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DIVERSIFY

Hormonal treatments to induce spawning

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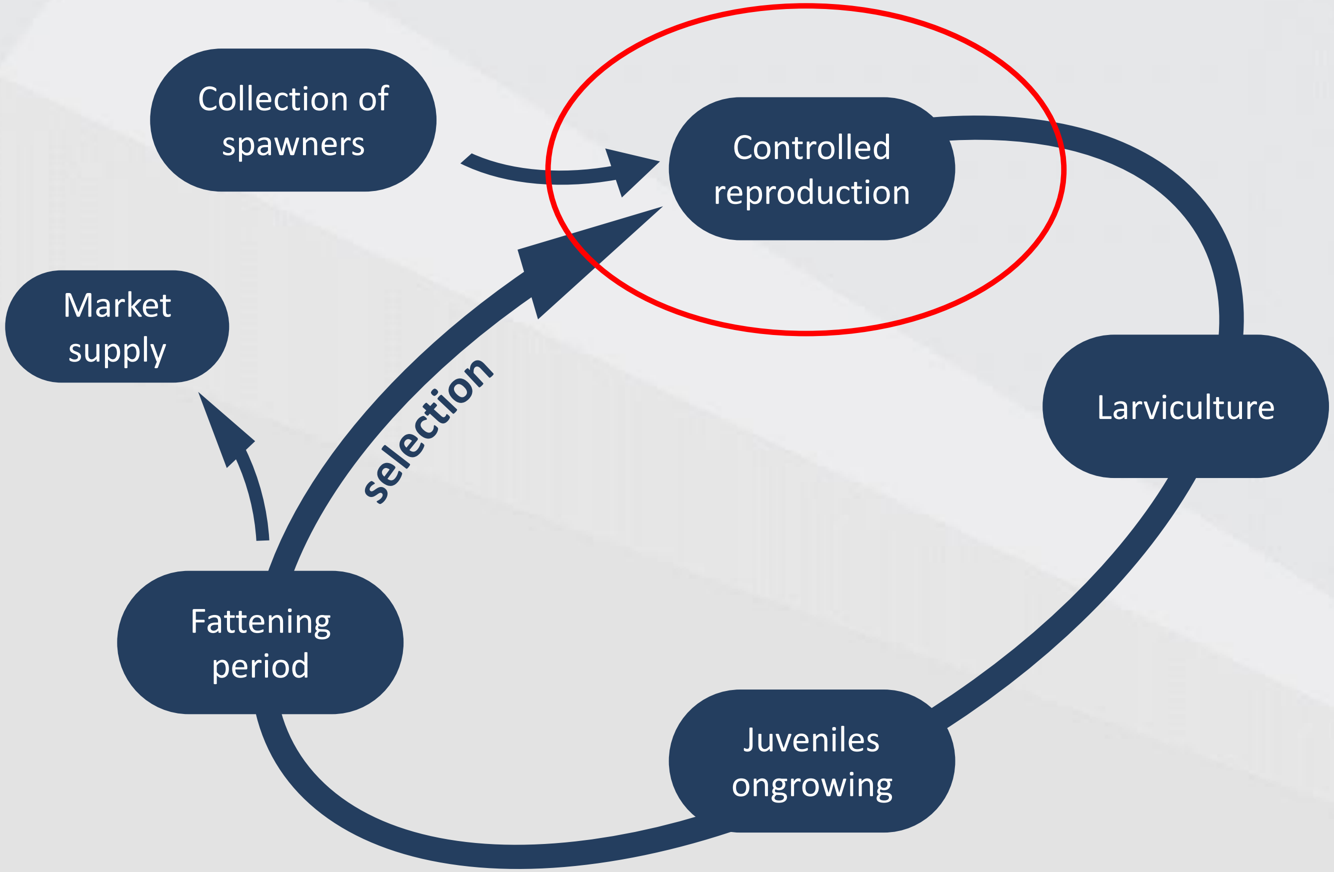
Pikeperch, *Sander lucioperca*

- Presently, one of the most required freshwater species for intensive aquaculture
- High commercial reputation and interest
- Aquaculture production is still limited due to lack of clear production technology
- Reproduction is among the main constraints

Serious obstacles:

- Low and variable egg quality
- Synchronization of spawning

Controlled reproduction



Intensive aquaculture
(production cycle)

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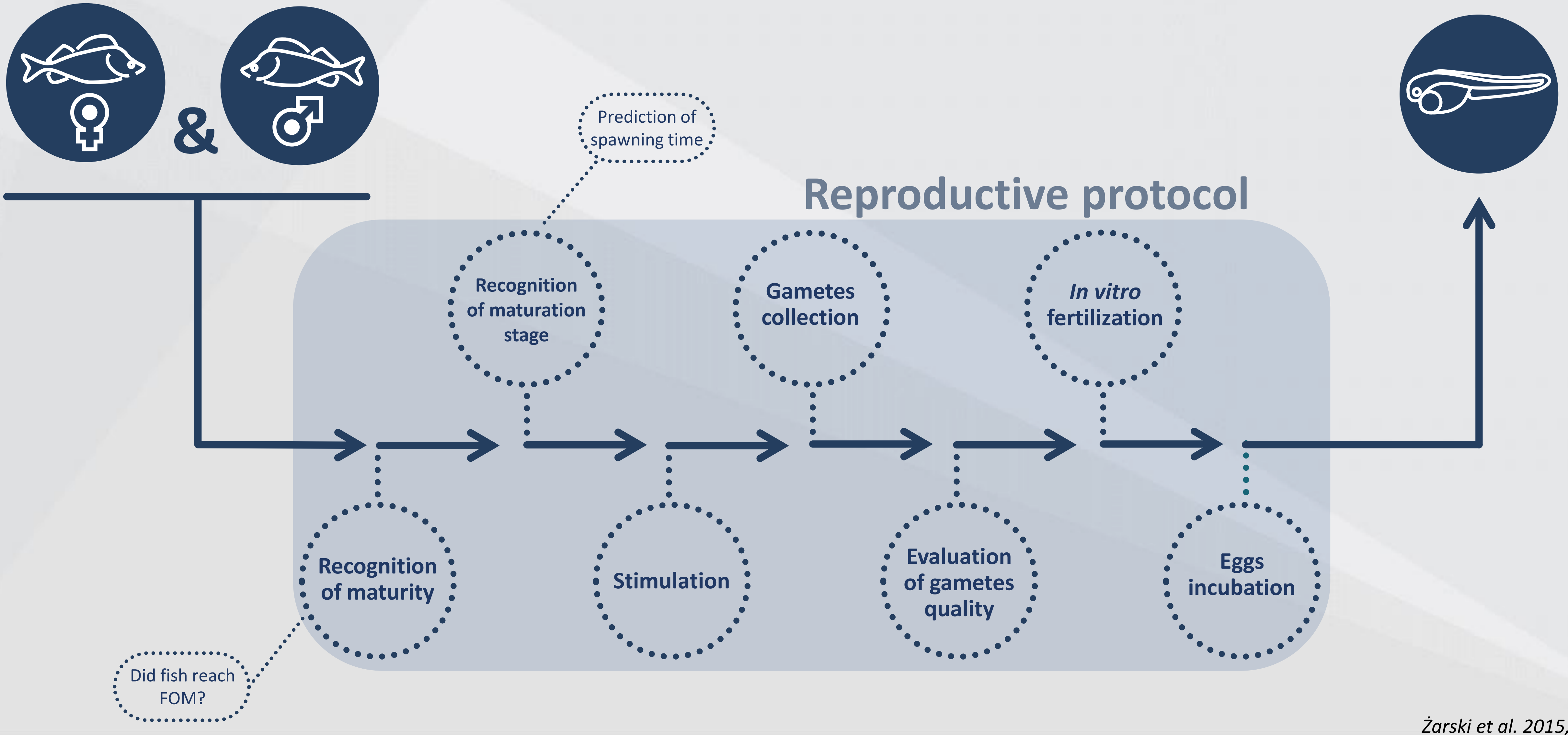
Controlled reproduction



Żarski et al. 2015, 2016

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Hormonal stimulation in pikeperch – is it really a must?

	Without hormonal treatment	With hormonal treatment
Duration of spawning operation	Up to 12 weeks	Up to 8 days
Synchronization	Incidental / hardly predictable	Down to 2 days
Labor intensity	High	Low
Egg quality	Variable	Variable

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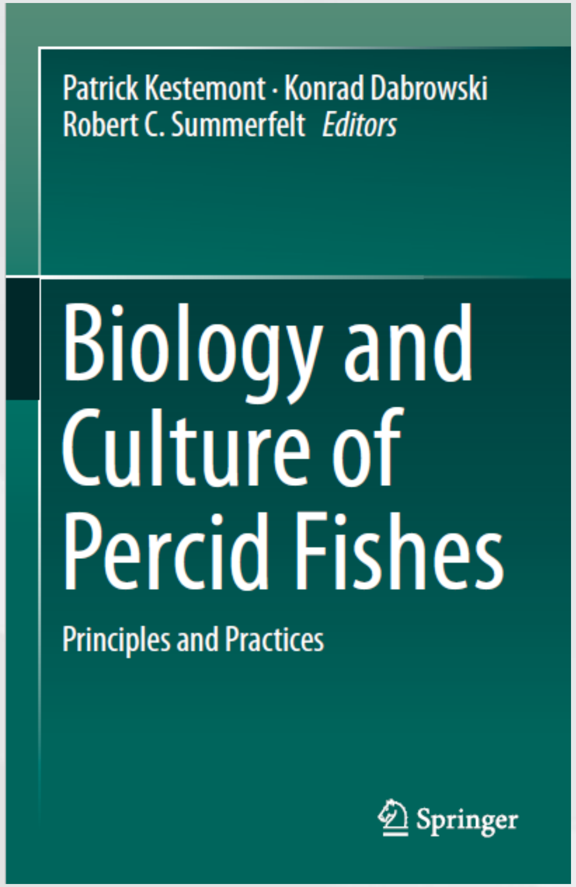
State-of-the-art

Table 4.3 Hormonal preparations, general reproductive methods applied and results of artificial reproduction of pikeperch, *Sander lucioperca*

Spawning agent	The dose (per kg of body weight)		Dopamine antagonist dose (per kg of body weight) [d]	Interval between injections (h)	Method of injection	Temperature (°C)[f]	Photoperiod	Maturity stage prior to hormonal stimulation	Spawning method	Ovulation rate (%)	Latency time (h)[g]	Embryonic survival (%)	Reference
	I injection	II injection											
hCG	500 IU	–	–	–	IP	13	12L:12D	3–4 [S6]	CS	100	96–120	88.3[h]	1
	500 IU	–	–	–	IP	15	12L:12D	3–4 [S6]	CS	100	72–96	84.4[h]	1
	500 IU	–	–	–	IP	12	nd	2 [S6]	CS	75	78–98	71.3[h]	2
	500 IU	–	–	–	IP	12	nd	3 [S6]	CS	100	57–78	73.3[h]	2
	500 IU	–	–	–	IP	12	nd	4 [S6]	CS	100	48–58	77[h]	2
	500 IU	–	–	–	IP	12	nd	5 [S6]	CS	83	32–49	76.5[h]	2
	500 IU	–	–	–	IP	12	nd	6 [S6]	CS	80	5–30	79.5[h]	2
	200 IU	200 IU	–	24	IP	14.5	nd	1–2 [S4]	CS	83.3	75	72.4[i]	3
	200 IU	500 IU	–	24	IP	14.5	nd	1–2 [S4]	CS	100	77	68[i]	3
	600 IU	–	–	–	IP	nd	nd	2 [S4]	CS/TS	100	60	nd	4
	150 IU	500 IU	–	48	IP	nd	nd	2 [S4]	CS/TS	100	133	nd	4
	250 IU	–	–	–	IM	15	14L:10D	2–4 [S4]	CS	71	85	70.9[k]	5
	500 IU	–	–	–	IM	16	14L:10D	2–4 [S4]	CS	100	78.1	84.2[k]	5
	750 IU	–	–	–	IM	17	14L:10D	2–4 [S4]	CS	100	78.6	86.8[k]	5
	1000 IU	–	–	–	IM	18	14L:10D	2–4 [S4]	CS	83	88	52.5[k]	5
PG–600	500 IU	–	–	–	IP	13	12L:12D	3–4 [S6]	CS	83	96–120	80.3[h]	1
	500 IU	–	–	–	IP	15	12L:12D	3–4 [S6]	CS	83	72–96	78.3[h]	1
hCG+CPE	200 IU[b]	3 mg	–	24	IM	15.5–16.7	12L:12D	nd	CS/TS	100	94	91[j]	6
CPE	4 mg	–	–	–	IP	13	12L:12D	3–4 [S6]	CS	67	72–96	78.2[h]	1
	4 mg	–	–	–	IP	15	12L:12D	3–4 [S6]	CS	67	48–72	74.9[h]	1
	3 mg[b]	3 mg	–	24	IM	15.5–16.6	12L:12D	nd	CS/TS	100	96	93[j]	6
	3 mg[b, c]	3 mg	–	24	IM	15.5–16.6	12L:12D	nd	CS/TS	94	81.5	86.5[j]	6
mGnRHa	5 µg	10 µg	2.5+5 mg	24	IP	14.5	nd	1–2 [S4]	CS	0	–	–	3
	5 µg	20 µg	2.5 mg+10 mg	24	IP	14.5	nd	1–2 [S4]	CS	50	94	3.2[i]	3
	1 µg	–	–	–	IM	19	14L:10D	2–4 [S4]	CS	86	89.3	52.3[k]	5
	2.5 µg	–	–	–	IM	20	14L:10D	2–4 [S4]	CS	71	84.42	65.5[k]	5
	5 µg	–	–	–	IM	21	14L:10D	2–4 [S4]	CS	71	83.9	51.1[k]	5
	10 µg	–	–	–	IM	22	14L:10D	2–4 [S4]	CS	71	79.4	52.2[k]	5
	25 µg	–	–	–	IM	23	14L:10D	2–4 [S4]	CS	100	93	60.5[k]	5
	50 µg	–	–	–	IM	24	14L:10D	2–4 [S4]	CS	86	86.5	22.1[k]	5
	40 µg[a]	–	20 mg	–	IP	13	12L:12D	3–4 [S6]	CS	67	96–120	80.3[h]	1
	40 µg[a]	–	20 mg	–	IP	15	12L:12D	3–4 [S6]	CS	67	72–96	72.3[h]	1
	2 µg[a, b, c]	2 µg	1+1 mg	24	IM	15.5–16.7	12L:12D	nd	CS/TS	85	102	72[j]	6
O–GnRHa	5 µg[b]	15 µg	–	24	IM	15.5–16.5	12L:12D	nd	CS/TS	100	110	43[j]	6
	5 µg[b]	15 µg	10 mg[e]	24	IM	15.5–16.5	12L:12D	nd	CS/TS	50	117	56[j]	6
NaCl	+	–	–	–	IP	13	12L:12D	3–4 [S6]	CS	17	144	–	1
	+	–	–	–	IP	15	12L:12D	3–4 [S6]	CS	–	–	–	1
	+	+	–	24	IP	14.5	nd	1–2 [S4]	CS	50	100	70.5[i]	3
	+	–	–	–	IM	25	14L:10D	2–4 [S4]	CS	0	–	–	5



