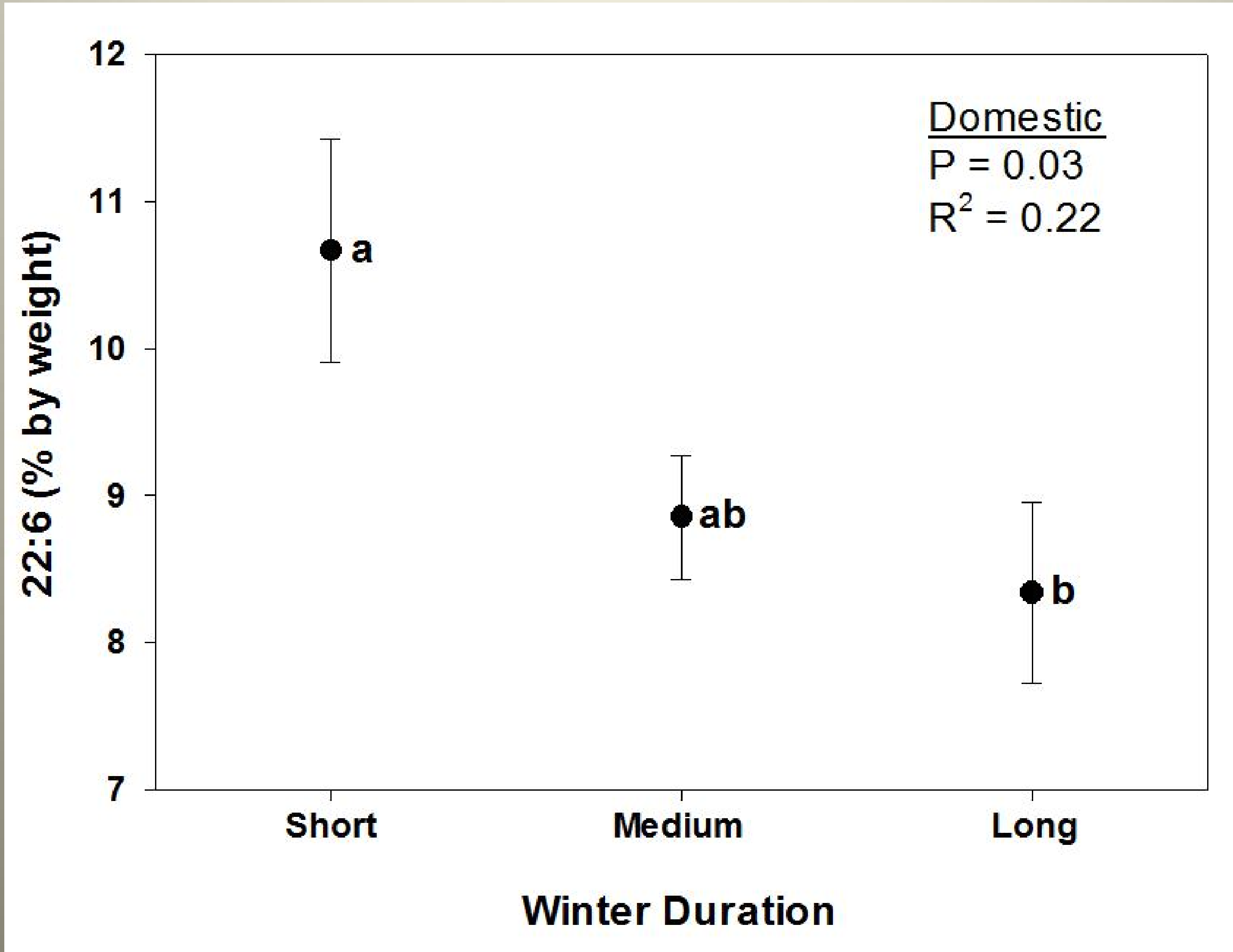


Non-injected ●
Injected ○

Docosahexaenoic (DHA) 22:6n3

Domestic females:

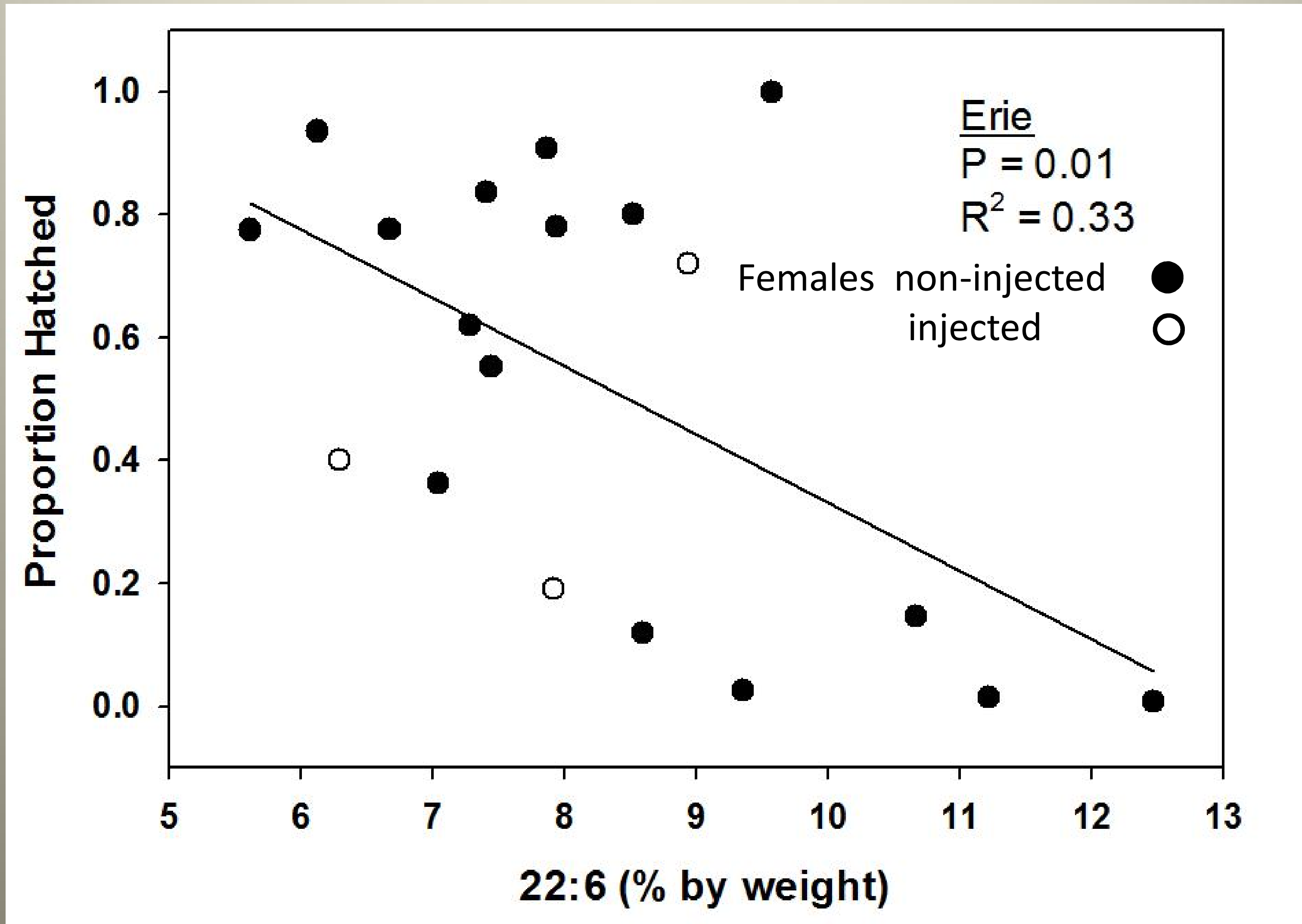
Declining essential fatty acid as winter duration increases



DHA C22:6n3

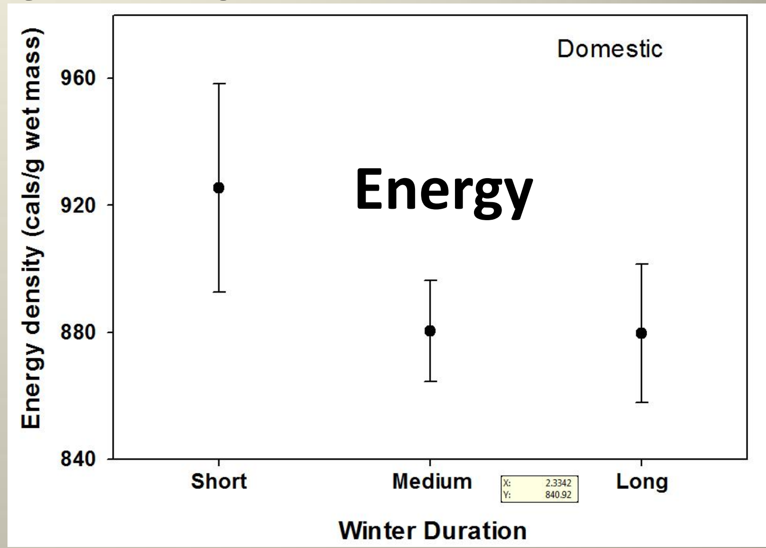
Erie females:

