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in České Budějovice

Importance and innovation of pikeperch (*Sander lucioperca*) controlled reproduction

Azin Samarin, Tomáš Policar and Oleksandr Malinovskyi

Importance of pikeperch

-highly priced and perspective economic species,

-High market demand, attractiveness as a game fish,

-source of high-quality muscle without tiny bones,

-biomeliorative tool in ponds, reservoirs, etc.,

-a marketing tool for selling market carp, (do you want pikeperch? Only with carp in addition, pikeperch + carp in high-scale sales)

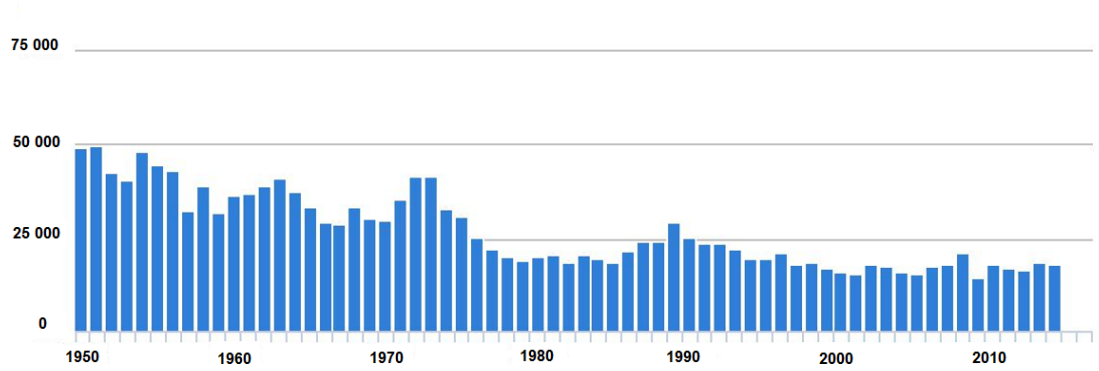
-production does not cover market demands,

-high retail prices = possibly high culture profitability,

- high request of stocking material to intensive farming (RAS)

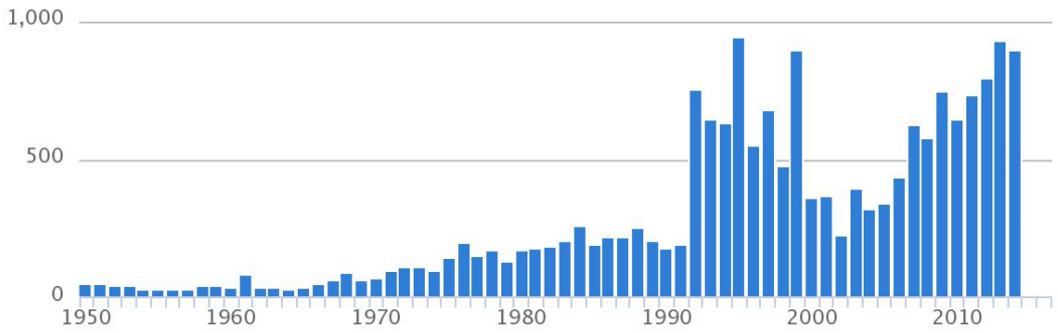
Current pikeperch production

Global capture production of pikeprech (*Sander lucioperca*) - tonnes



Source: FAO FishStat

Global aquaculture production of pikeperch *(Sander lucioperca)* in tonnes



Source: FAO FishStat

Future development of pikeperch production

In open waters decline in pikeperch population occurs because of:

- Barely managed catches in Ukraine, Russia, Kazakhstan, etc.,

- Huge fishing pressure,

- Lack of stocking to open waters, stocking of unappropriated age of the fish.

- Eutrophication processes led to low-oxygen conditions in the waters,

- Poorly implemented fishery management.

Future development of pikeperch production

Pikeperch breeding in Europe is gaining importance in terms of production

- **The main goal is producing larger volumes:**

1) Market fish for consumption or stocking to open waters,
2) Stoking material for intensive culture; stocking into open water.

- ***Price for pikeperch is varying:***

1) Market fish: 11-14 EUR per 1 kg (275 - 350 CZK per 1 kg)
2) Juvenile stocking material adapted for pellets and RAS (W = 10 - 30 g): 0,8 - 1,5 EUR per piece (20 - 37,5 CZK each)
3) Juveniles stocking material (not adapted, W = 10 - 30 g): 0,3 - 0,45 EUR per piece (7,5 - 11,2 CZK each)

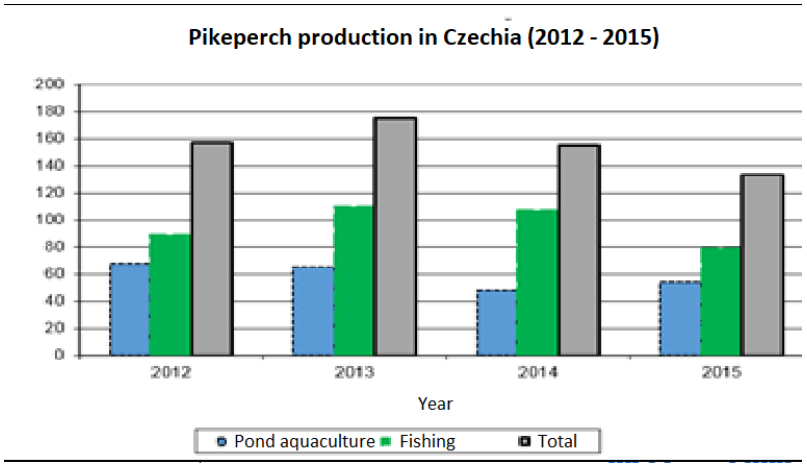


Future development of pikeperch production

In Europe market sized pikeperch is produced in two ways:

Pond way:

- Classical polyculture of pikeperch and other predators, and above all with carp fish,
- In the Czech Republic, the annual production of pikeperch from pond culture of 50 - 70 tons (80 -110 tons is from fishing),
- Pikeperch in pond aquaculture accounts for only 0.3% of the fish stock,
- Production in ponds on the level of 6.1 kg.ha-1,
- In Europe, pond aquaculture is produced annually: 500-1,500 tones (the main producers are: Czech Republic, Hungary, Serbia, Poland, Germany, etc.)..