Żarski et al.
CHAPTER 4:
Artificial reproduction of percid fishes

What did we learn?

- hCG was the most widely tested spawning agent – variable/ambiguous results
- The hormonal treatment was tested mostly in wild or pond-reared fish
- Non-treated fish were characterized by low spawning quality
- GnRH is effective without dopamine antagonists
- Mostly mammalian GnRH was tested
- Application of mGnRHa result in dose-dependent adverse effect (the higher the worse)

What can we conclude?

Application of the hormones can be beneficiary in wild and pond-reared fish

And what about domesticated fish?

State-of-the-art

Zakęś et al. 2013. Aquacult. Int. 21: 801-810.

Table 1 Characteristics of the pikeperch groups and injection applied for out-of-season stripping experiments (see "Materials and methods" section for explanation of groups)

Group	Age (year class)	N (indiv.)	Body weight (BW; kg) Mean	SD	Min.	Max.	Injection
Females							
Bg-4	4+	6	3.07	0.25	2.77	3.38	300 IU hCG kg^{-1} BW
Pl-4	4+	9	2.93	0.47	2.17	3.56	300 IU hCG kg^{-1} BW
Pl-3	3+	6	1.41	0.10	1.32	1.52	300 IU hCG kg^{-1} BW
Sr-3	3+	6	1.53	0.19	1.34	1.83	100 IU hCG + 200 IU PMSG kg^{-1} BW
Pg-3	3+	6	1.46	0.24	1.18	1.80	100 IU hCG + 200 IU PMSG kg^{-1} BW
Pl-2	2+	10	0.96	0.14	0.73	1.17	300 IU hCG kg^{-1} BW
Pl-5	5+	7	3.93	0.51	3.27	4.51	300 IU hCG kg^{-1} BW
C-2	2+	7	0.91	0.17	0.71	1.21	0.2 ml 0.9 % NaCl kg^{-1} BW
C-3	3+	6	1.35	0.23	1.15	1.72	0.2 ml 0.9 % NaCl kg^{-1} BW
Males							
Pl	2+	10	0.91	0.20	0.59	1.36	200 IU hCG kg^{-1} BW
Pl	3+	19	1.08	0.23	0.80	1.67	200 IU hCG kg^{-1} BW
Pl	4+	11	2.74	0.34	2.29	3.48	200 IU hCG kg^{-1} BW
Pl	5+	7	2.95	0.19	2.73	3.16	200 IU hCG kg^{-1} BW

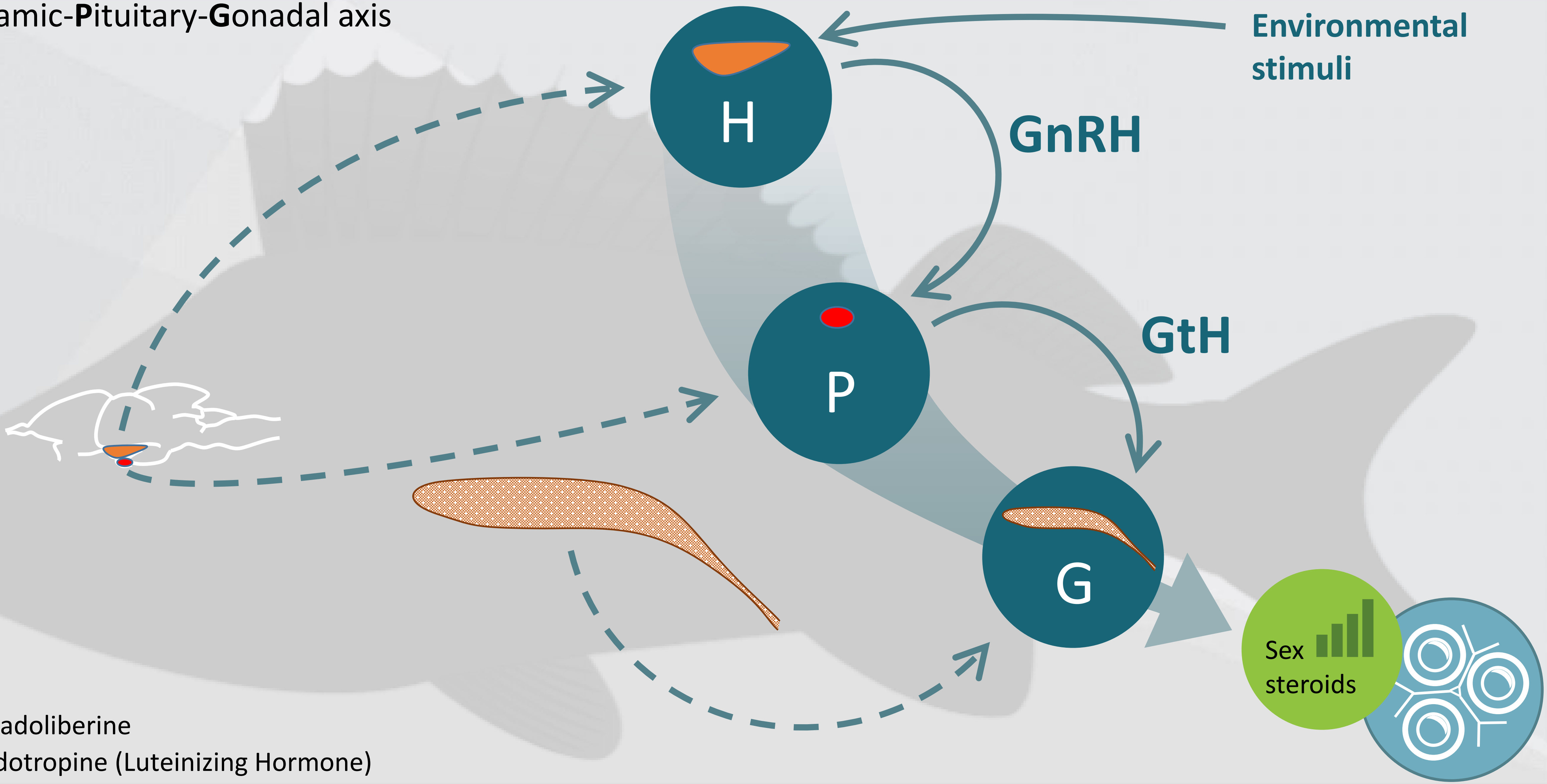
Table 2 Effect of tested preparations containing mammalian gonadotropins on cultured pikeperch out-of-season stripping (for explanation of fish groups, see Table 1)

Group	Stripped females (%)	Working fecundity (% BW)		Latency time (h)				Eyed-egg survival (%)		Female mortality (%)
		Mean	SD	Mean	SD	Min.	Max.	Mean	SD	
Experiment I										
Pl-4	100	11.16	1.90	74.75	7.79	65	84	77.33	6.50	0
Bg-4	100	10.86	1.26	65.73	8.76	57	81	70.67	7.18	0
Experiment II										
Pl-3	100	10.39	1.11	80.20^{a}	3.35	77	85	70.40	13.90	16.7
Sr-3	100	11.20	3.21	95.60^{b}	11.08	83	109	75.40	9.79	16.7
Pg-3	100	11.04	2.84	96.20^{b}	11.61	84	107	74.20	13.10	0
C-3	0	–	–	–	–	–	–	–	–	0
Experiment III										
Pl-2	80	5.40^{a}	1.67	102.44^{a}	8.08	82	107	47.11^{a}	14.87	10.0
Pl-3	100	10.39^{b}	1.11	80.20^{b}	3.35	77	85	70.40^{b}	13.90	16.7
Pl-4	100	11.16^{b}	1.90	74.75^{b}	7.79	65	84	77.33^{b}	6.50	0
Pl-5	100	10.87^{b}	1.63	77.14^{b}	7.15	68	87	70.29^{b}	11.28	14.3
C-2	0	–	–	–	–	–	–	–	–	14.3
C-3	0	–	–	–	–	–	–	–	–	0

Values in the same column with different superscript letters are significantly different ($P \leq 0.05$)

Hormonal regulation of ovulation

Hypothalamic-**P**ituitary-**G**onadal axis



GnRH – gonadoliberine

GtH – gonadotropine (Luteinizing Hormone)

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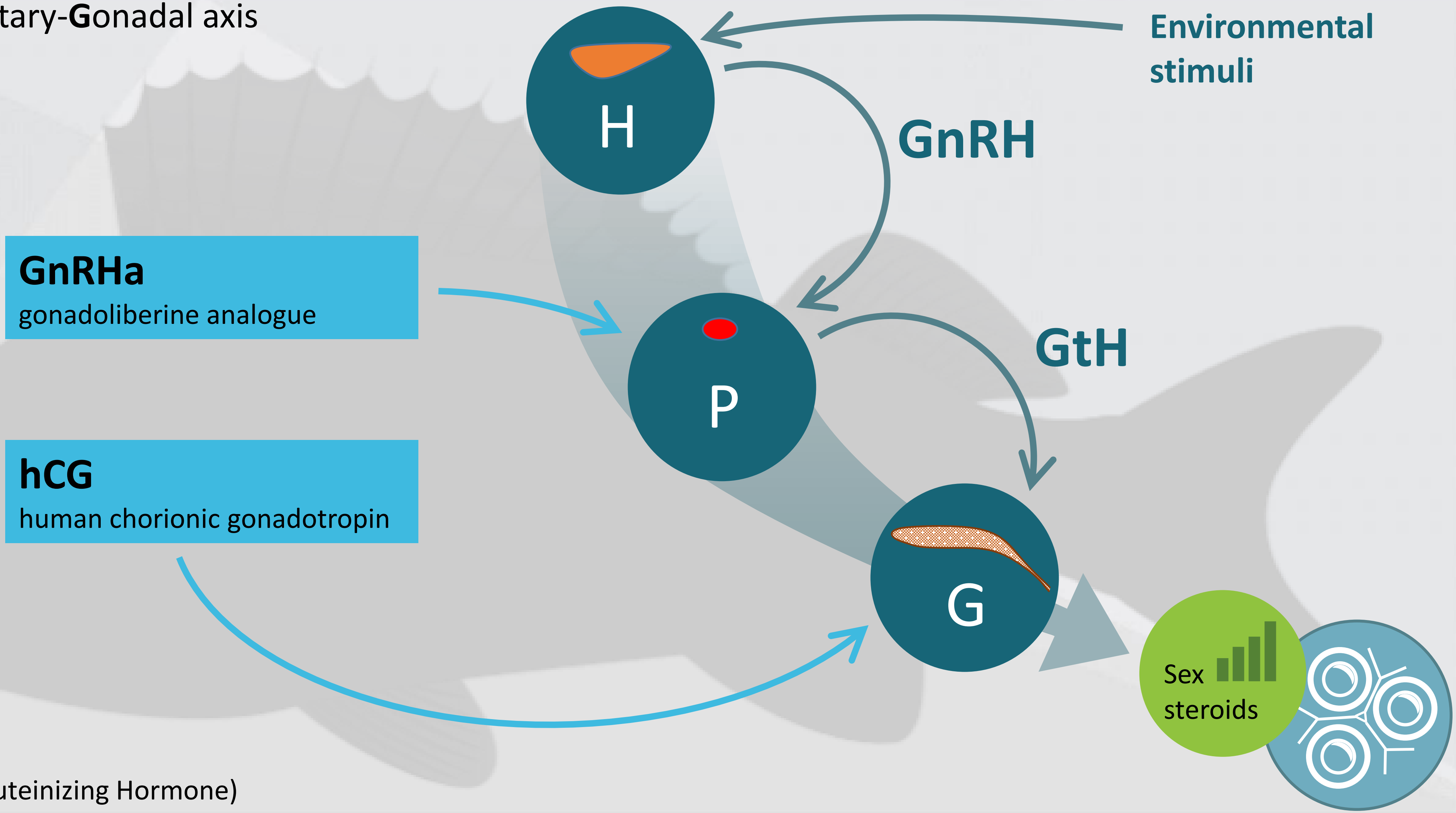
Hormonal regulation of ovulation