Declining hatching success as DHA fatty acid increases

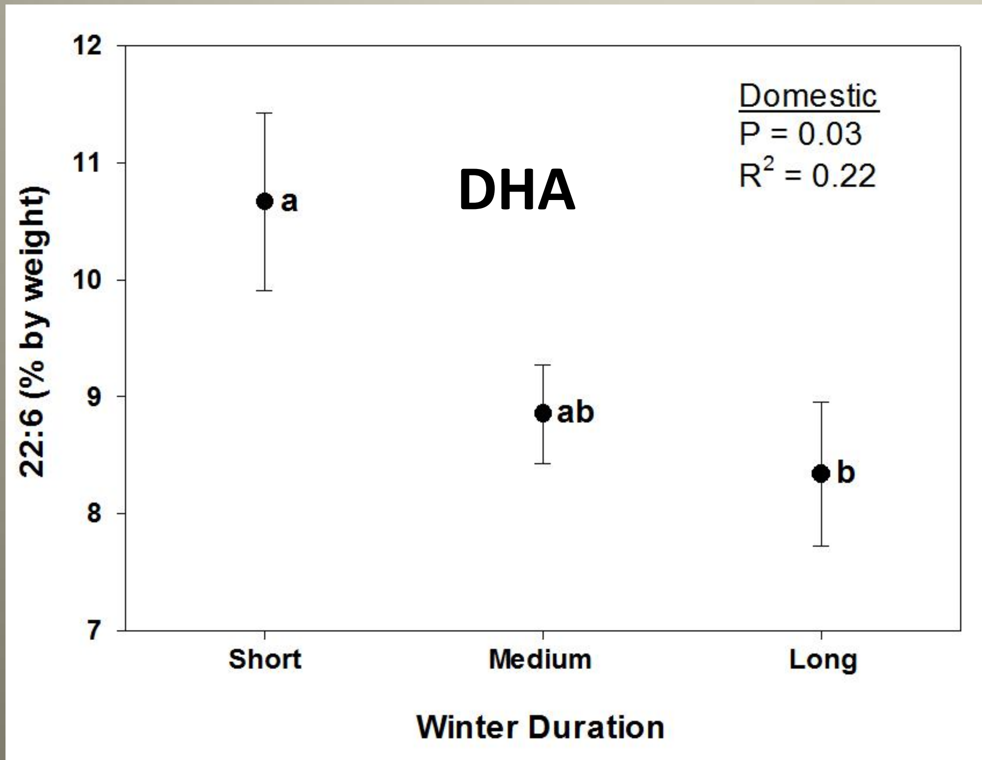


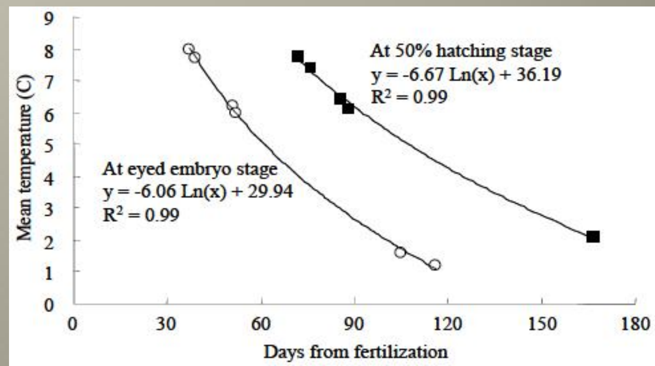
Conclusions

Extended period of low water temperatures results in increased sum of degree-days, thermal exposure at low temperatures enhances stress on membranes integrity and lipid oxidative cascade is initiated.



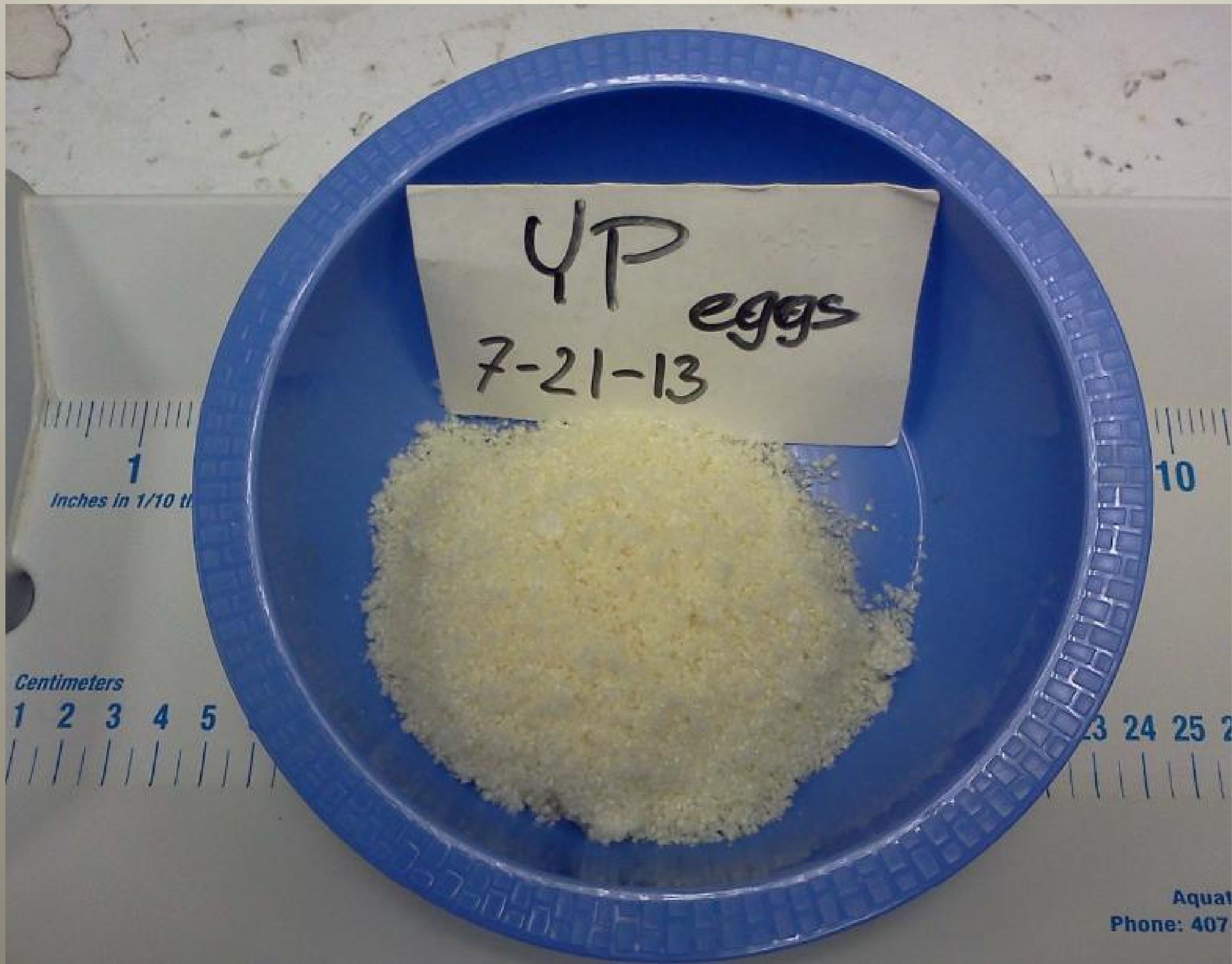
Docosahexaenoic acid (C22:6n3)
Essential PUFA





B.J. Lee in lake trout embryos at 1-2°C

Ovulated, hydrated (unfertilized) and freeze-dried eggs of yellow perch. Domesticated, fed commercial (Aquamax) diet. Collected in June 2013 (n=6 females)