Future development of pikeperch production

In Europe market sized pikeperch is produced in two ways:

Intensive way

- Mainly, in the Netherlands (100 to 150 tons) and Denmark (50-100 tons)
- Specialized farms with intensive aquaculture using RAS,
- Most of the farms are with closed reproduction cycle (juvenile fish weighing 10 - 20 grams),
- They use domesticated fish, out-off-seasonal spawning, culturing larvae in RAS using rotifers and artemia, adaptation to pellets and subsequent rearing in different market sizes,
- Market fish around 1 - 2 kg in 1 - 2 years of breeding.

	2005	2006	2007	2008	2009	2010	2011
Denmark	49	36	47	55	106	105	105
Netherlands	100	100	100	100	115	100	120

Future development of pikeperch production

In Europe market sized pikeperch is produced in two ways:

Intensive way

- In Central Europe, the intensive farming economy is enhanced by a combination of pond and RAS farming, where fish from ponds are used.
- Fish are spawned under controlled conditions, larvae are stocked in ponds, where they get juvenile stage.
- Pond is harvested and juveniles then get adapted to pellets and RAS.
- They are cultured in controlled condition to market size.

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Innovation of controlled reproduction in pikeperch

Breeding of pikeperch broodstock

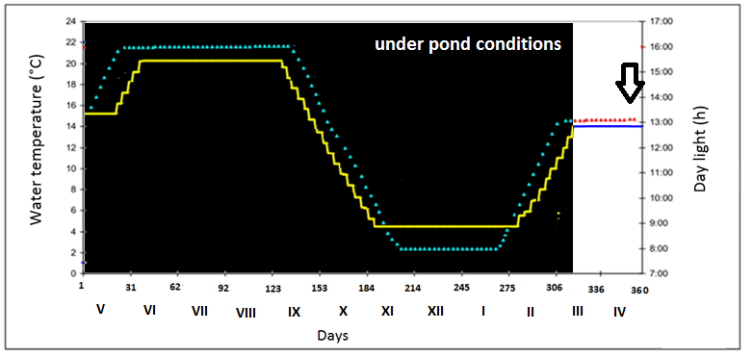
Classic pond culture:

- Polyculture approach, with carp in ponds of 10-100 ha,
- Three to four years of production cycle,
- Natural food in the form of small cyprinid fish - high quality of gametes and fertilizing ability X higher stress and mortality during handling,
- In autumn (or spring) catching fish and holding them in small earthen ponds with high exchange rate or in RAS (protection against the fish eating mammals - otter) with plenty of forage fish.

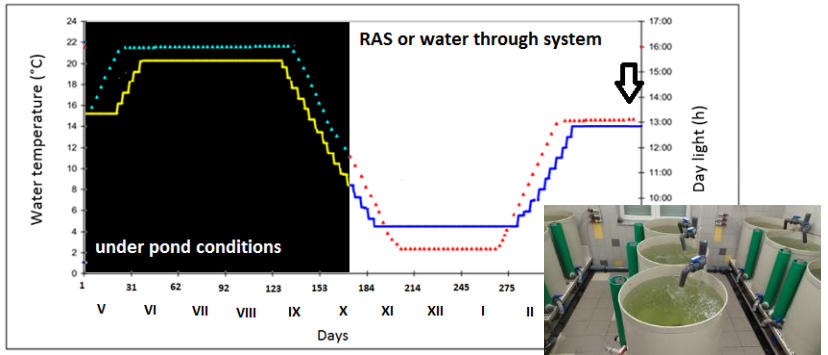
Autumn harvest – keeping of broodstock in small ponds

- Getting the fish in the autumn - an easier way
- Keeping fish in small ponds and forage fish (Roach or Topmouth Gudgeon): 5 pieces per broodstock fish per day,
- Protection from predators, possibly chloramine bath (against bacterial infections),

- Possibility for fish to get transferred into RAS and affect term spawning (February / March)
- The need for fish to be exposed to a low water temperature of 7 - 4 ° C and shorter photoperiod 8L: 16D,
- Fish under constant conditions shows low GSI or sterility without gonadal development,



- Fish density of 30 - 35 kg.m-3, feed with forage fish,
- Possibility to use salt bath (3 g L-1)
- High survival from harvest to spawning - 95 - 100%,



Broodstock management related to pond cultured fish

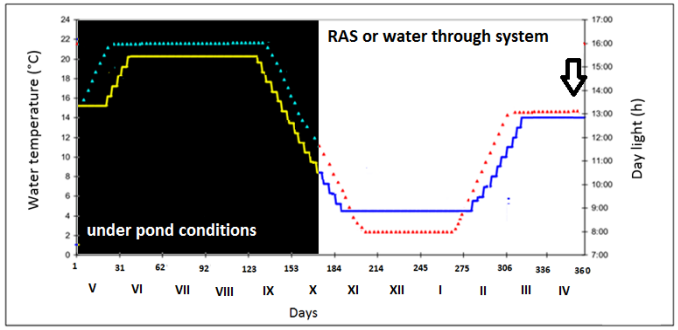
- Natural environmental stimulation for gonadal development and maturation (6 periods before spawning) – control and cutting of summer or wintering periods for different terms of spawning (from January till June),

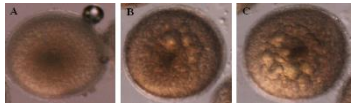
- Broodstock culture under POND and RAS conditions with stable and good supply of prey fish (15 kg of prey fish. kg^{-1} of broodstock per year),

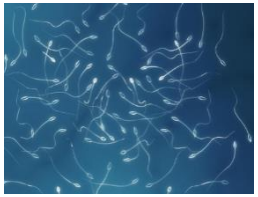
- Broodstock protection in ponds against otters and cormorants,

- Selection of the best broodstock 40 days before spawning according conditions,

- Checking of maturation stages of oocytes and selection of maturated broodstock for final hormonal stimulation.







Broodstock management related to pond cultured fish

In RAS

- More demanding and higher production costs,
- Artificial pelleted feed, domesticated fish,
- Easier handling x lower fertility and gamete quality,
- Controlled environmental conditions - several groups of broods
  - several spawning's per year,
- In the Czech Republic, Anapartners s.r.o.,
- Abroad: France - Asialor Ltd., Denmark - AquaPri Ltd., Netherlands - Excellance BV, FISH 2Be BV and others.