Hypothalamic-**P**ituitary-**G**onadal axis



GnRH – gonadoliberine

GtH – gonadotropine (Luteinizing Hormone)

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Differences between hCG and GnRH

hCG
human chorionic gonadotropin

+ Long half-life in the blood (up to few days) = single injection is enough
  Wide range of doses were found to be effective

− Immune response
  High stress response after application of hCG

GnRHa
gonadoliberine analogue

+ Stimulates endogenous gonadotropins (GtH) - lack of immune response
  No evidence for stress response after application of GnRH alone

− Short half-life (several hours?)
  Induces release of dopamine (?) which inhibits release of GtHs

Application of GnRH in induction of ovulation

In non-percid freshwater finfishes

- Usually **not effective when administered alone** = need for administration of **dopamine antagonists** (DA)
- Due to the short half-life it is necessary to **repeat the injection**
- Very good spawning results

In percids

- **Effective when administered alone** (no need to apply DA)
- Very often administered together with dopamine antagonists (?)
- Tested in **double injection** mostly **with DA**
- Only **mGnRHa** was tested so far

What else can be improved?

Table 1. Amino acid composition of naturally occurring GnRH forms and GnRH analogues used in hormonal therapies in Cyprinidae

GnRH forms	Amino acid sequences	
	1 2 3 4 5 6	7 8 9 10
Native forms		
sGnRH	pGlu – His – Trp – Ser – Tyr – Gly	– Trp – Leu – Pro – Gly-NH_2
cGnRH-II	pGlu – His – Trp – Ser – His – Gly	– Trp – Gln – Pro – Gly-NH_2
Synthetic analogues		
mGnRHa	pGlu – His – Trp – Ser – Tyr – D-Ala	– Leu – Arg – Pro – Net
	pGlu – His – Trp – Ser – Tyr – D-Tle	– Leu – Arg – Pro – Net
	pGlu – His – Trp – Ser – Tyr – D-Trp	– Leu – Arg – Pro – Net
	pGlu – His – Trp – Ser – Tyr – [D-Nal(2)]	– Leu – Arg – Pro – aza-Gly
	pGlu – His – Trp – Ser – Tyr – [D-Ser(t-Bu)]	– Leu – Arg – Pro – Net
sGnRHa	pGlu – His – Trp – Ser – Tyr – D-Arg	– Trp – Leu – Pro – Net

Podhorec and Kouril 2009. Czech J Anim Sci

What else can be improved?

Aquaculture, 74 (1988) 1-10 1
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

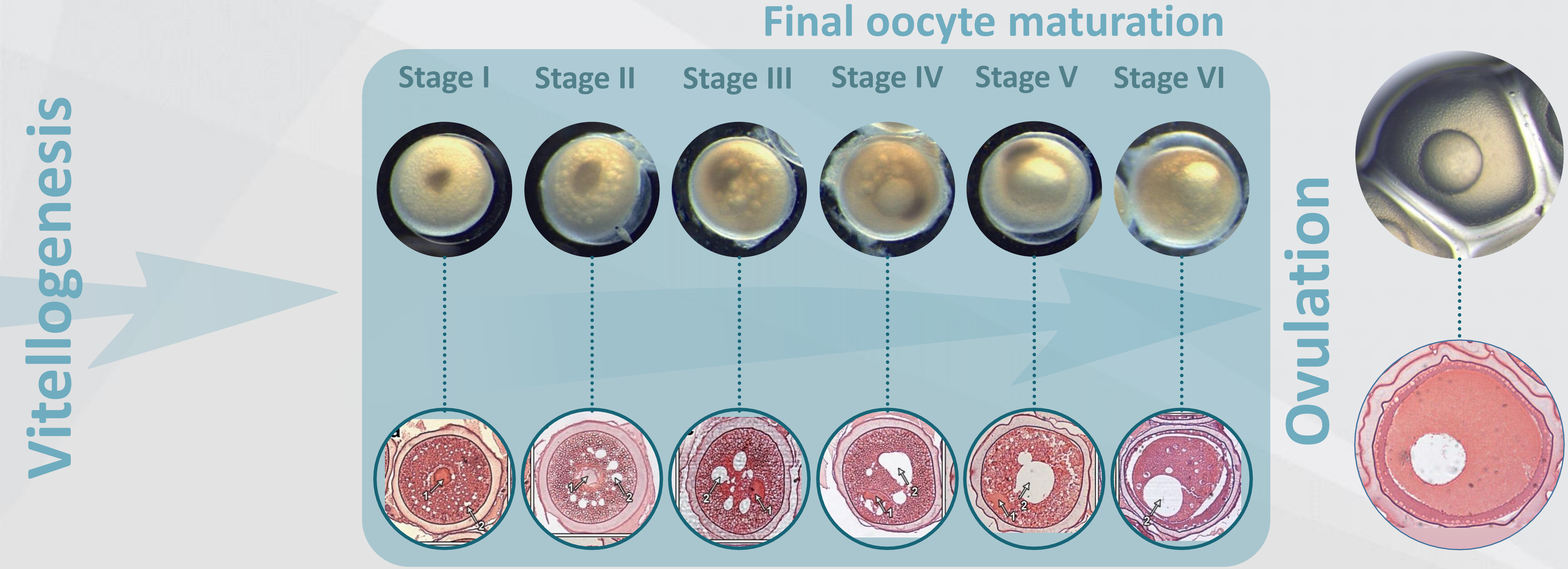
Induced Ovulation and Spawning of Cultured Freshwater Fish in China: Advances in Application of GnRH Analogues and Dopamine Antagonists

RICHARD E. PETER[1], HAO-REN LIN[2] and GLEN VAN DER KRAAK[1]
[1]*Department of Zoology, University of Alberta, Edmonton, Alta. T6G 2E9 (Canada)*
[2]*Department of Biology, Zhongshan University, Guangzhou (China)*

(Accepted 13 June 1988)

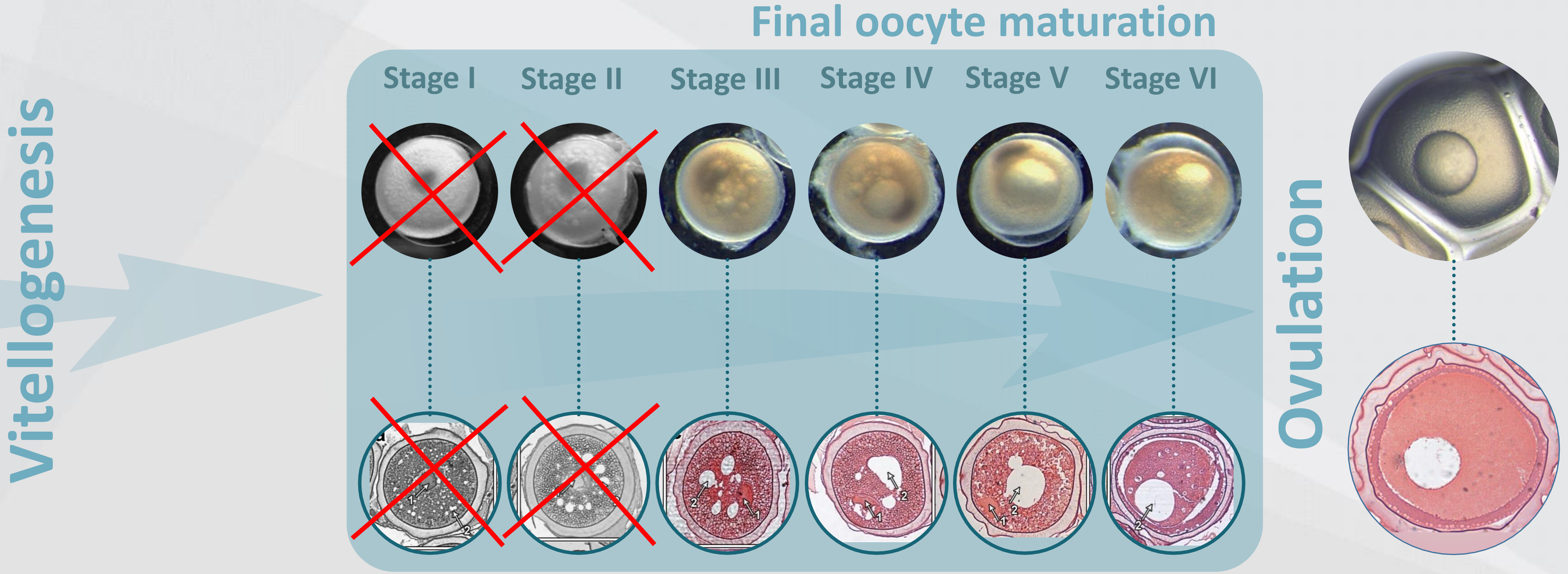
„(…) an analogue of a teleost GnRH [D-**Arg**^{6}, Trp^{7}, Leu^{8}, Pro^{9}- NEt]-LHRH **(sGnRHa)** was found to be **more potent** than ([D-**Ala**^{6}, Pro^{9}-NEt] – *edit*.) LHRH-A in inducing ovulation in goldfish (…)"

Final oocyte maturation (FOM)



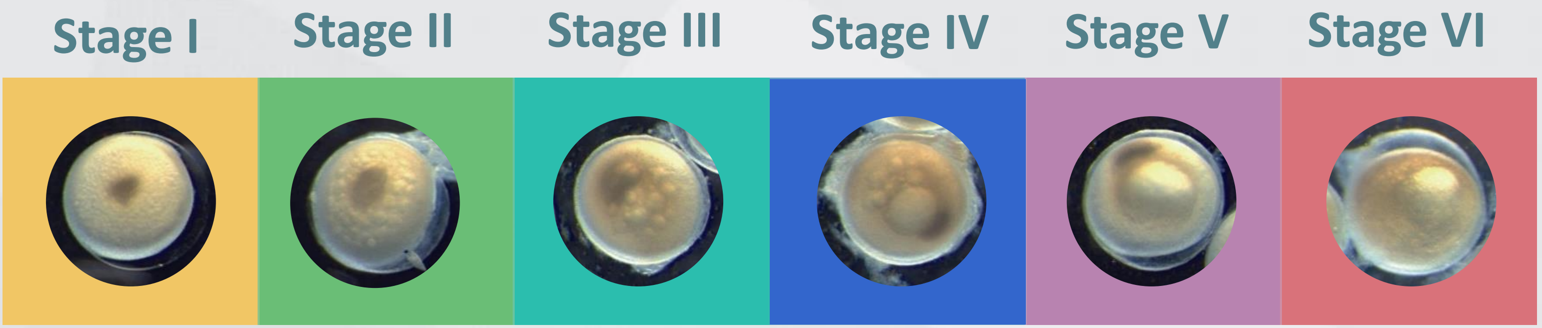
Żarski et al. 2011, Reproductive Biology 11: 194-209
Żarski et al. 2012, Aquaculture 364-365: 103–110