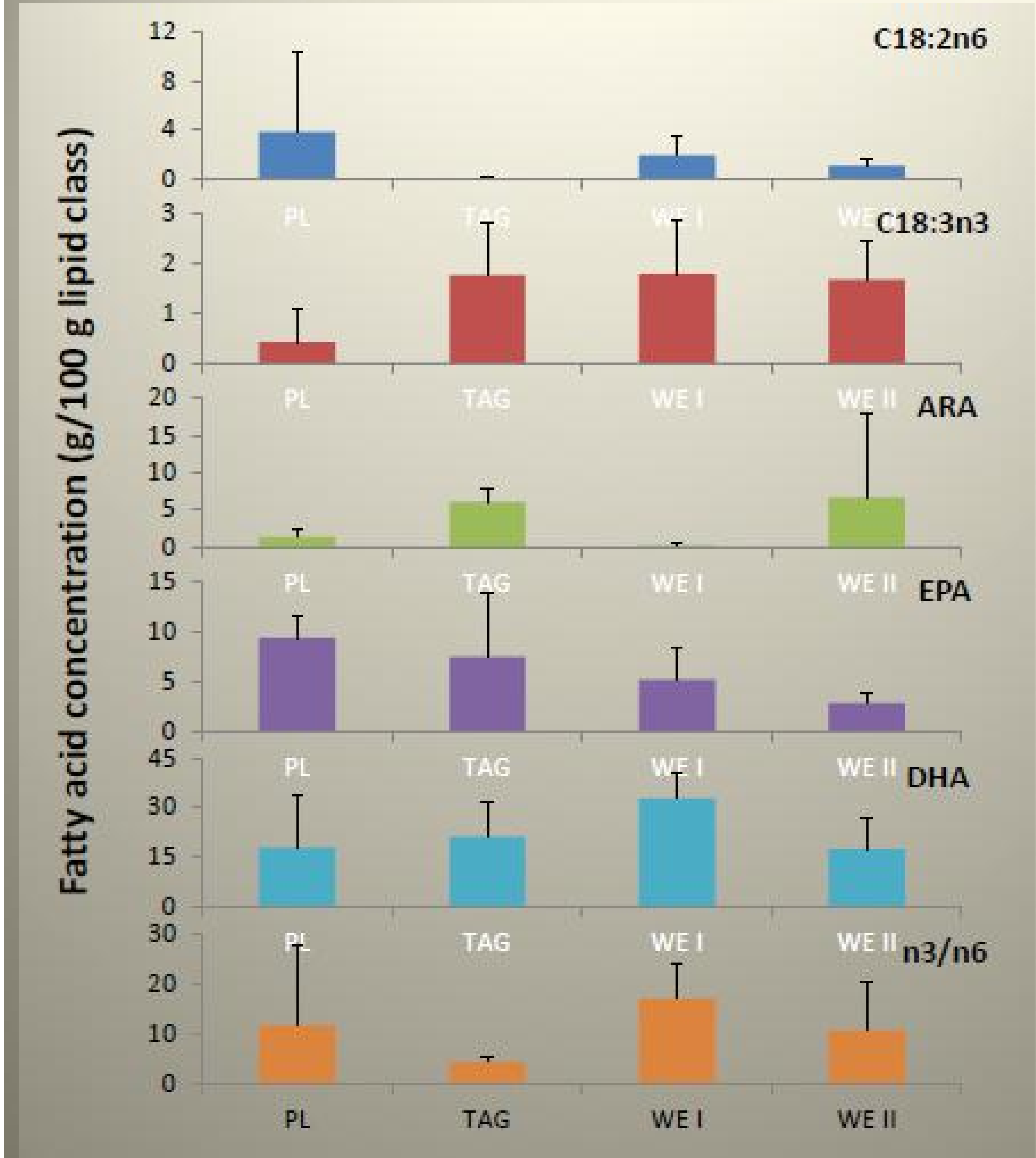


Recovery of the lipid classes after separation of NL fraction of yellow perch egg lipids on ion-exchange column

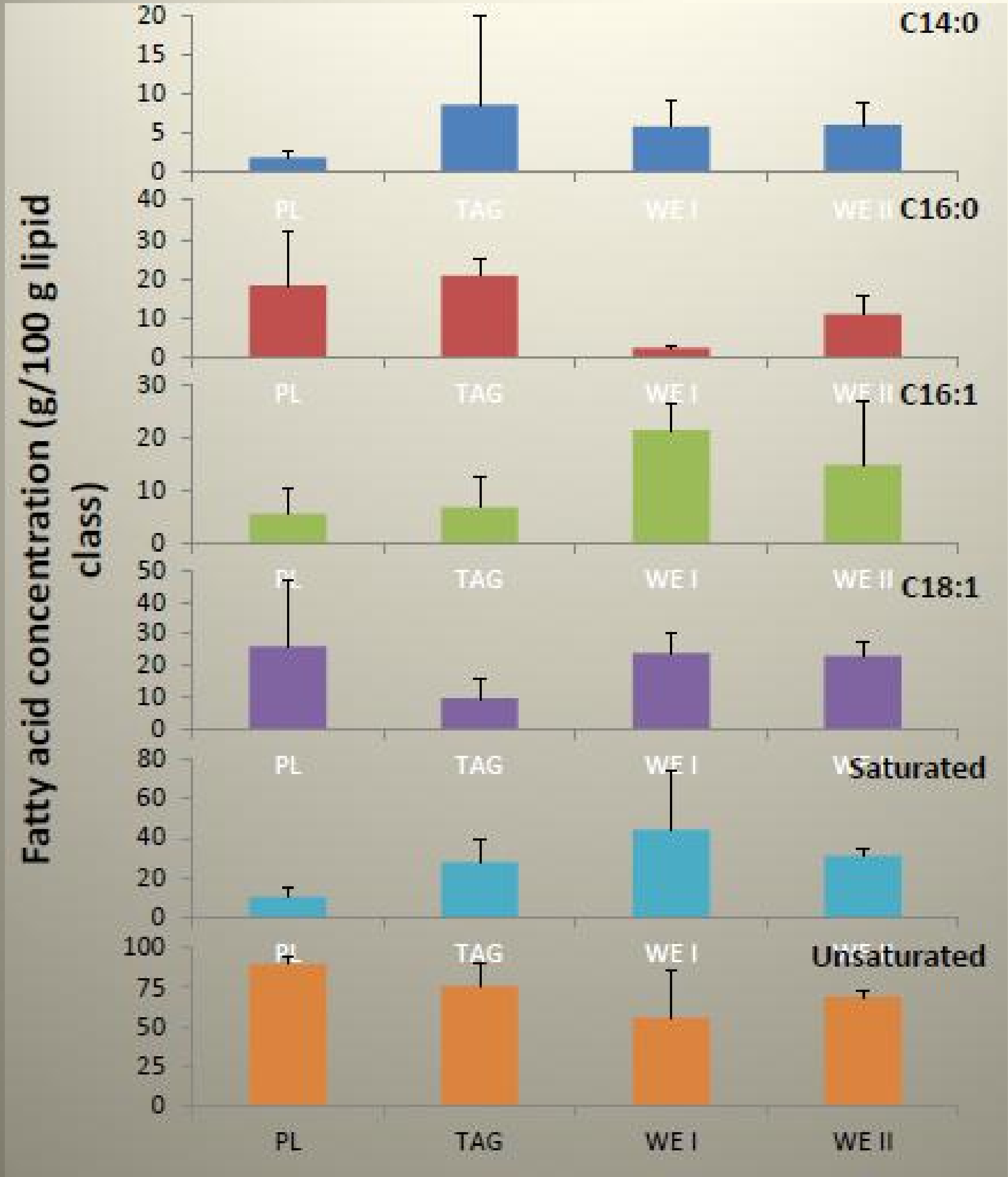
	Total load [g]	WE1 [%]	WE2 [%]	TAG [%]	PL [%]	Recovery rate [%]
LE YP eggs	0.300	74.64	4.64	3.33	1.48	84.09
LE YP eggs 2	0.315	75.96	3.18	3.71	1.55	84.40
Ovulated YP eggs	0.330	64.93	8.79	1.74	1.43	76.89

Fatty acid composition of neutral (NL) and polar (PL) fraction of yellow perch eggs

	NL fraction	PL fraction
C14:0	0.40	2.43
C14:1	0.14	1.05
C16:0	5.16	29.05
C16:1[n-9]	23.51	8.27
C18:0	0.01	0.04
C18:1[n-9c]	25.27	12.98
C18:1[n-7]	2.35	4.46
C18:2[n-6c]	1.12	2.71
C18:3[n-3]	0.33	0.26
C20:0 #1	0.46	0.11
C20:1[n-9]	0.40	0.25
C20:2[n-6]	0.42	2.77
C20:4[n-6]	0.03	1.14
C20:3[n-3]	0.09	0.25
C20:5[n-3]	9.22	9.64
C22:0	0.94	0.21
C22:1	0.35	0.63
C22:6[n-3]	29.79	23.76