Selection and size of broodstock

- We have used pond-cultured broodstock with TL= 520 – 570 mm, W= 1200 – 1800 g not bigger,

- Better and easier manipulation,

- Lower egg fecundity and better egg distribution on the artificial nests providing good incubation conditions and high hatching rate and larval production,

- Lower demanding for tanks capacity and culture place,

- We can use higher number of fish for higher genetic diversification.

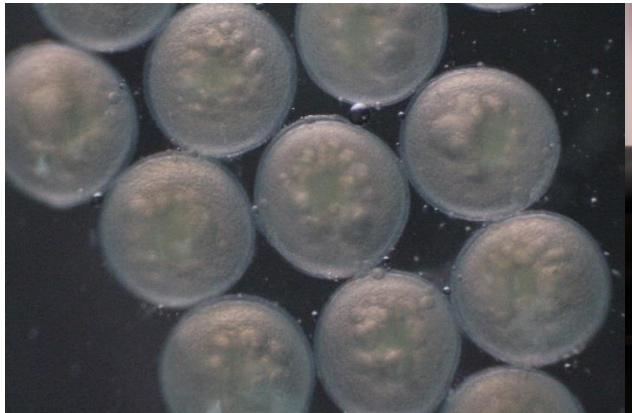
Spawning of pikeperch

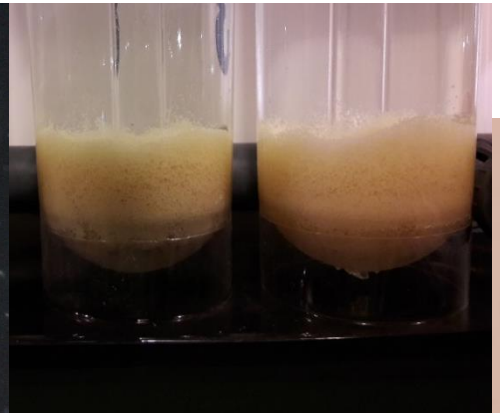
- Spawn in pairs, once a year,
- For the final oocytes maturation (FOM) in controlled conditions, thermal and light stimulation is required
- Spawning period from April to May,
- At a water temperature of 10-15 ° C,
- Male plays a dominant role selecting and cleaning the place of spawning – nest preparation,
- The male prepares the so-called spawning nest (pikeperch - litophilic or phytophilic species).



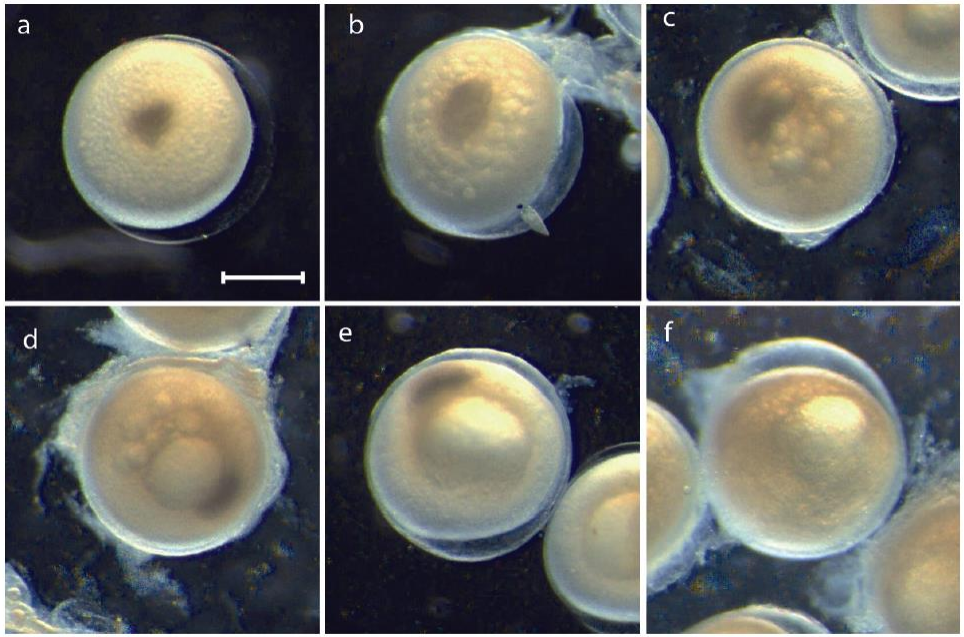
Spawning of pikeperch

- After spawning, the male is very aggressive chasing all the fish from the nest, including females. In limited space female could be killed by male.
- Relative fecundity is 100,000-200,000 fish eggs / kg,
- Incubation time is 120-150 D° (day-degree),
- The eggs are very small and adhesive,
- Size of larvae after hatching is 3,6 – 4 mm.





Before final oocytes maturation, control oocytes stage with catheter is used

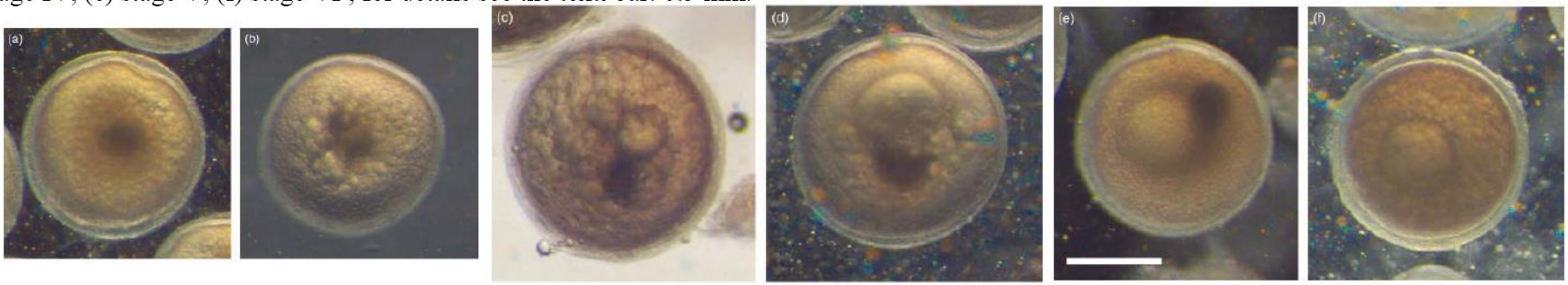


Proposed oocyte maturation stages: (a) stage I, (b) stage II, (c) stage III, (d) stage IV, (e) stage V, (f) stage VI ; for details see the text: bar: 0.5 mm.

to use hormonal stimulation only for fish with oocytes of the 3rd and higher stages

x

Low fertilization rate



Preovulatory oocyte maturation stages in pikeperch, *Sander lucioperca* (L.) after cytoplasm clarification: (a) stage I, (b) stage II, (c) stage III, (d) stage IV, (e) stage V and (f) stage VI. Bar represents 0.5 mm.