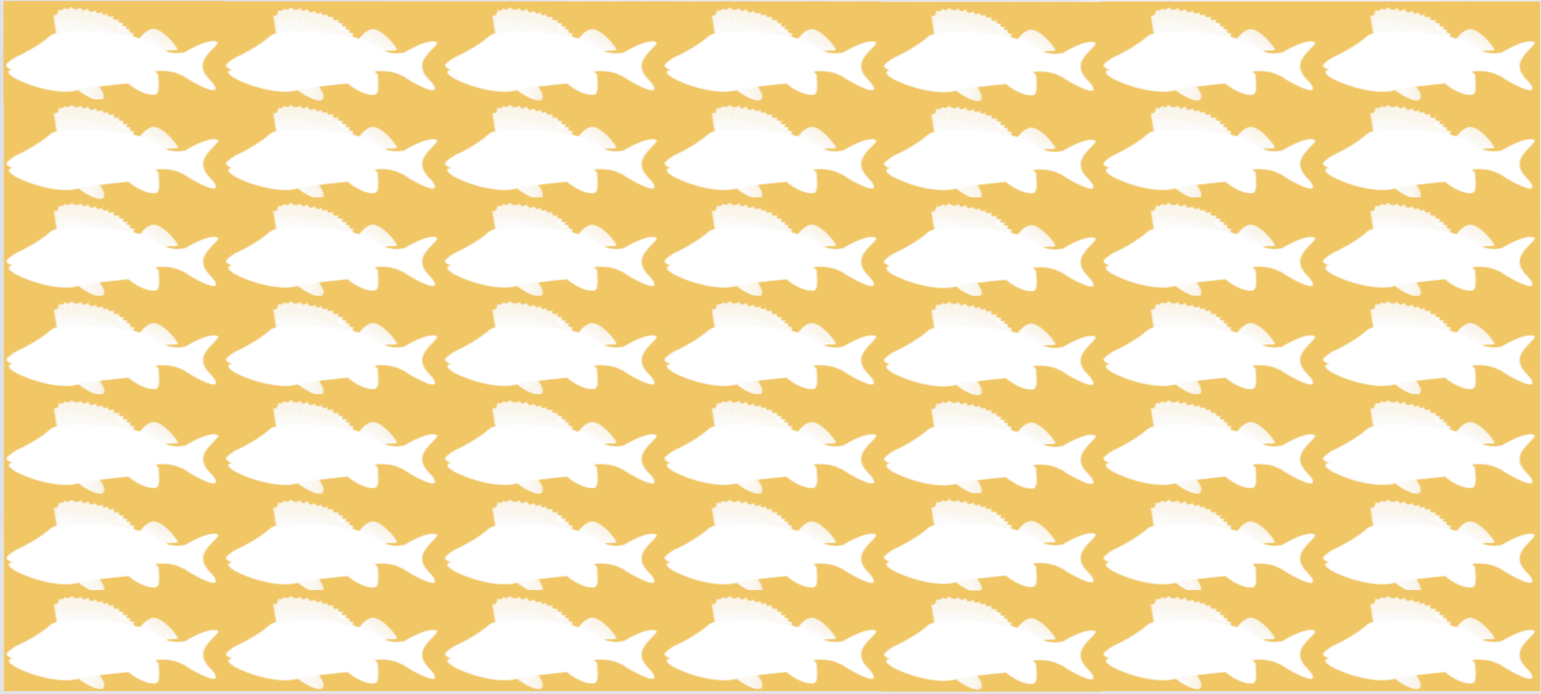
Final oocyte maturation (FOM)



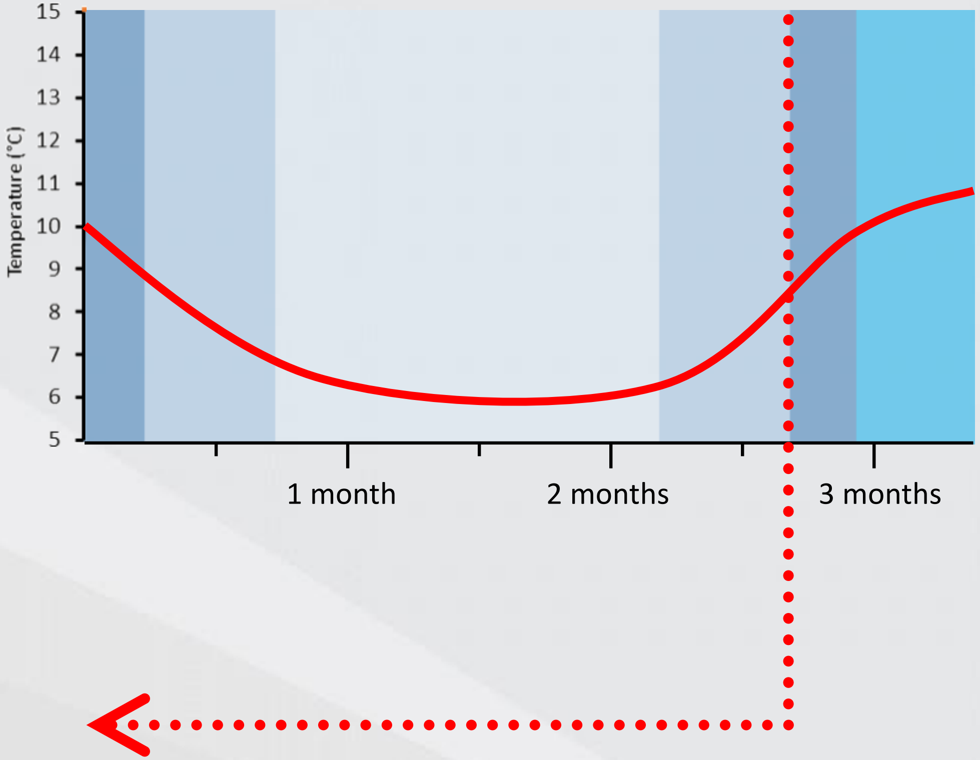
Żarski et al. 2011, *Reproductive Biology 11: 194-209*
Żarski et al. 2012, *Aquaculture 364-365: 103–110*

Typical kinetics of FOM in percids





Wintering period



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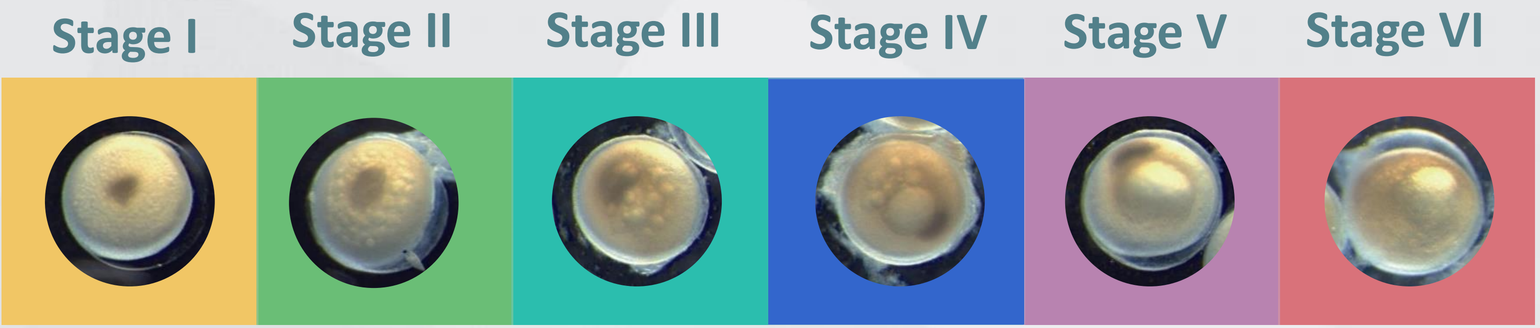
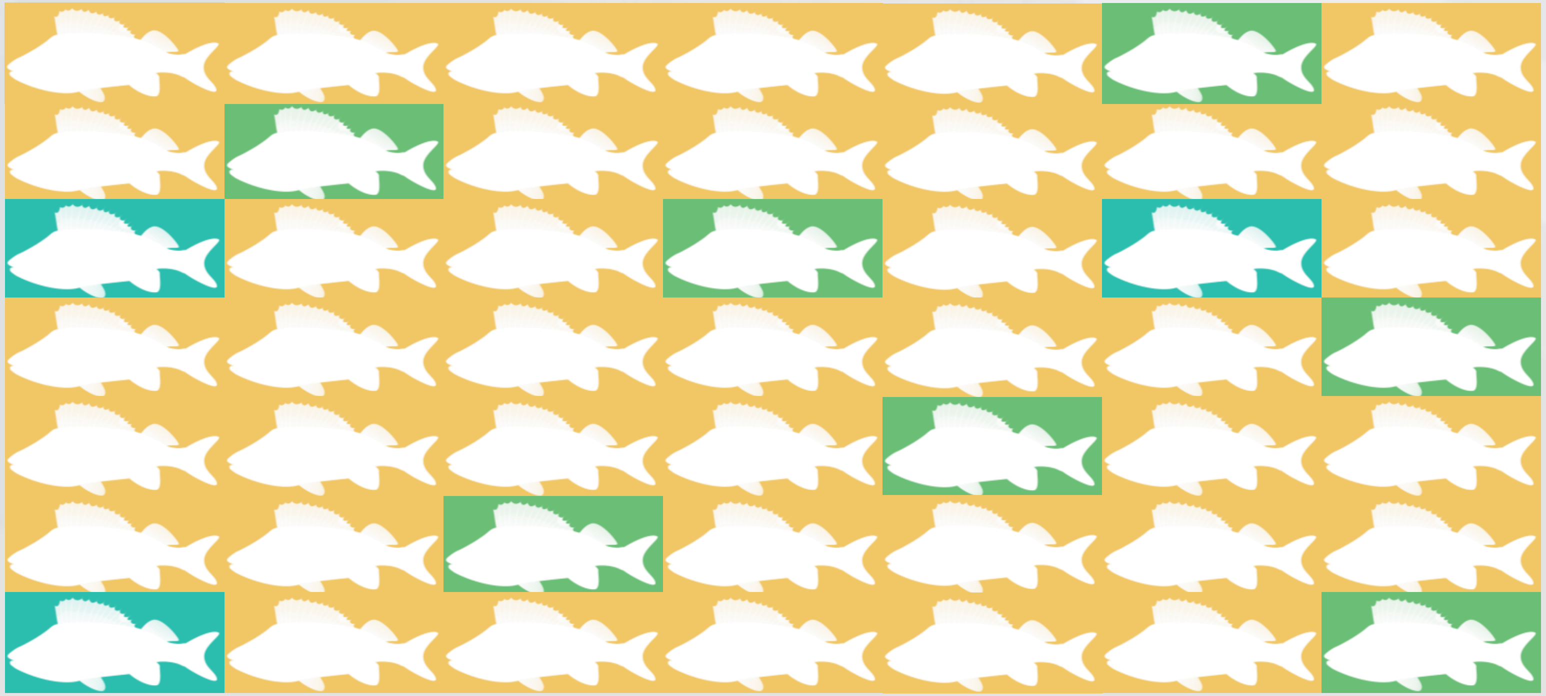
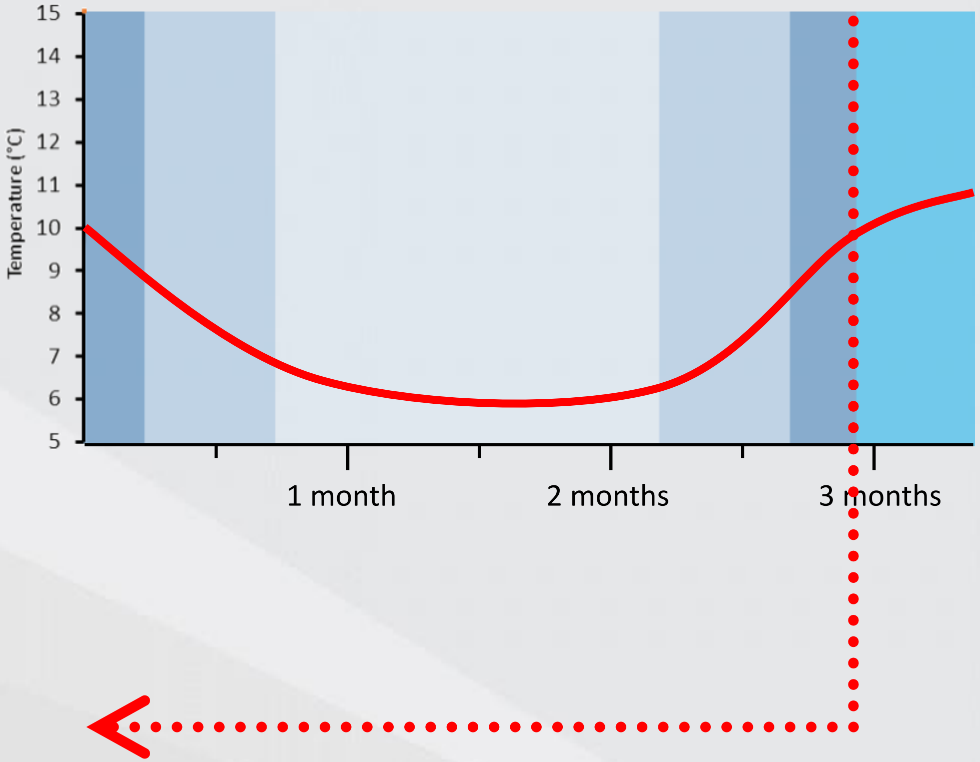
Typical kinetics of FOM in percids