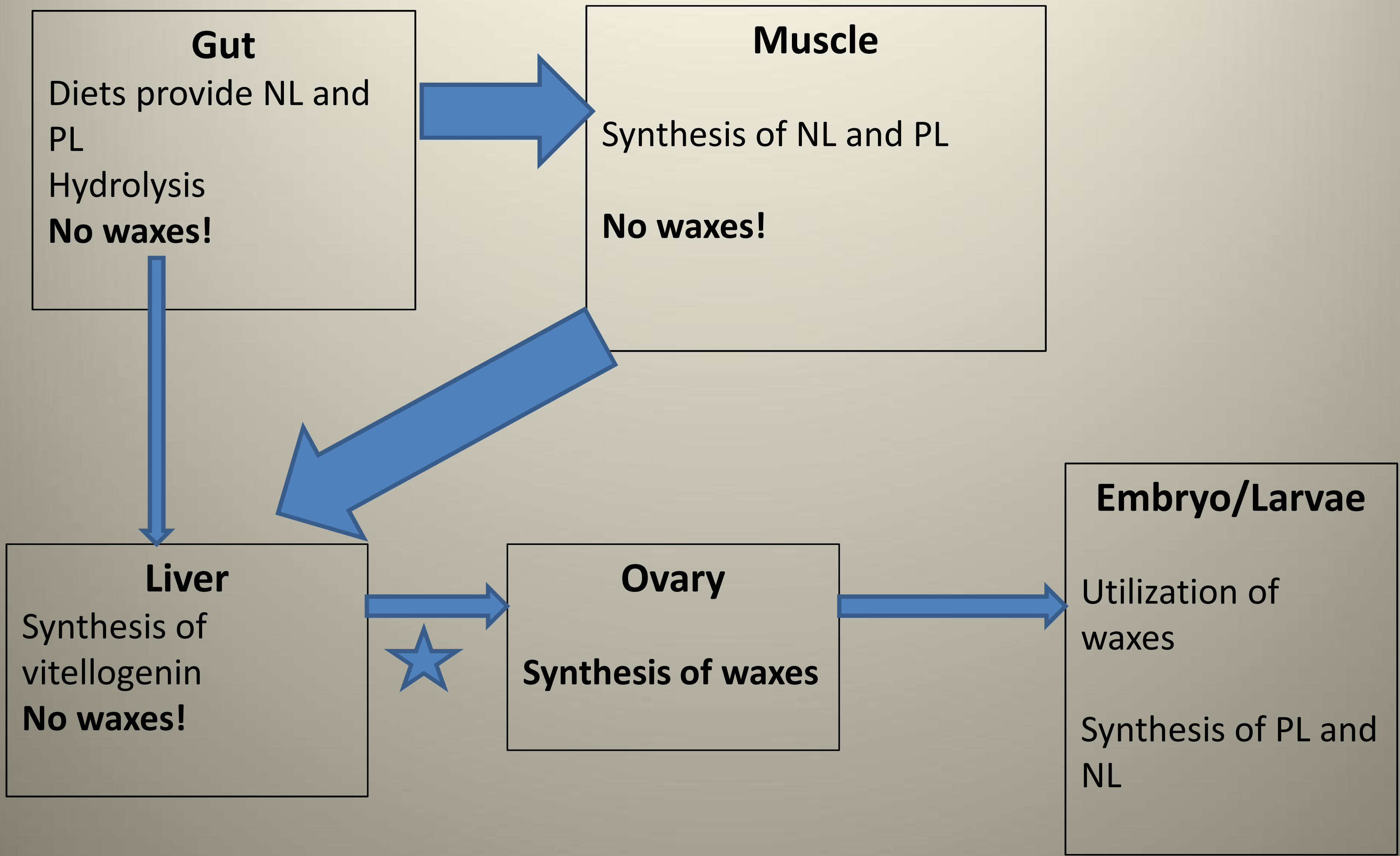


Fatty acid profiles of different lipid classes in non-ovulated, matured eggs of yellow perch (GSI 22-28%) – 2



Fatty acid profiles of different lipid classes in non-ovulated, matured eggs of yellow perch (GSI 22-28%) – 1

Uptake, transport and metabolism of lipids in the life cycle of yellow perch



Final conclusions

1. Short winter for gametogenesis does not mean bad for reproduction
2. Short winter means good for essential fatty acid profiles in eggs/embryos
3. Long period of exposure in low temperatures can mean higher utilization rate (loss) of DHA (PUFA) to protect cell membranes
4. Long winter may mean higher demand for antioxidants (vitamins)!

Final conclusions - 2

5. Lipid and fatty acid profiles in ovulated eggs show great promise in predicting viability of embryos and (perhaps) larvae

6. Extensive transformation of lipid classes takes place following ovulation, endogenous and mixed feeding phases

7. Wax esters may provide advantages in respect to water/ion balance, microbial detachment, swim bladder inflation in early life of freshwater fish

8. Enrichment of the first larval diets with specific wax esters should be examined.

Acknowledgements

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Carey Knight
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Sherr Vue
Nick Agins

ODOW - Sandusky

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Chris Vandergoot
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Travis Hartman
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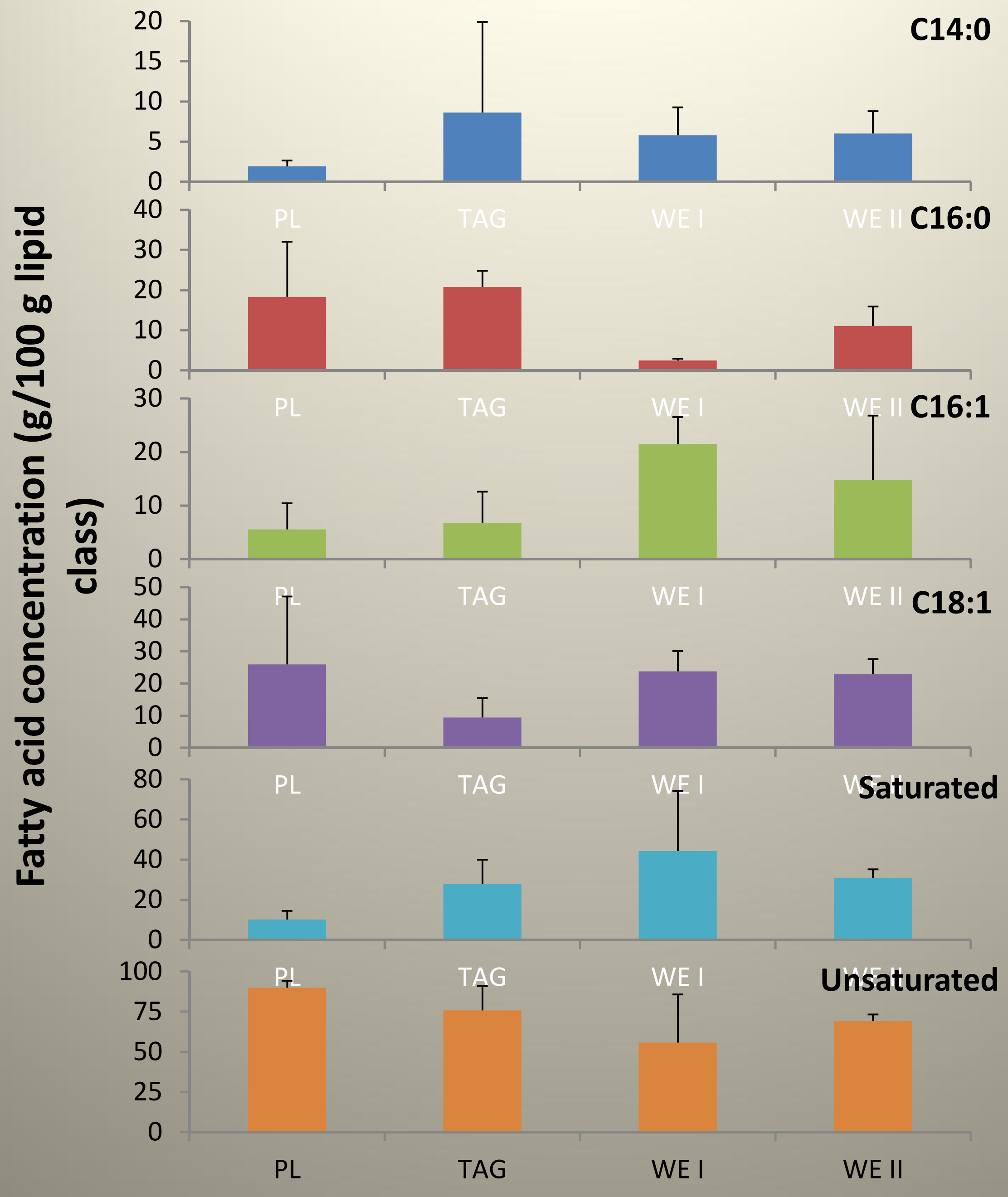
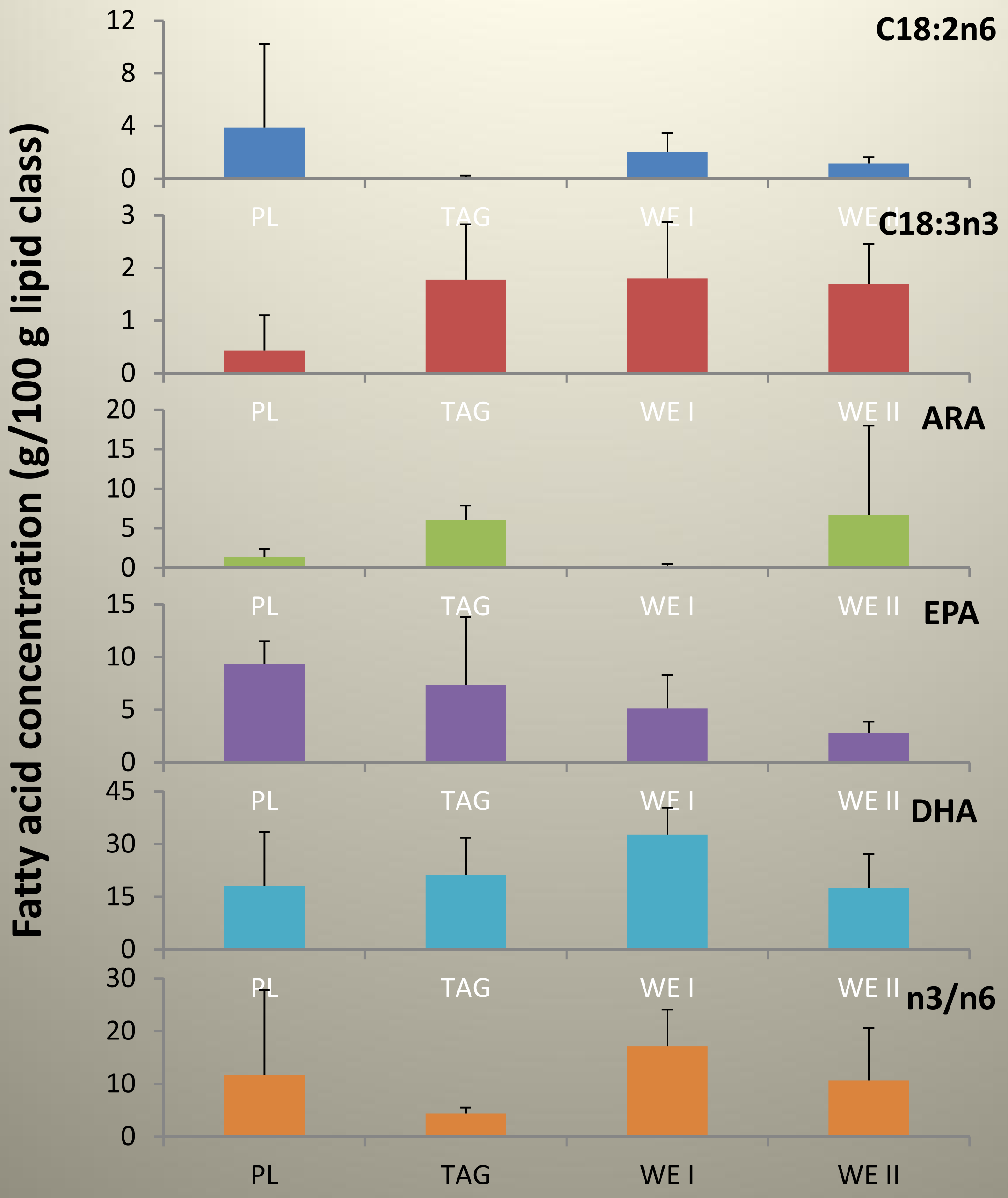
Dale Hall
Tim Cherry
Brandon Giesler