Hormonal induction of spawning

-Semi-artificial spawning on prepared nest or artificial spawning under controlled conditions in natural or off-season terms,

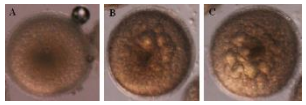
-Fish size: TL = 521 - 571 mm, W = 1200 - 1800 grams,

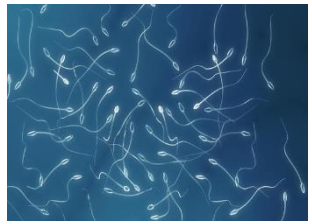
-Individual spawning in pairs (pre-selected fish)

-Intramuscular hormonal induction of spawning:

HCG (Chorulon) at a single dose of 500 IU.kg-1,

GnRa (Supergestran) in a single dose of 25 mg.kg-1.





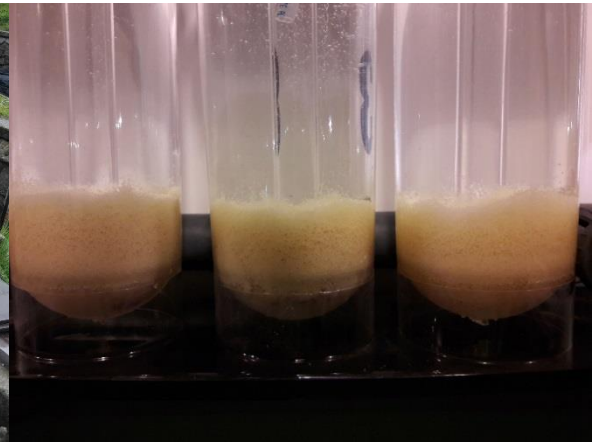
Hormonal agent	Dosage
Supergestran	20-50 µg GnRH.kg^{-1}
Carp pituitary	2-6 mg. kg^{-1}
Chorulon	400-600 IU hCG. kg^{-1}
Ovopel	1,2-2 pelets. kg^{-1}

Four methods of spawning

Natural spawning

Semi-artificial reproduction

Artificial reproduction



Out of season spawning

Natural spawning in ponds

- The stocking of broodstock in production ponds in order to obtain a fry,
- 1 - 2 pairs per 1 ha area,
- Very uncertain and uncontrollable outcome.
- Primitive way is not often used today,
- Today – stocking of hatched larvae in the pond before beginning of exogenous nutrition.

Semi-artificial reproduction

Where to perform semi-artificial reproduction

Flow-through systems, small ponds or RAS – for incubation of eggs on the nests is important to have a source of high quality water, and to conduct an antifungal bath - high hatching larvae.

Why?

- To synchronize spawning hormonal stimulation is required,
- A long tradition in the Czech Republic,
- A simple way of preproduction,
- Easy control of spawning,
- In the smaller tanks after spawning, removing of female is necessary (otherwise a problem with her injury or killing).

Semi-artificial reproduction

Why?

- There was no positive effect on larvae confirmed if the male protecting the nest.
- 2 days before mass hatching - stop water flow in the tank,
- Easy harvesting and counting of hatched larvae.

Typical spawning behavior on the nest

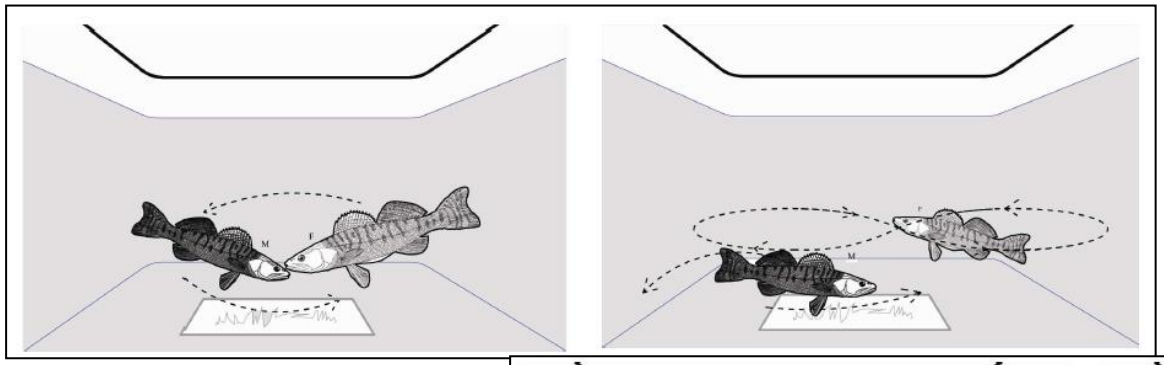
- The male selects the swab substrate, prepares a swab nest of size (diameter) of 0.5-1.5 m,

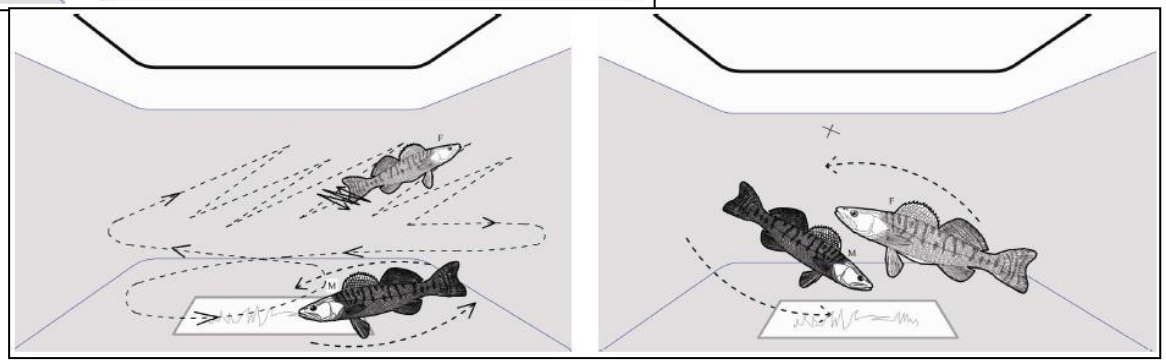
-The ideal material for the nest are fine and long whiskers,