Wintering period



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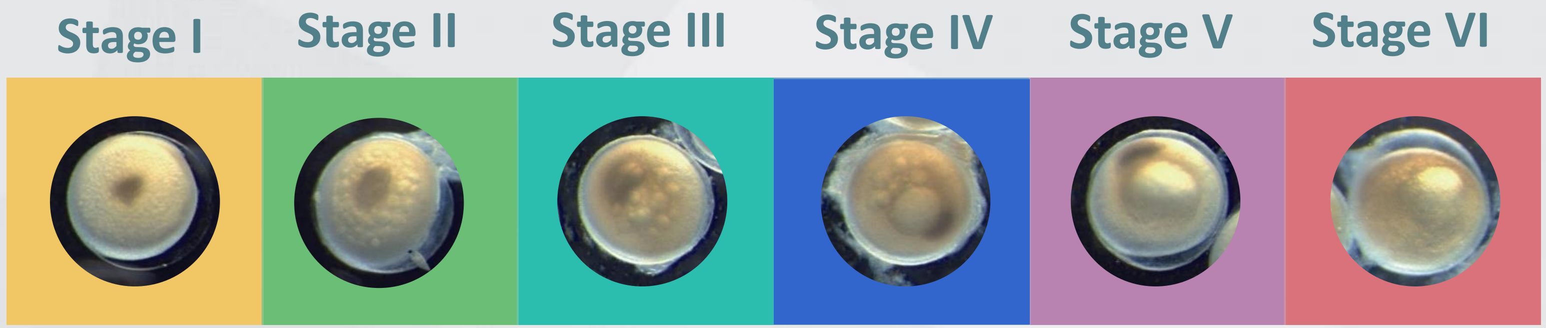
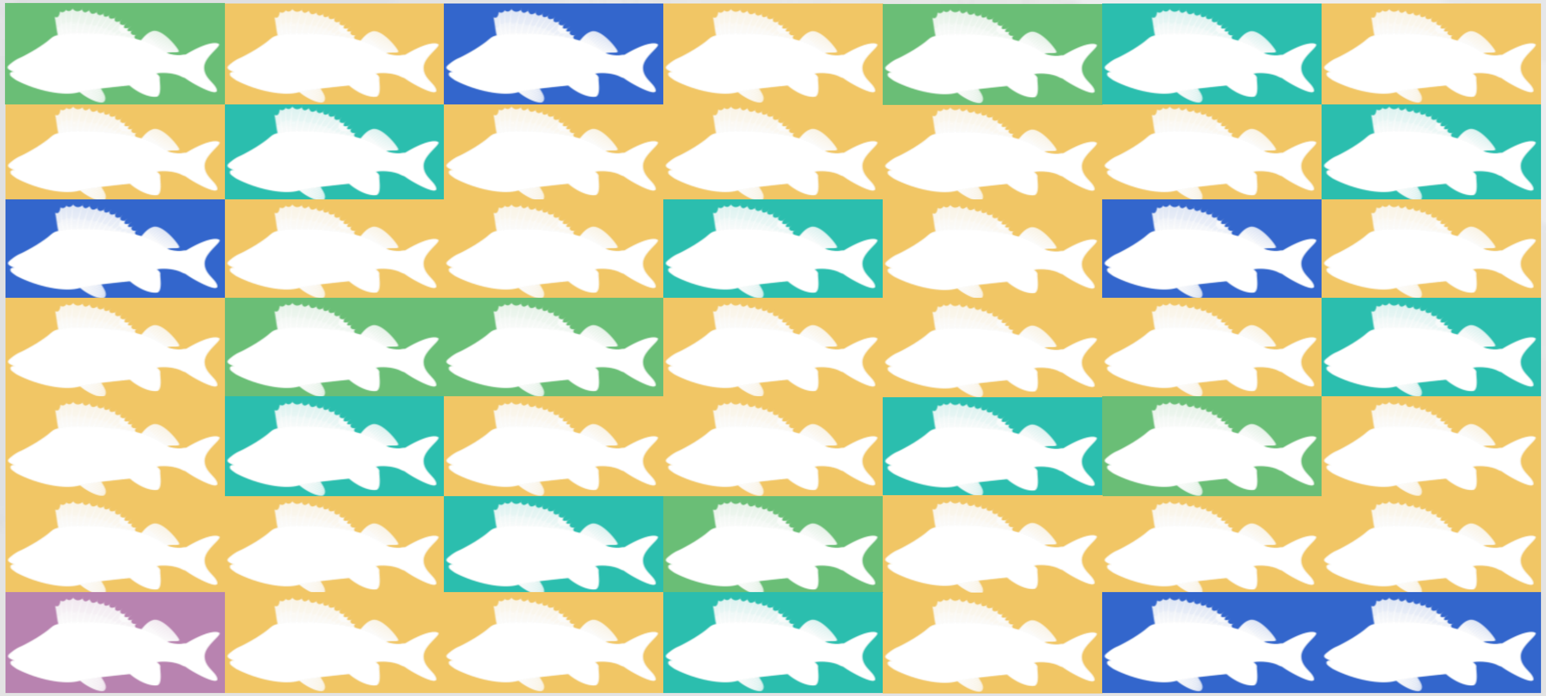
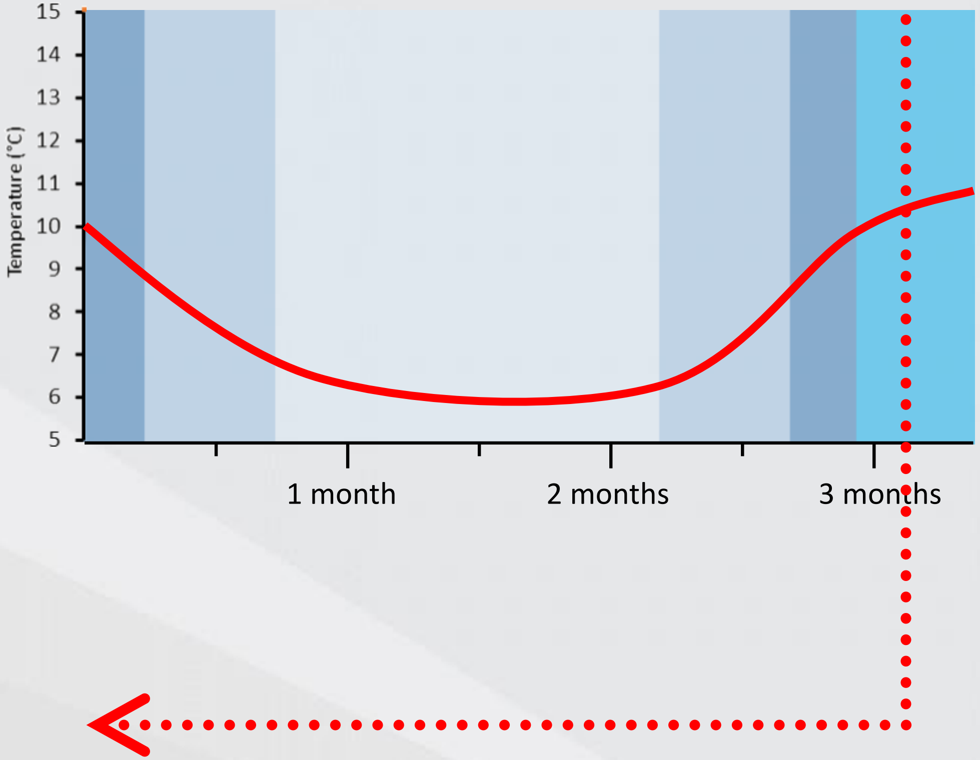
Typical kinetics of FOM in percids





Wintering period



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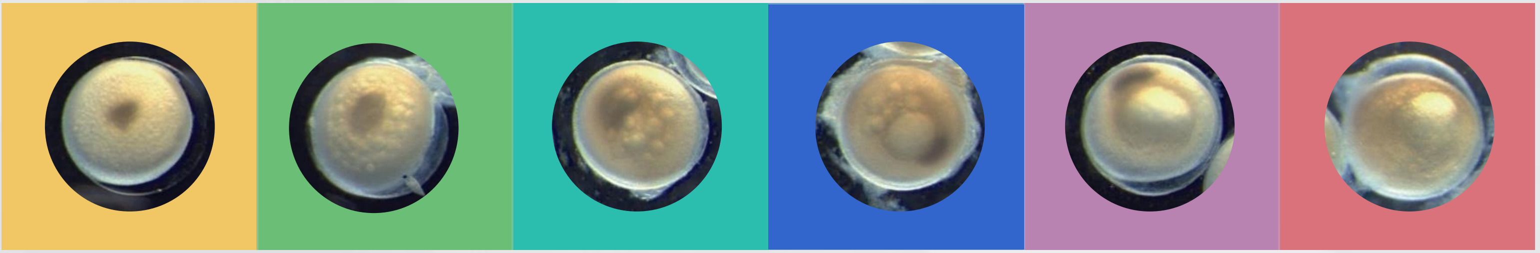
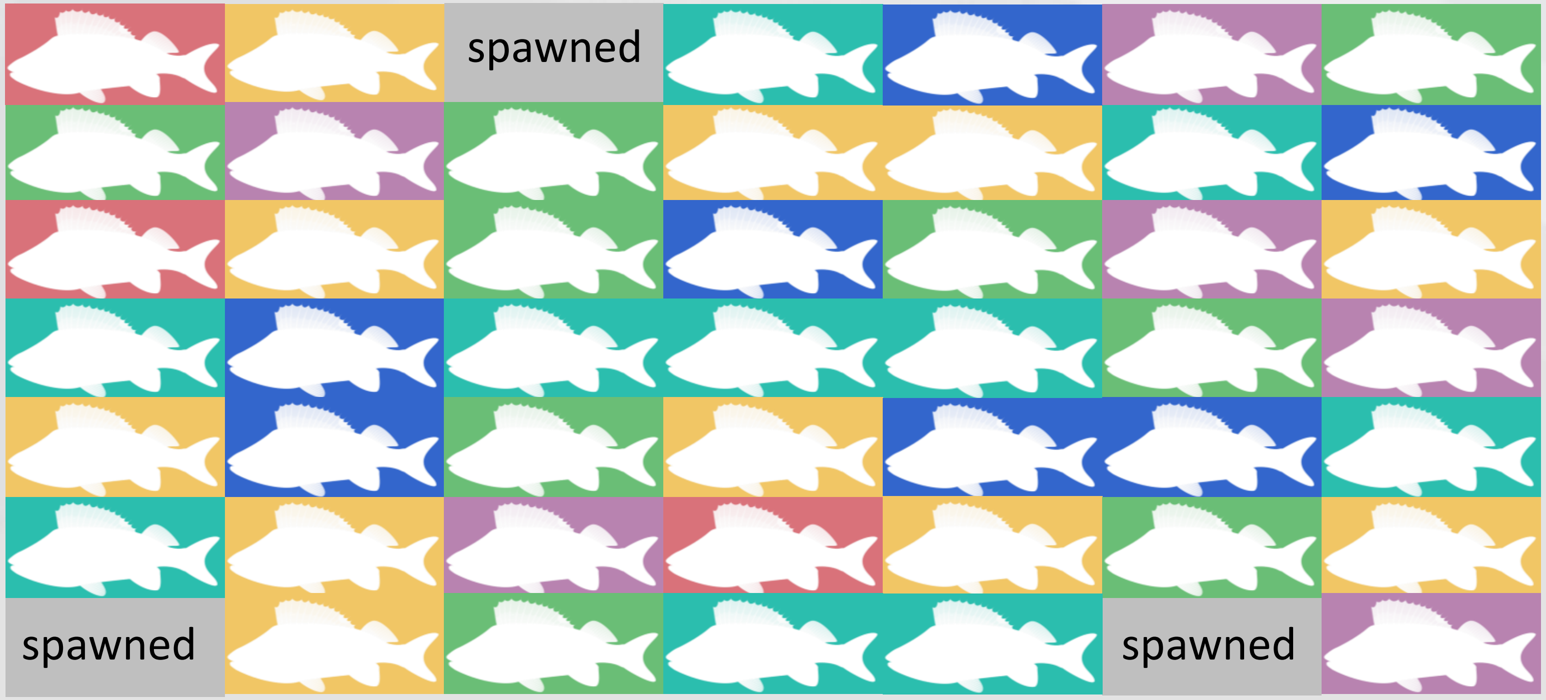
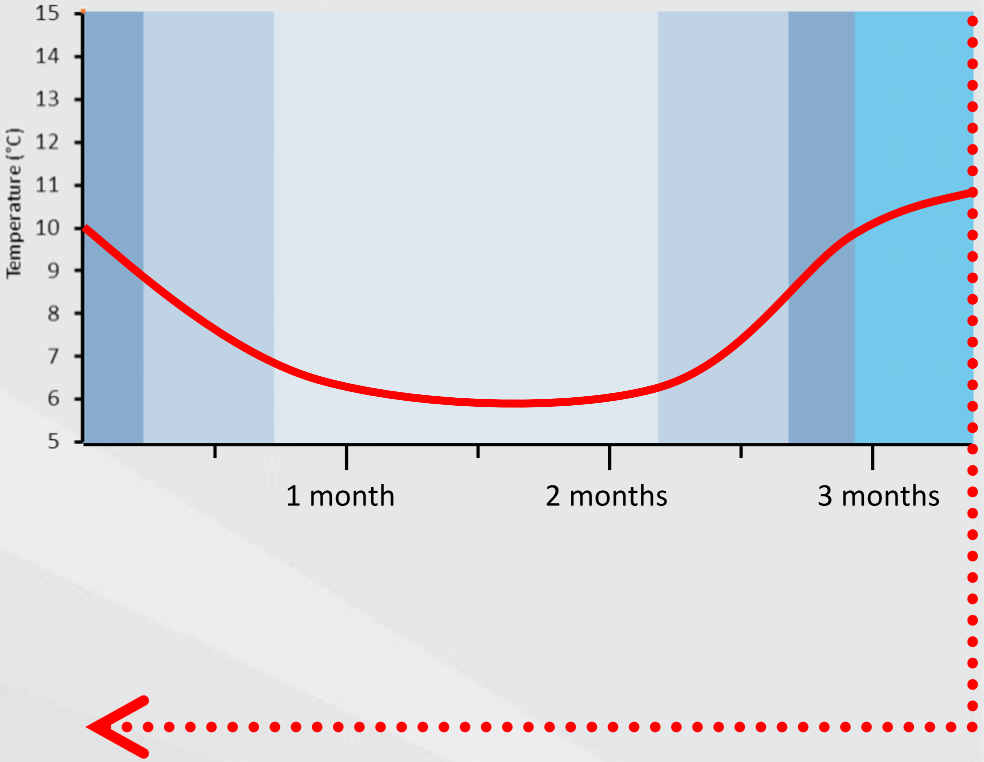
Typical kinetics of FOM in percids