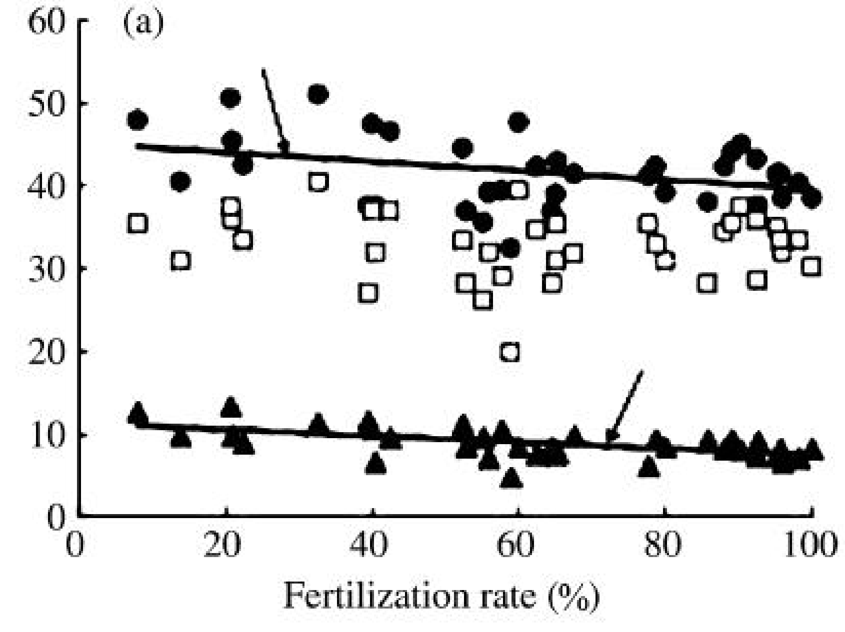


Fatty acid concentration (g/100 g lipid class)
C14:0
20
15
10
5
0
C16:0
40
30
20
10
0
C16:1
30
20
10
0
C18:1
50
40
30
20
10
0
Saturated
80
60
40
20
0
Unsaturated
100
75
50
25
0
PL
TAG
WE I
WE II



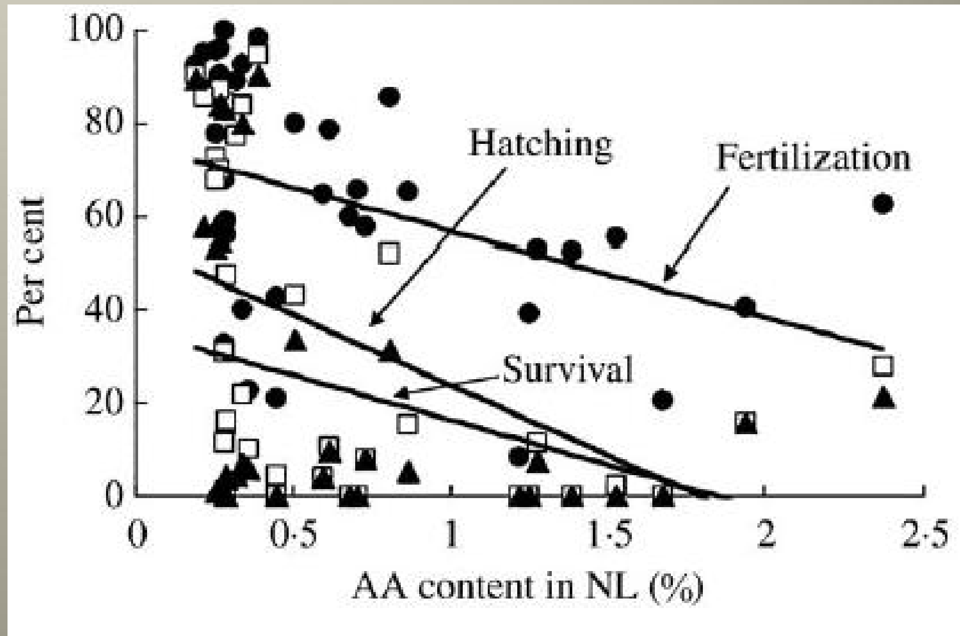
Fatty acid concentration (g/100 g lipid class)
C18:2n6
C18:3n3
ARA
EPA
DHA
n3/n6
PL
TAG
WE I
WE II
12
8
4
0
3
2
1
20
15
10
5
45
30

Effect of lipid and fatty acid composition on embryo viability of Japanese eel (*Anguilla japonica*) (Furuita et al. 2006. J. Fish Biol. 69: 1178)



NL (triglycerides)

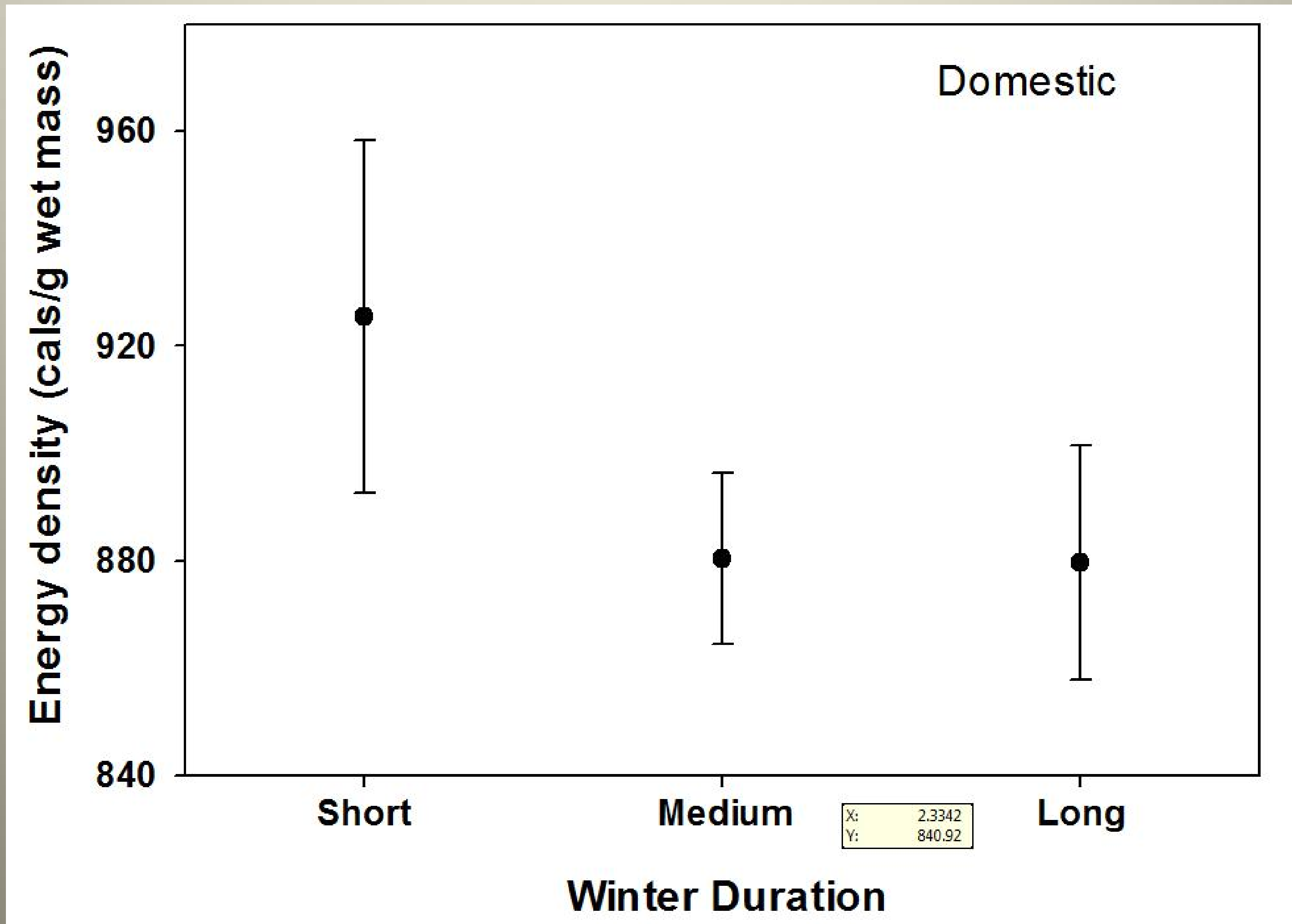
PL



Results on a per egg basis

Example using caloric density:

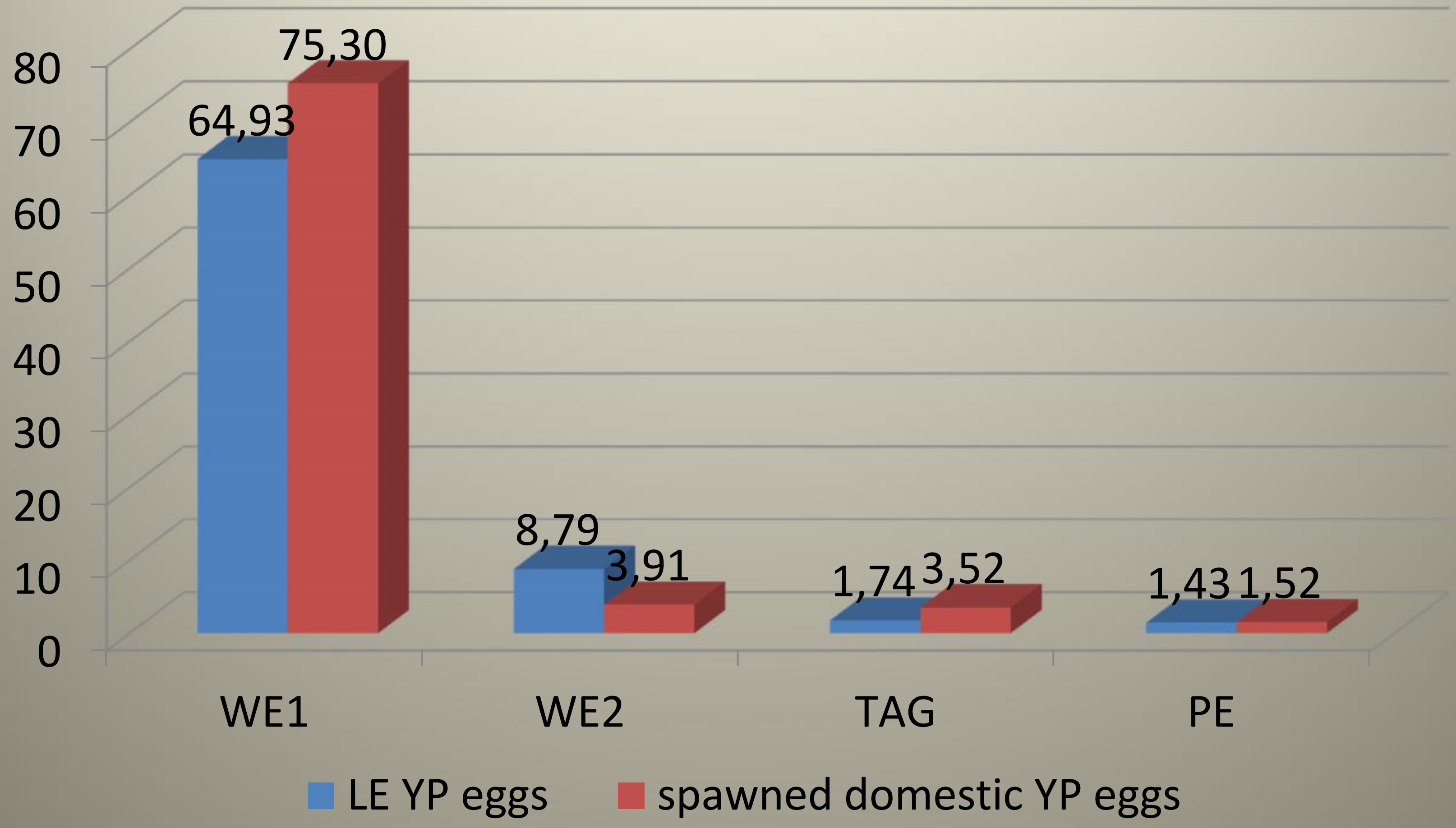
Energy density declines as winter duration increases



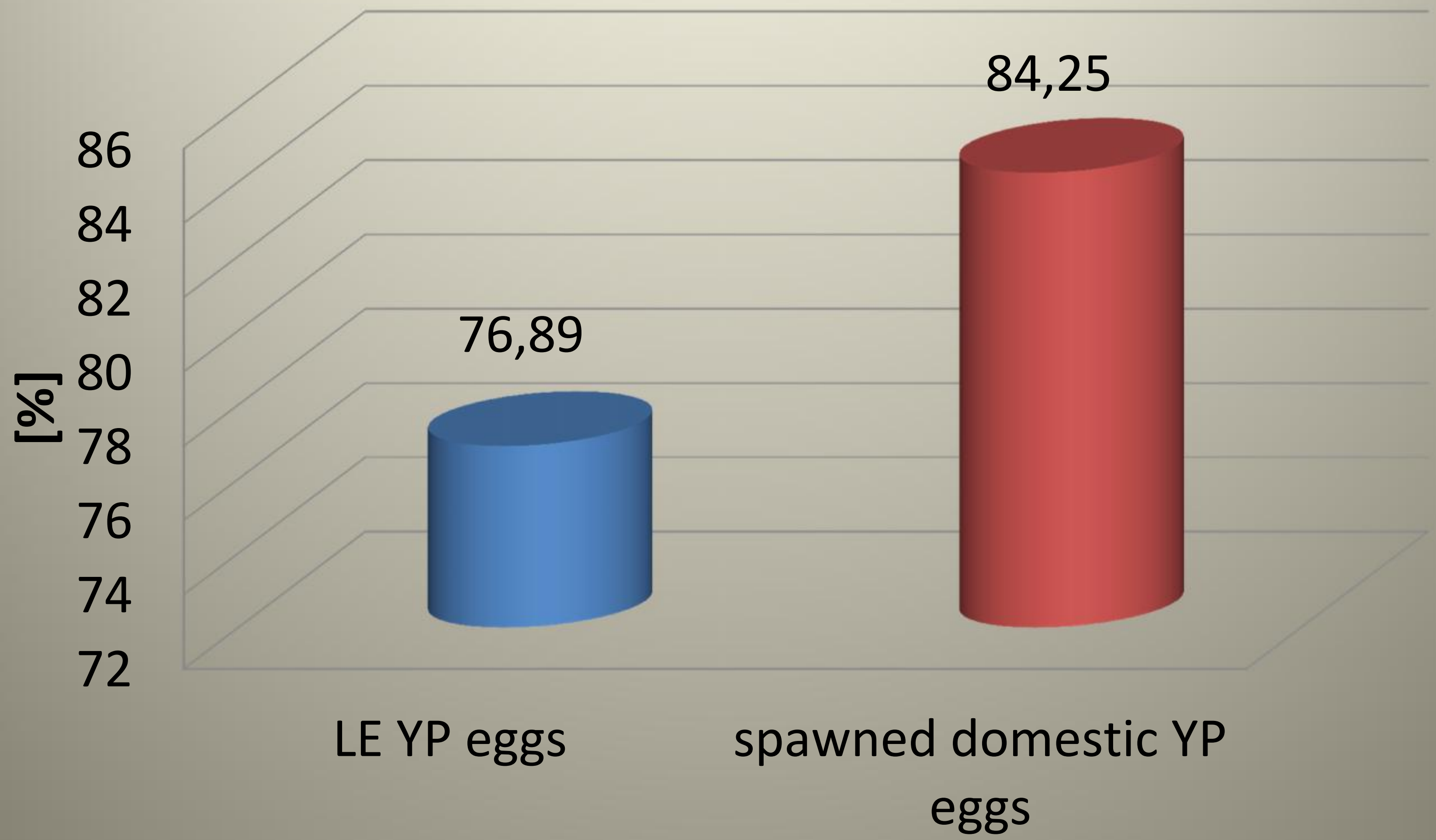
Lipid classes and fatty acid composition of perch food (fathead minnows) offered in the course of gametogenesis 2011-2012

Category	Mean % ± StdErr
Total Lipids	3.5 % ± 0.3
Neutral Lipids	65 % ± 2.5
Phospholipids	35 % ± 2.4
Linoleic C18:2 (n6)	0.04 % ± 0.01
Linolenic C18:3 (n3)	0.40 % ± 0.07
Arachidonic C20:5 (n3)	5.4 % ± 1.4
EPA C20:4 (n6)	0.02 % ± 0.09
DHA C22:6 (n3)	5.5 % ± 1.7
n3 /n6	8.3 : 1.0

Recovery of the lipid classes after separation of NL fraction of yellow perch egg lipids on ion-exchange column