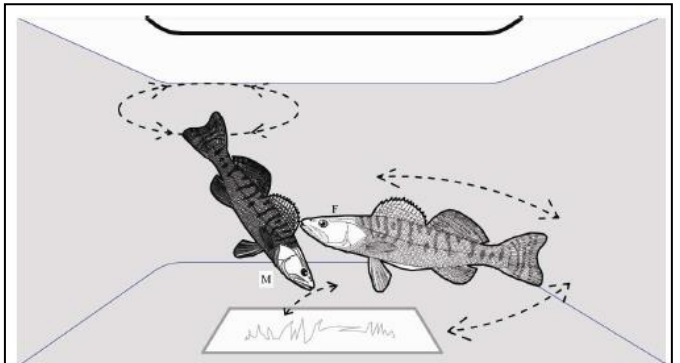
-It attracts the female to the nest,

-At this point, the typical “spawning movement" begins.

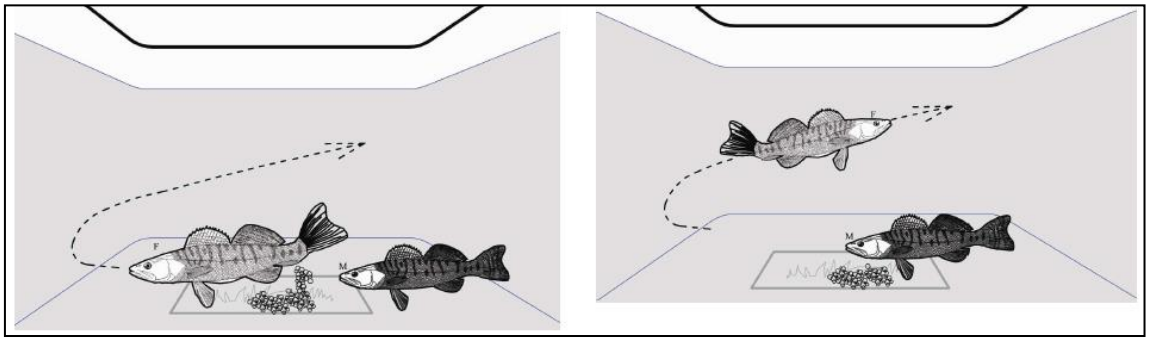
Semi-artificial reproduction – behavioral aspects of spawning

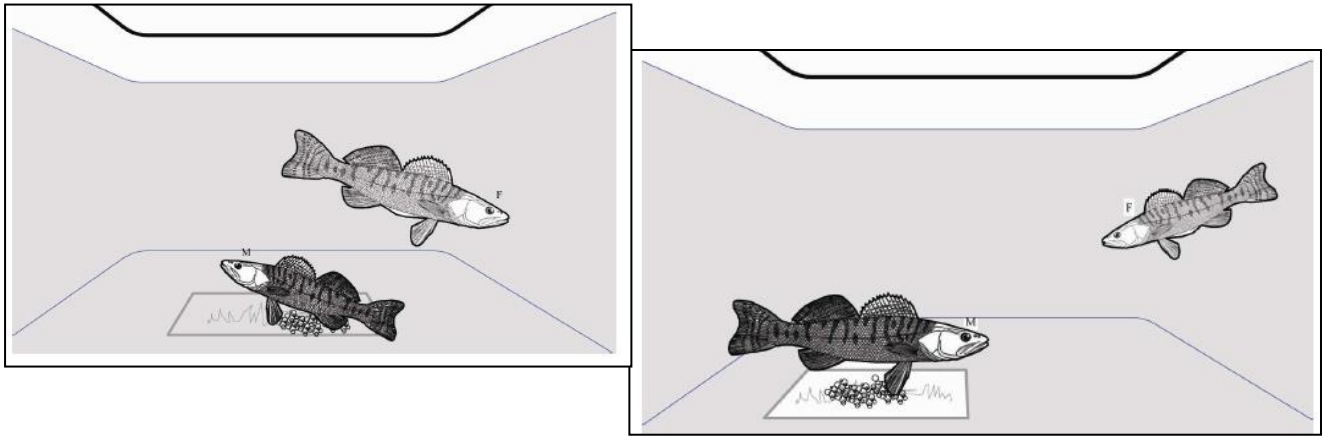






Semi-artificial reproduction – behavioral aspects of spawning





Spawning substrate selection for semi-controlled reproduction of pikeperch

- Three small ponds (repetitions),
- Three types of the substrate, each fish was able to select one particular type (18 nests for 6 pairs of pikeperch)
- Six pairs of fish per pond, without hormonal stimulation.

Followed parameters:

- Substrate preference
- Latency time from stocking of the fish till firs male occupation and spawning.
- Number of hatched larvae per one nest
- Hatching rate

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Substrate selection and semiartificial spawning in pikeperch

Brush

Artificial turf

Smooth plastic

Nest material	Pairs	Use of nest (%)	Hatching rate (%)[a]	Larvae, (thousands)[b]	Time from occupation — spawning, hr[c]	Time from stocking — spawning, hr[c]
Brush	11	61.1 ± 9.6	71.8 ± 23.1	200.3 ± 92.6	96.5 ± 45.5	167.4 ± 72.8
Artificial turf	6	33.3 ± 0	72.8 ± 21.4	180.2 + 104.7	98.5 ± 17.9	147.2 ± 46.3
Smooth plastic	0	0.0	–	–	–	–
No spawning	1	5.6	–	–	–	–
Total	18	100.0				

Semi-controlled reproduction – hormonal induction of spawning

Hormonal induction of spawning during semi-controlled reproduction:

YES of NO??

Is it necessary to stimulate both of the sexes or only female is enough?