Stage I | Stage II | Stage III | Stage IV | Stage V | Stage VI



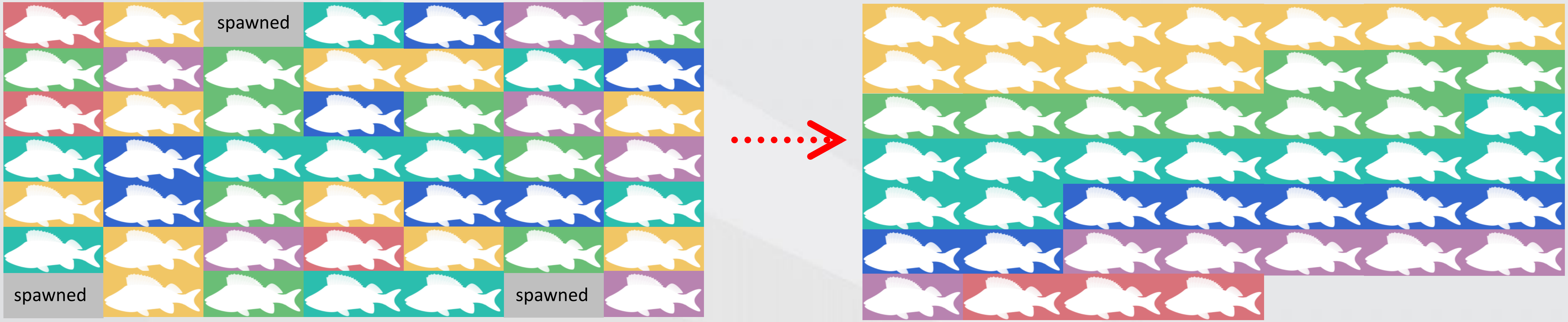


Wintering period



Beginning of the spawning ‚season' ?

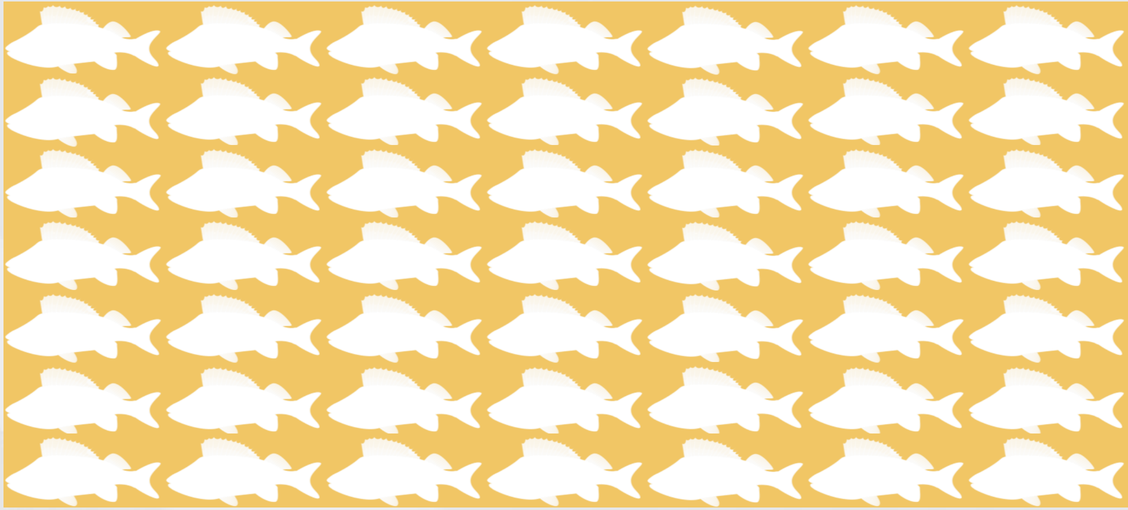
Without hormonal stimulation



- Separation of fish according to their maturation stage (reducing stress caused by checking)
- Development of procedures for controlling them for ovulation (exact timing)
- Recording spawning kinetics over years and possibly make suitable selection?
- High number of females and use only part of them?

labor + time + cost

With hormonal stimulation



- Egg quality is the highest concern
- Good protocol is needed

Objectives

The aim

To evaluate the effectiveness of sGnRHa in comparison to hCG in controlled reproduction of domesticated pikeperch females

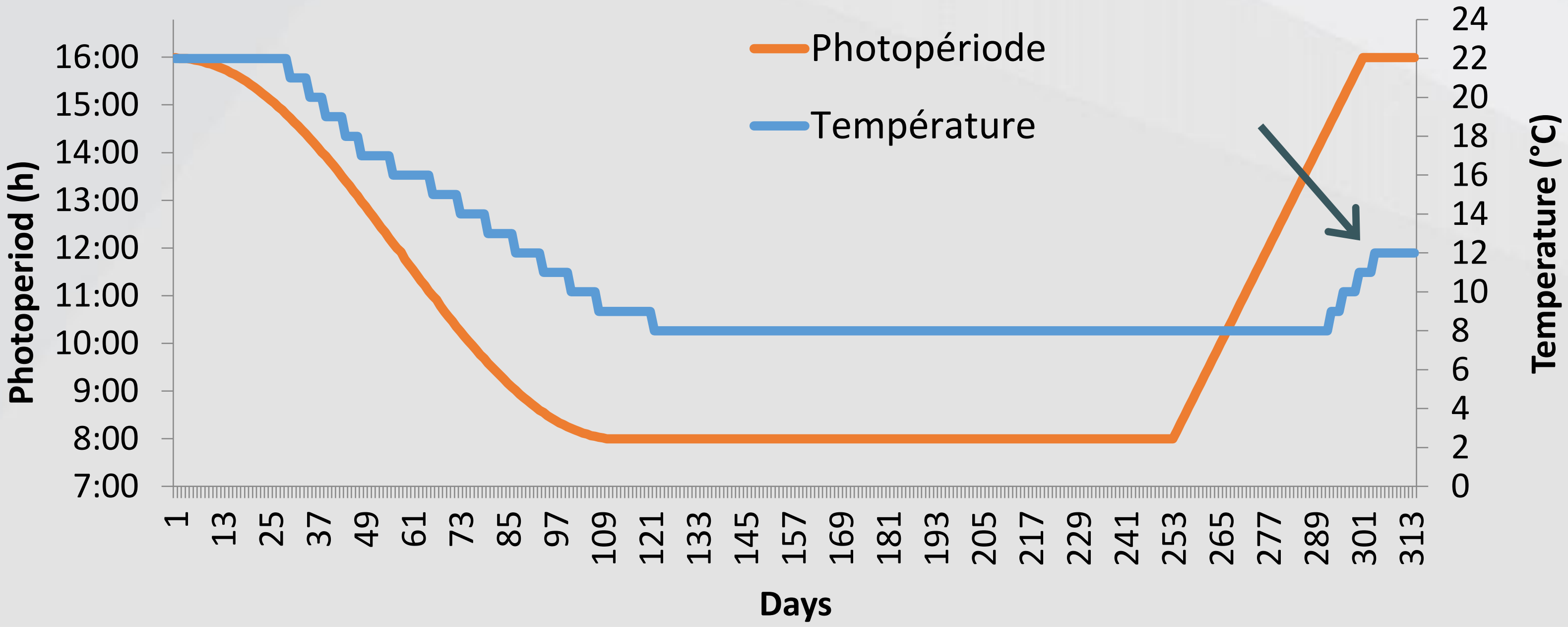
Experimental strategy

Three experiments:

1. 2016: Screening of various doses of hCG and sGnRHa
2. 2017: Verification of double injection strategy of sGnRHa (as for *P. fluviatilis*)
3. 2018: „Large" scale validation of the best treatments

Experimental conditions

- Domesticated broodstock
- 3+, 4+ and 5+
- Second, third and fourth spawning
- Only fish at maturation stage I were used *(Żarski et al. 2012)*



Following protocol for perch (modified)
afte Abdulfatah (2010)

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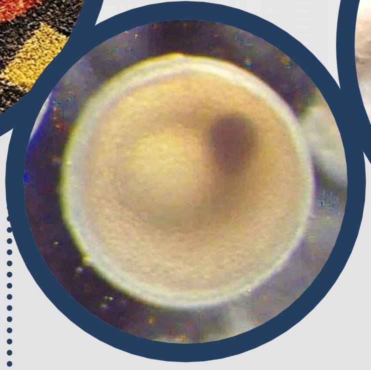
Material and methods

Hormonal injection

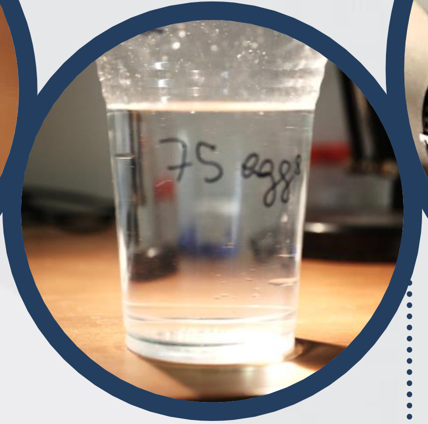


Progress of maturation stage of oocytes was monitored

Eggs stripping

Sperm stripping

In vitro fertilization on a Petri dish



Incubation in a ‚beakers' (11-13 °C)

Fertilization rate (72 h post fertilization), hatching rate and larval deformity rate were counted under the microscope

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Material and methods

Experiment 1 (2016)

Group	Control	GnRH-10	GnRH-25	GnRH-50	hCG-250	hCG-500	hCG-1000
Type of hormone	NaCl	sGnRHa			hCG (Chorulon)		
Dose (per kg)	1 ml	10 µg	25 µg	50 µg	250 IU	500 IU	1000 IU

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Material and methods

Experiment 1 (2016)

Group	Control	GnRH-10	GnRH-25	GnRH-50	hCG-250	hCG-500	hCG-1000
Type of hormone	NaCl	sGnRHa			hCG (Chorulon)		
Dose (per kg)	1 ml	10 µg	25 µg	**50 µg**	250 IU	**500 IU**	1000 IU

Material and methods

Experiment 1 (2016)

Group	Control	GnRH-10	GnRH-25	GnRH-50	hCG-250	hCG-500	hCG-1000
Type of hormone	NaCl	sGnRHa			hCG (Chorulon)		
Dose (per kg)	1 ml	10 µg	25 µg	**50 µg**	250 IU	**500 IU**	1000 IU

Experiment 2 (2017)

Group	Control	5+25	10+25	25+25	50	hCG
Type of hormone	NaCl	sGnRHa				hCG (Chorulon)
Dose of 1st injection (per kg)	1 ml	5 µg	10 µg	25 µg	50 µg	500 IU
Dose of 2nd injection (per kg)*	-	25 µg			-	-