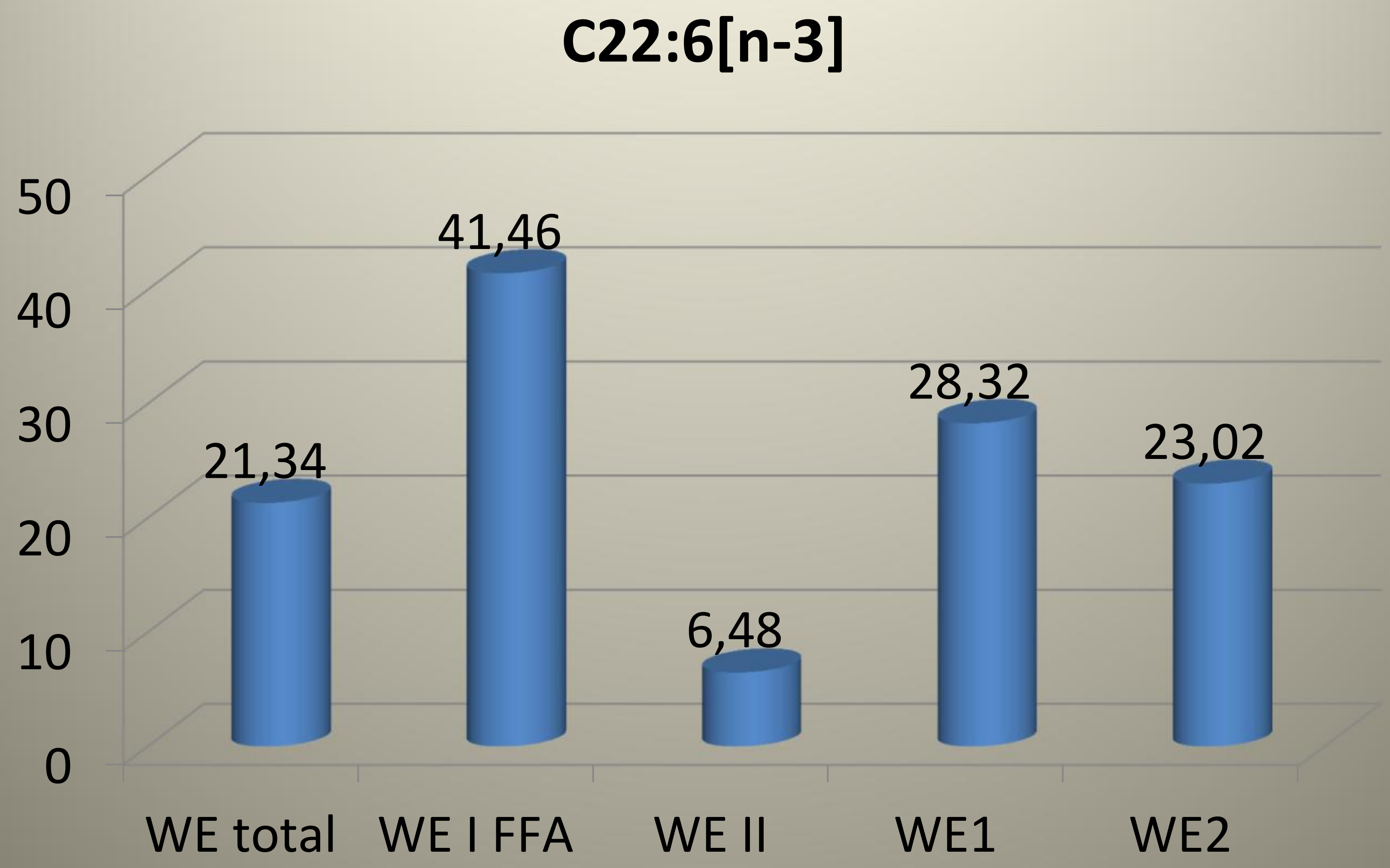
Lipids recovery after ion-echange separation of NL from yellow perch eggs

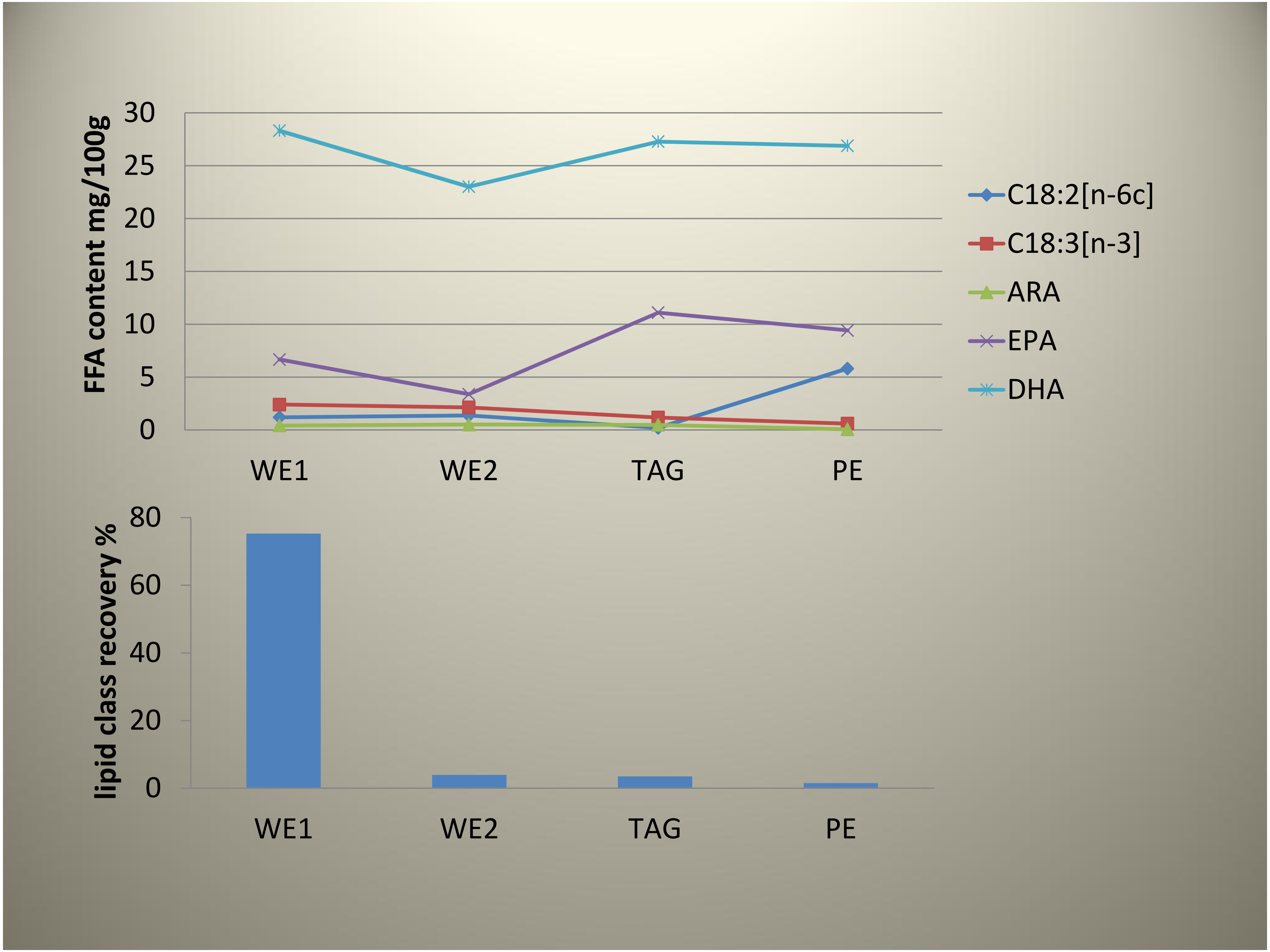


Cumulative water temperature from initiation of gametogenesis (October) to ovulation (degree-days, ^oD)

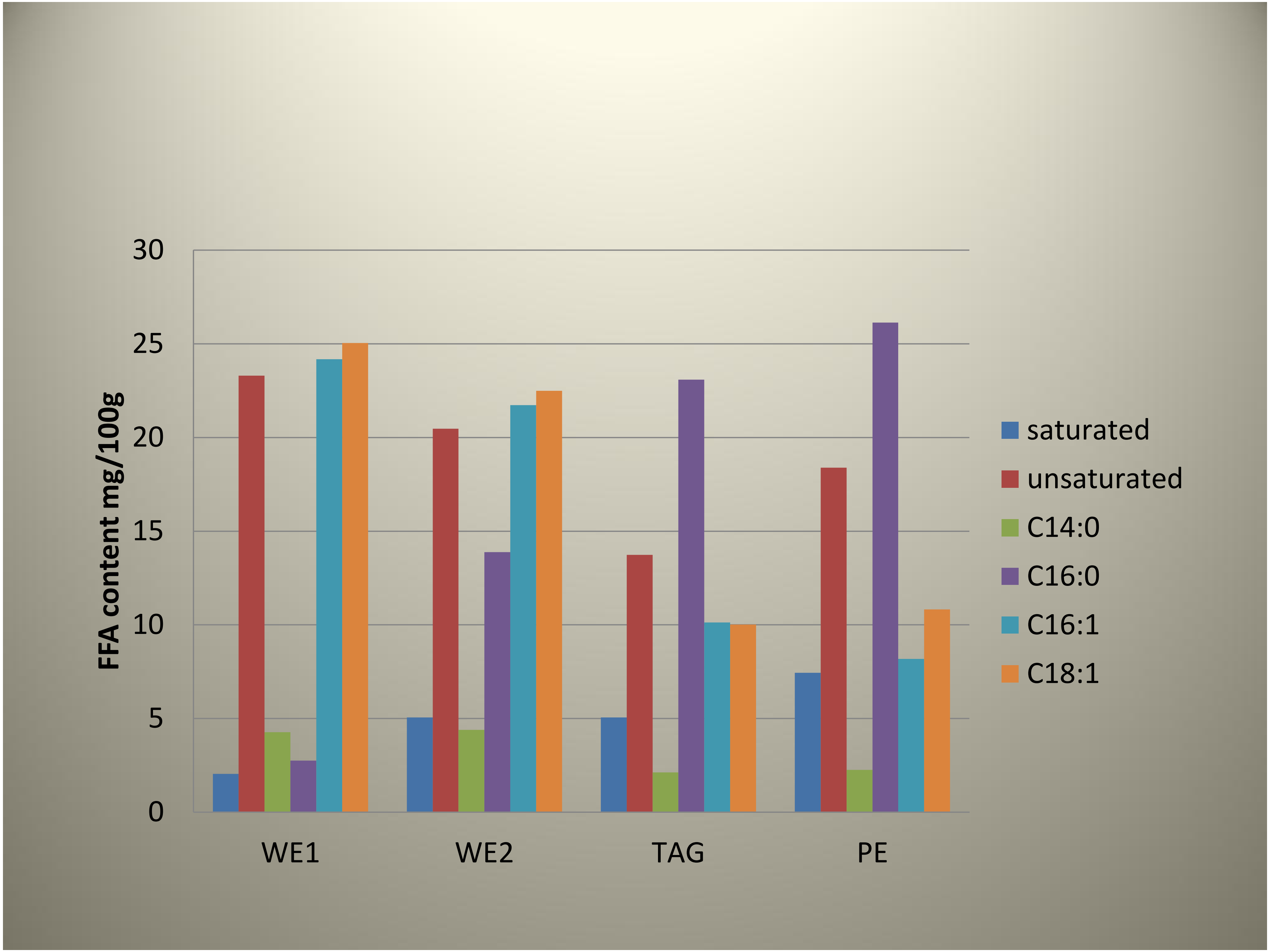
Origin	Erie		Domestic		
Winter duration	Short	Long	Short	Medium	Long
Degree-days	**2,158**	**1,771**	**2,165**	**1,938**	**1,890**
Difference		18%		11%	13%