Semi-controlled reproduction – hormonal induction of spawning

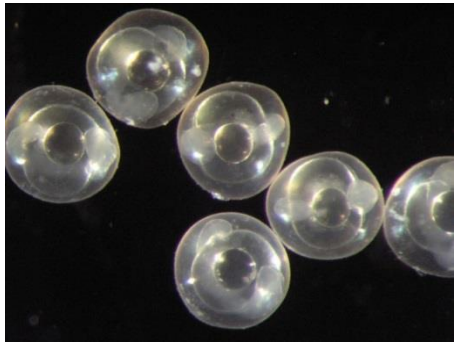
Design of the experiment

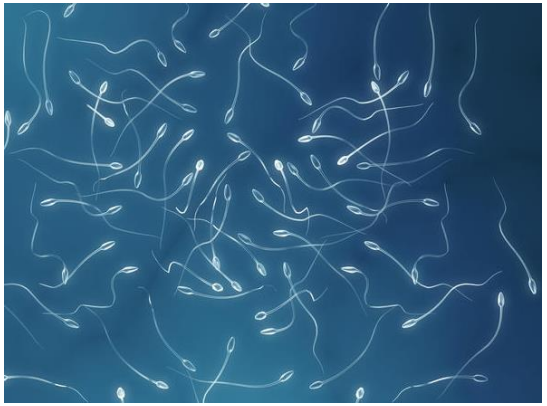
2 groups

- Only **females** are injected with hormone – 500 IU hCG/kg
- **Both** of the sexes are injected – 500 IU hCG/kg

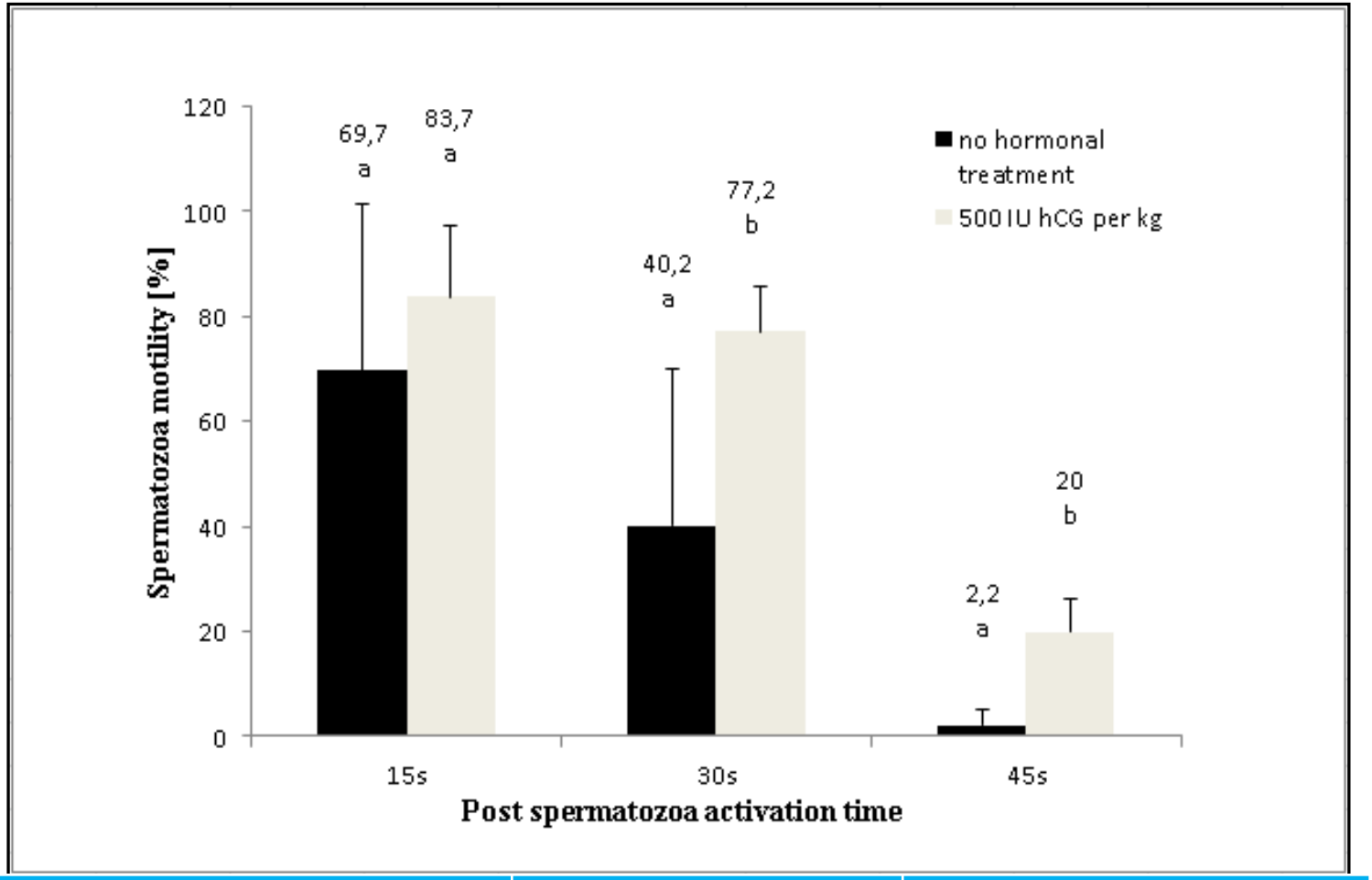
Assessed parameters

- Spawning success
- Latency time to spawning
- Fertilization rate
- Hatching rate
- Incubation time
- Larvae production per one female
- Sperm quality





Semi-controlled reproduction – hormonal induction of spawning. **Sperm quality.**



Sperm quality parameter	With hormonal induction	Without hormonal induction
Volume of collected sperm (ml)	0,16±0,09a	0,64±0,26b
Spermatozoa concentration (mld.ml-1)	37,6±8,3a	19,3±3,9b
Motility duration (s)	59,5±31,8a	97,7±7,1b

Semi-controlled reproduction – hormonal induction of spawning

Quality of spawning and larvae production

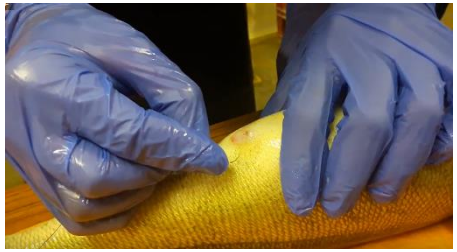
Parameters	Only females injected	Both of the sexes injected
Latency (hours)	96,4±9a	92,4±1,5a
Spawning success (%)	57,1	71,4
Fertilization rate (%)	59,5±17,9a	80,4±9b
Hatching rate (%)	51,2±17,7a	71,6±9,4b
Incubation time (day-degree)	89,9±7,8a	90,4±1,8a
Larvae rep female (pcs)	49 500±32 500a	122 000±15 000b

The positive effect of hormonal injection of both sexes occurred:

-Higher sperm production and longer sperm motility,
-Higher spawning success, fertilization rate, hatching rate,
-And above all, more than doubled production of larvae.