* 2nd injection given 48 h after the 1st injection

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Group	Control	GnRH-10	GnRH-25	GnRH-50	hCG-250	hCG-500	hCG-1000
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Group	Control	5+25	10+25	25+25	50	hCG
Type of hormone	NaCl	sGnRHa				hCG (Chorulon)
Dose of 1st injection (per kg)	1 ml	5 µg	10 µg	25 µg	**50 µg**	**500 IU**
Dose of 2nd injection (per kg)*	-	25 µg			-	-

* 2nd injection given 48 h after the 1st injection

Experiment 3 (2018)

Group	GnRHa	hCG
Type of hormone	sGnRHa	hCG (Chorulon)
Dose (per kg)	50 µg	500 IU

Results

Experiment 1 (2016)

Tab. 2.Results of controlled reproduction of pikeperch after application of different hormonal preparations at different doses

Group	n	Latency time (h)			Fertilization rate (%)			Hatching rate (%)		
		Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
GnRH-10	6	139	40	28	30.9	27.4	89	18.1	25.3	140
GnRH-25	7	137	53	39	14.9	26.2	176	11.8	25.5	216
GnRH-50	6	169	50	29	67.6	9.6	14	60.6	11.5	19
hCG-250	7	146	31	21	28.3	28.9	102	19.4	19.6	101
hCG-500	7	130	14	11	46.9	19.6	42	32.0	18.4	57
hCG-1000	7	113	23	20	48.5	37.8	78	37.4	34.4	92

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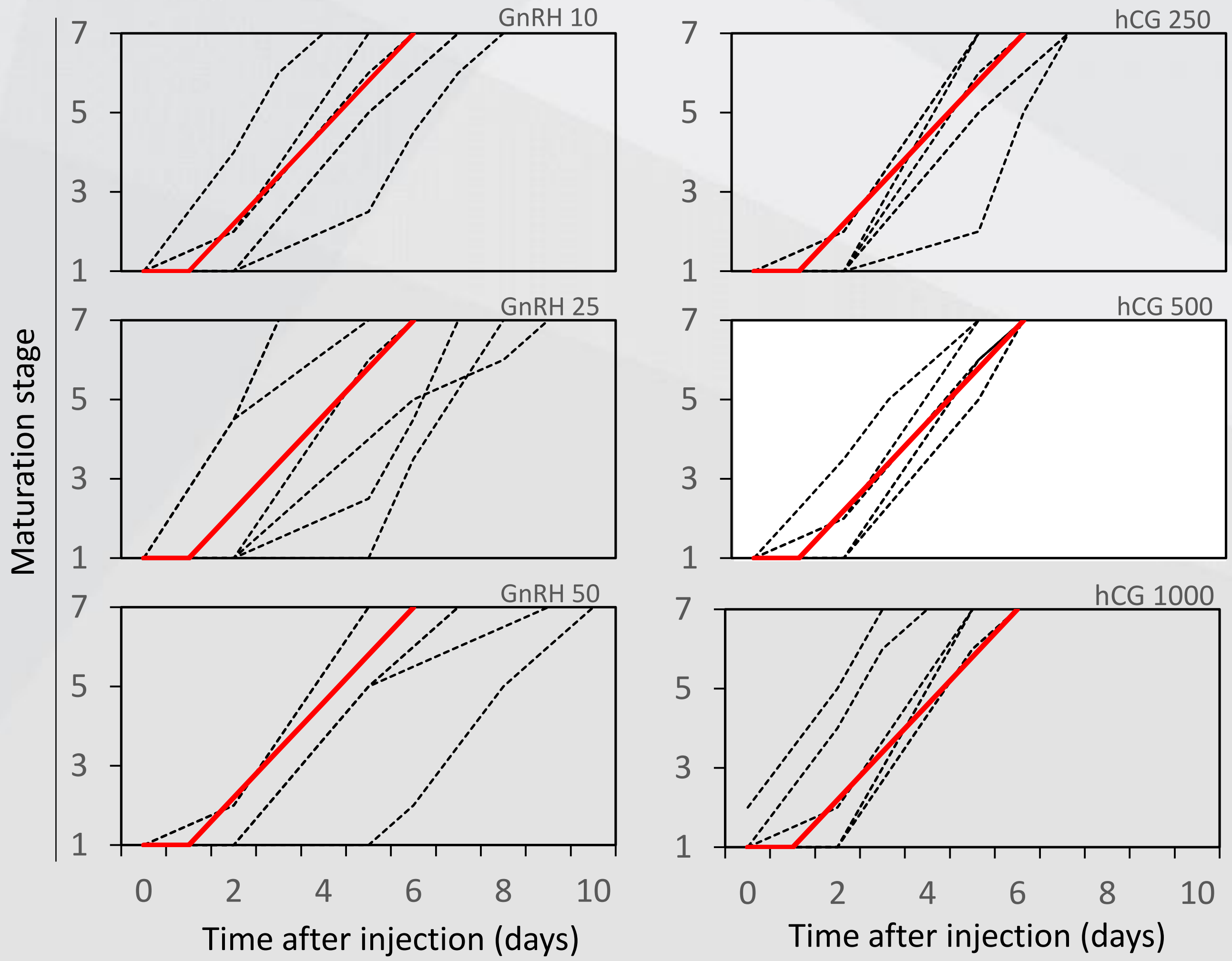
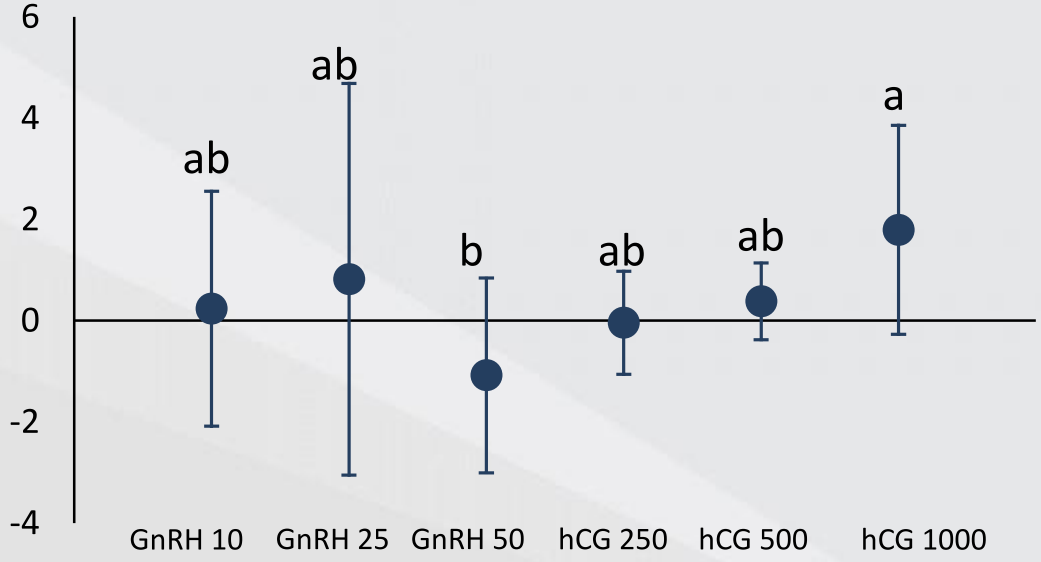
Experiment 1 (2016)



$$Z = \frac{\beta_1 - \beta_2}{\sqrt{(SE\beta_1)^2 + (SE\beta_2)^2}}$$



Clogg et al. (1995)
Paternoster et al. (1998)

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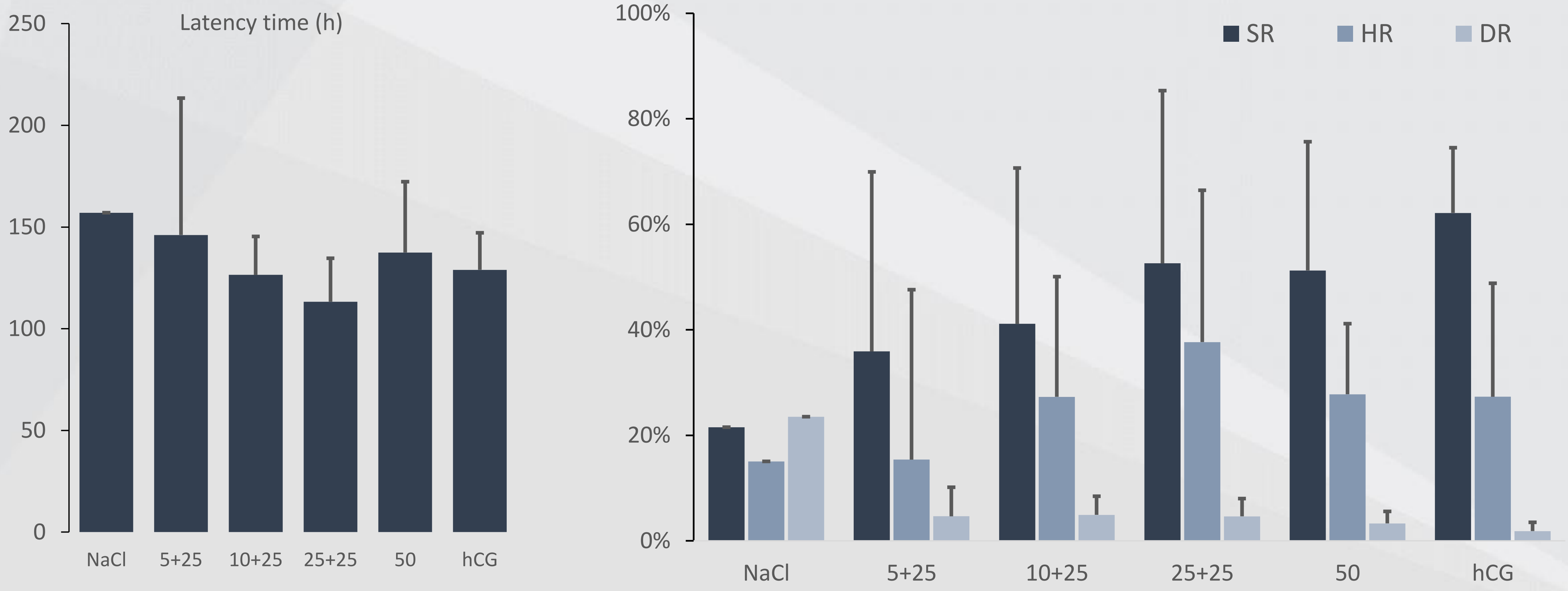
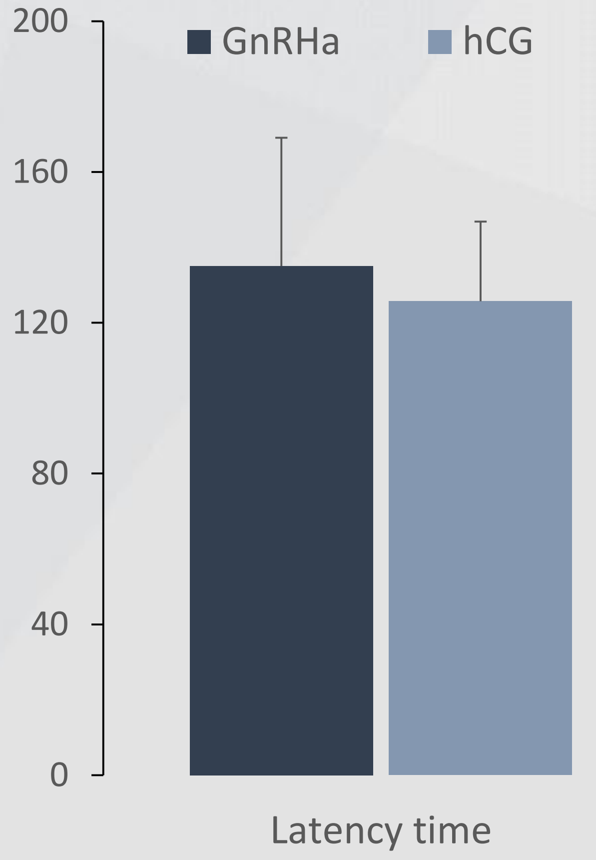
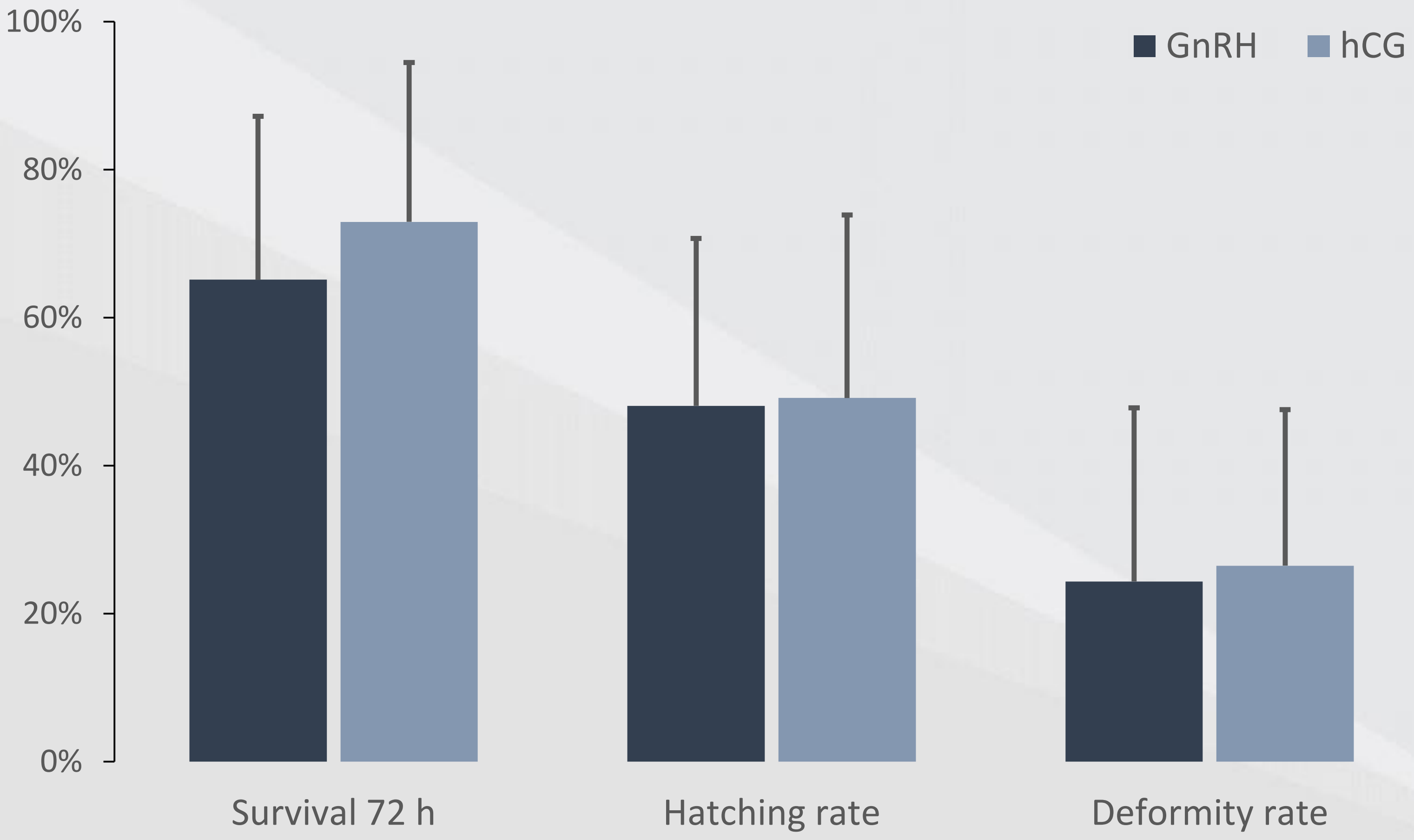
Experiment 2 (2017)