YP eggs Troy's





FFA content mg/100g
30
25
20
15
10
5
0
WE1
WE2
TAG
PE
C18:2[n-6c]
C18:3[n-3]
ARA
EPA
DHA
lipid class recovery %
80
60
40
20
0
WE1
WE2
TAG
PE

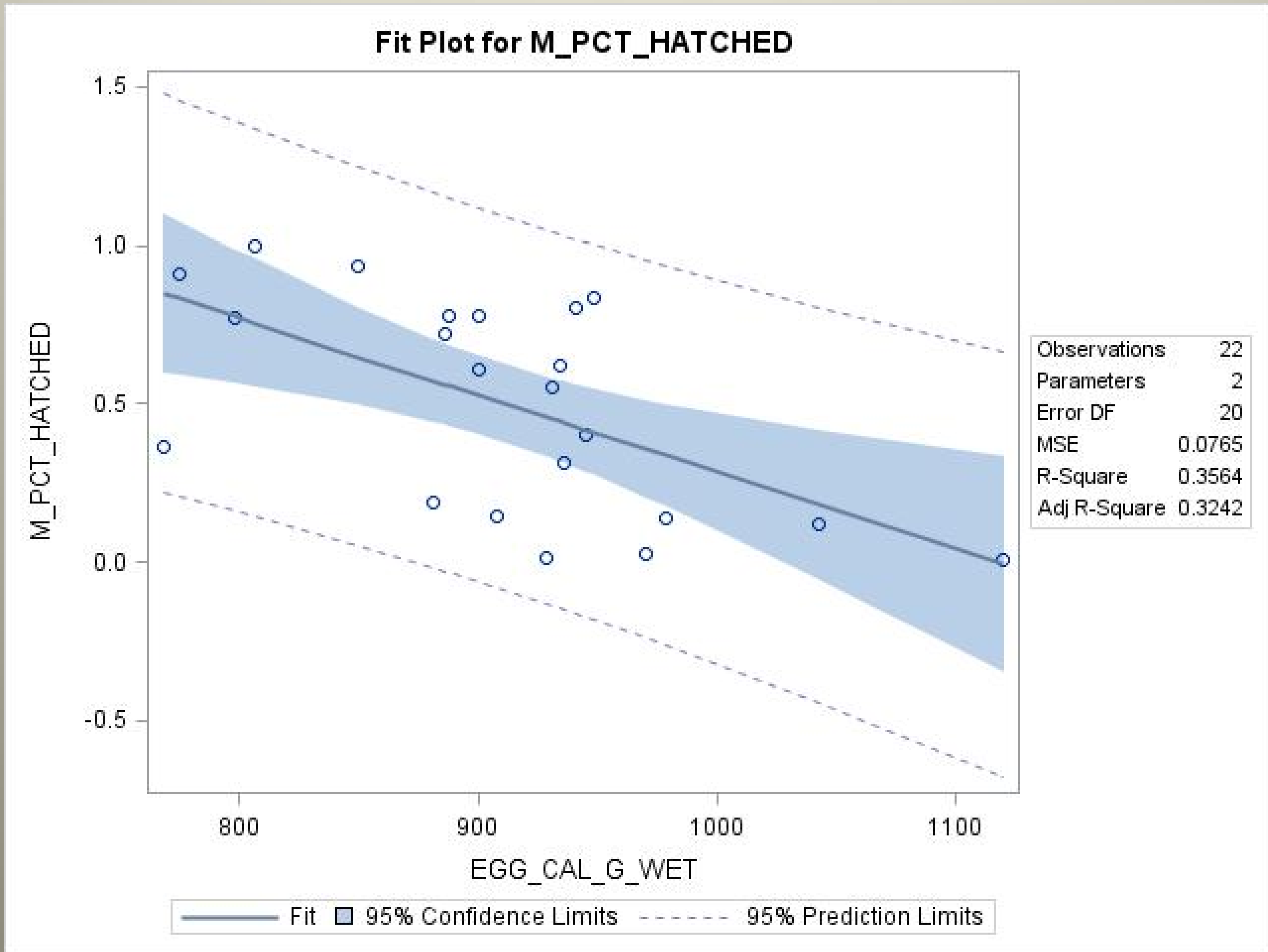


FFA content mg/100g
30
25
20
15
10
5
0
WE1
WE2
TAG
PE
saturated
unsaturated
C14:0
C16:0
C16:1
C18:1

Results on a per egg basis

Example using caloric density:

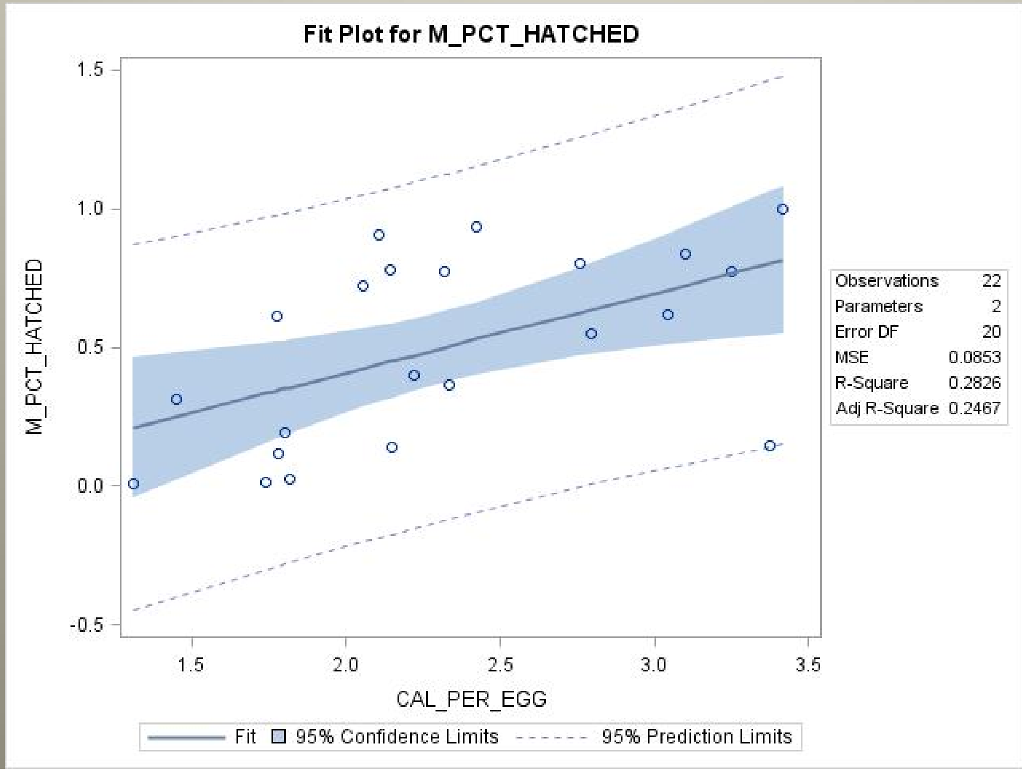
Hatching success declines with increasing energy density



Results on a per egg basis

But when results are scaled to calories per egg:

- *Hatching success increases as the calories per egg increase.*
- *This is because eggs/g (of ribbon) also decreased with increasing winter duration.*
- *Even though the density declines, fewer eggs/g translates into higher calories per egg.*



Time of divergence among teleost fishes

(Saito et al. 2011. PLOS)