Artificial spawning

- For synchronization of spawning, hormonal injection are necessary ,
- Spawning in RAS (stable water temperature of 14 ° C and a good water quality)
- Frequent monitoring of ovulation of females / sewing urogenital papilla,
- Lower fertilization of eggs and larvae hatching rate,
- Spontaneous loosening of eggs from the body of the female (possibility of sewing a papilla)
- Higher mortality of broodstock fish.



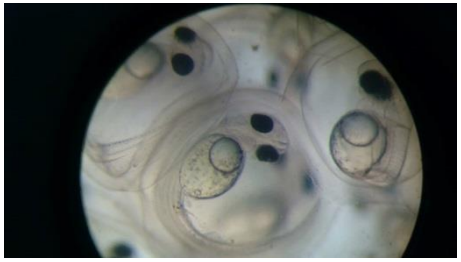
Hormonal agent	Dosage
Supergestran	20-50 µg GnRH/kg
Carp hypophysis	2-6 mg/kg
Chorulon	400-600 IU hCG/kg
Ovopel	1,2-2 pelets/kg

Artificial spawning

- More demanding method of operation (frequent checks: 1x 2-4 hours and at night),
- Gametes: per 100 g of egg 2 ml of milt from 3 males,
- Optimal ratio of 1 egg: 100,000 sperm,
- Necessary to remove egg adhesiveness after fertilization,

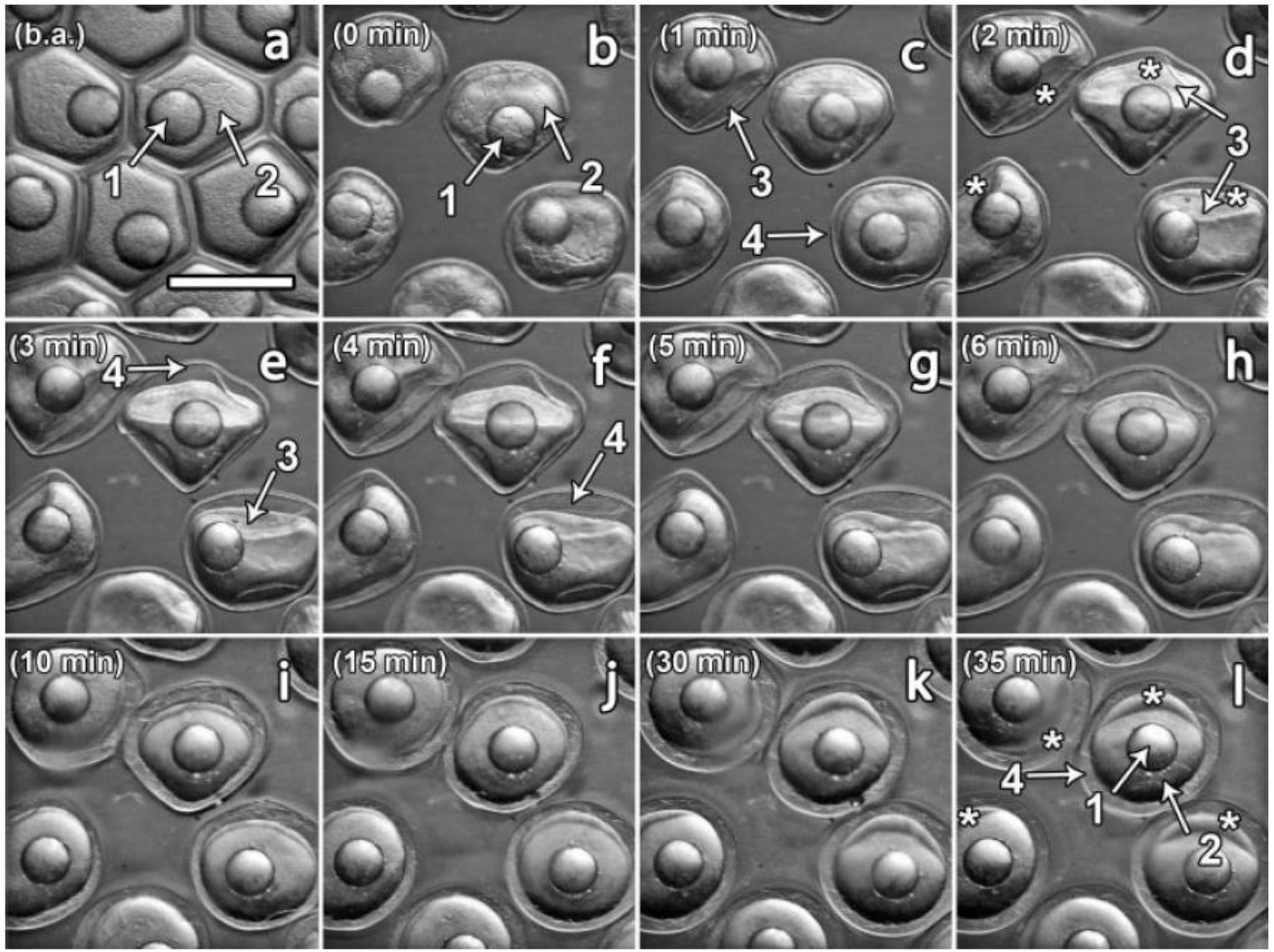
Agent	Concentration/proportion	Duration of application
Alkalase	1,5ml ALK + 998,5ml water	2 minutes
Whole milk + Talc	1:1	60-90 minutes

- Incubation of eggs in Zug jars (10 liters per 1 kg of eggs = 1.2 mil)
- Incubation time 6 - 7 days at 13 - 16 ° C,
- Appropriate method for research and triploidization,
- For field and mass production, semi-controlled spawning is generally recommended.