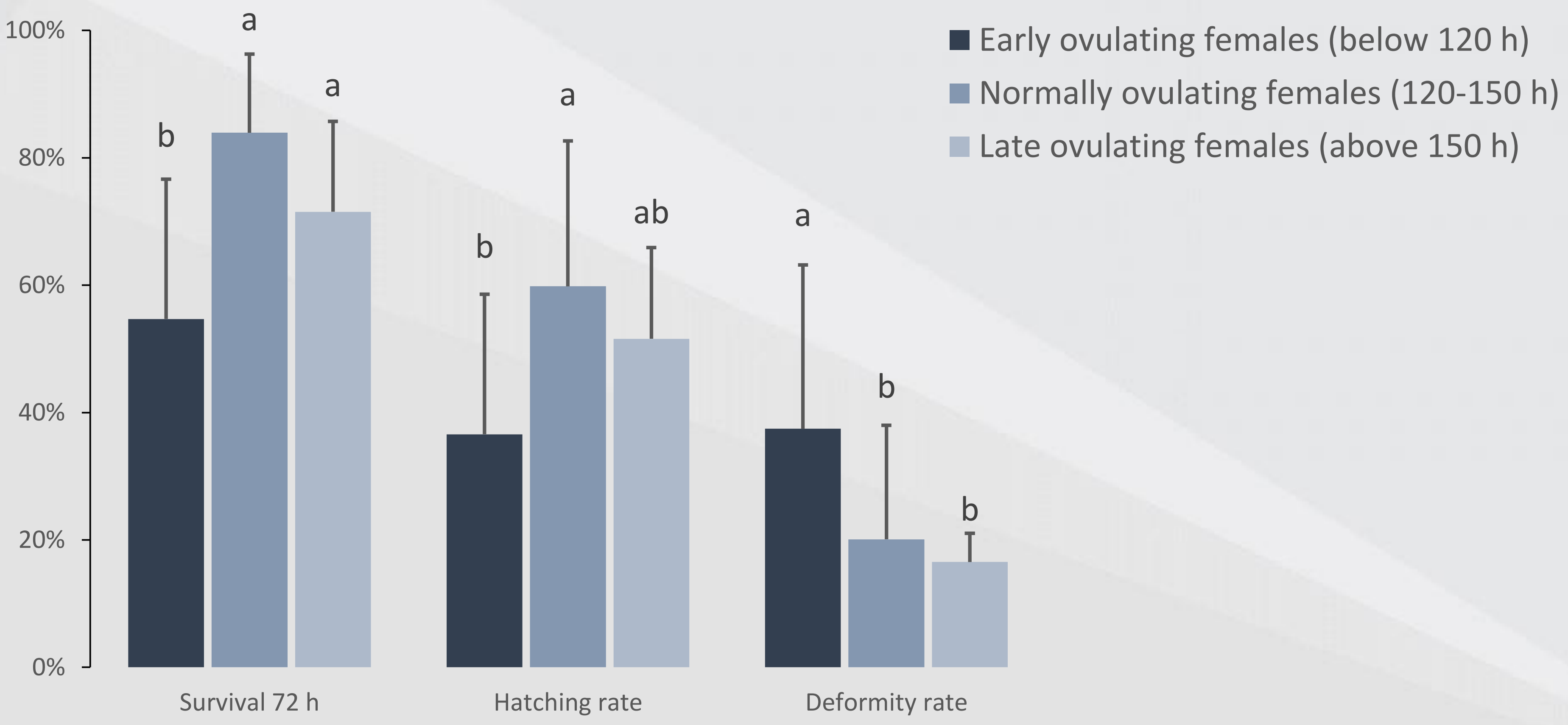
Results

Experiment 3 (2018)



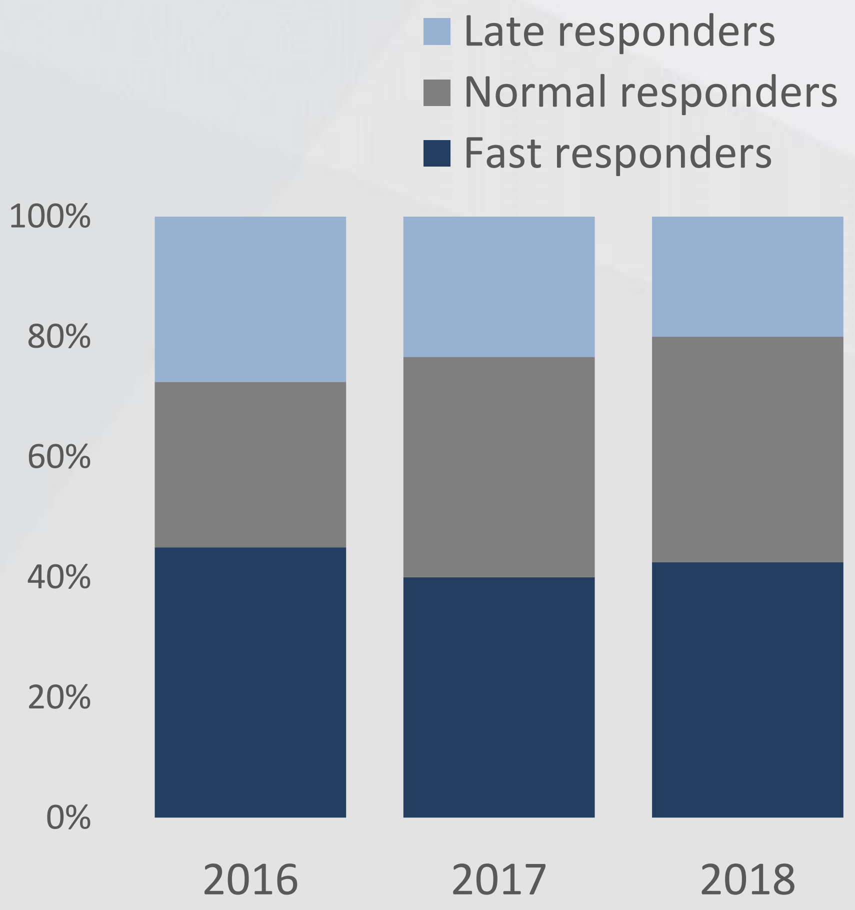


Experiment 3 (2018)

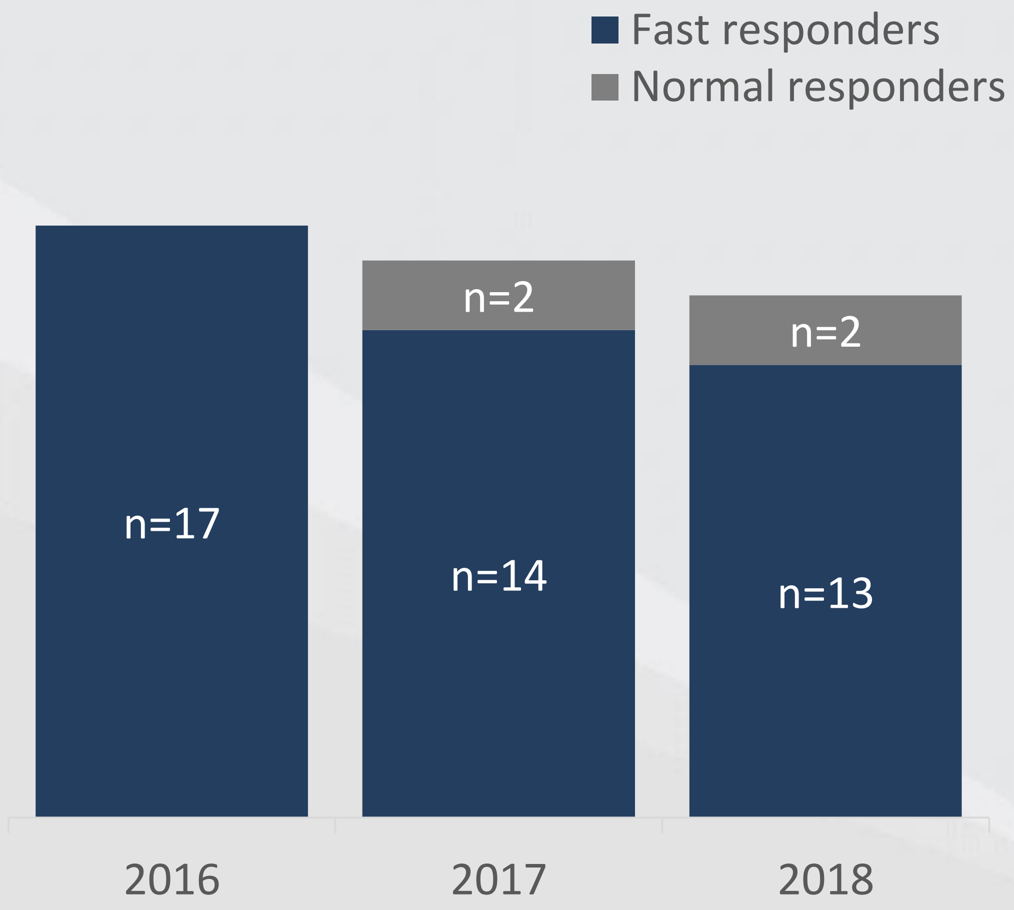


Results

Percentage share of differently responding fish (to hormonal treatment) over the years



„History" of „fast responders". Seventeen fish were followed for 3 years



Conclusions

1. Both hCG and sGnRHa were found to be efficient for induction of ovulation in pikeperch at various doses
2. A single-dose hormonal treatment mode is recommended for induction of ovulation in pikeperch at a doses:
   a) 500 IU of hCG per kg
   b) 50 µg of sGnRHa per kg
3. Fish responding to hormonal treatment fastly (i.e. below 120 h at 12°C) were constituting high proportion of fish yielding lowered egg quality

Future prospects

1. „Fast responders" (~40% of population) – what they really are and why they are like they are?
2. Can we enhance egg quality in „fast responders"?
3. Testing different forms of GnRHa (reduce costs, increase availability)
4. Thermal manipulation – the effect on spawning synchronization and egg quality
5. Can it be useful „trait" in selective breeding programs?
6. Reconsideration of selection according to „response to hormone" rather than „egg quality"

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Acknowledgments

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