Artificial spawning

Cortical reaction of oocytes in pikeperch – quality assessment:



Artificial spawning

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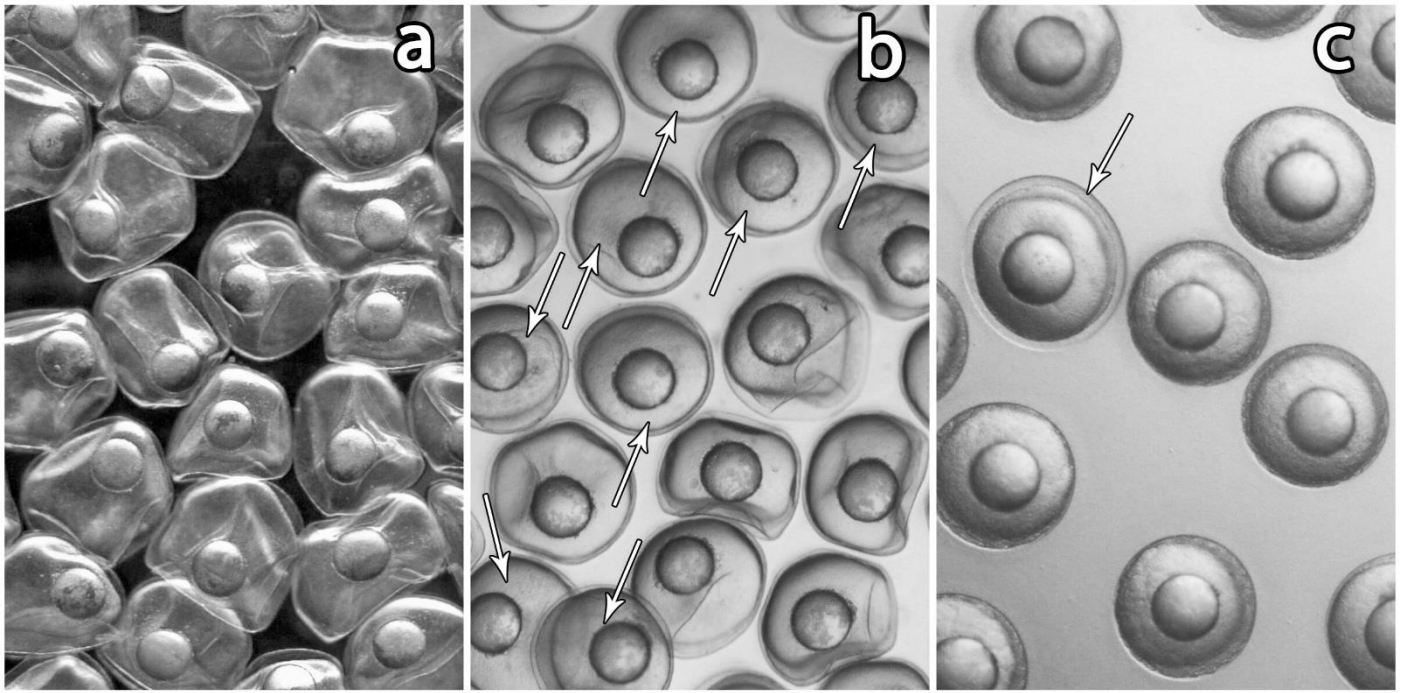


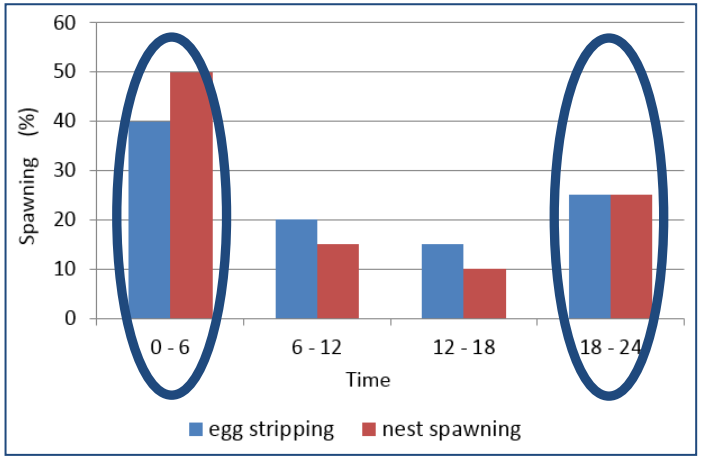
a) 100% deformation – high quality oocytes;
b) 50% deformation – mediocre quality;
c) without deformation – bad quality

Comparison of efficiency between stripping and semiartificial spawning




Parameter	Egg stripping (artificial fertilization)	Nest spawning (natural fertilization)
Spermation rate (%)	100_a	100_a
Ovulation rate (%)	$78.0 \pm 11_a$	$95.0 \pm 5.0_b$
Spontaneous spawning without fertilization (%)	35_b	0_a
Fertilization rate (%)	$78.7 \pm 10.5_a$	$91.5 \pm 7.0_b$
Hatching rate (%)	$63.5 \pm 14.5_a$	$75.6 \pm 11.7_b$
Production of larvae per female (thousands of pcs)	$81.6 \pm 16.0_a$	$97.3 \pm 23.8_b$

Diurnal spawning activity



Egg striping is more time consuming and provides lower fertilization and hatching rates and larval production.

Out-of-seasonal spawning

What is an out-off-seasonal spawning and why do we do it?

- Spawning independent of natural conditions and natural spawning season
- Continuous production of larvae and market fish in the RAS

The most important factors of successful out-off-seasonal spawning

- Controlled by adjusting the light period
- Thermal stimulation of broodstock
- Hormonal stimulation of broodstock before spawning

Out-of-seasonal spawning – necessary equipment

1) RAS-cultured broodstock fish:

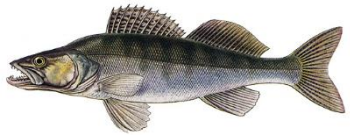
- Nutrition 44% protein, 16% fat
- 50 lux light mode, (18: 6) L: D
- temperature 21-23 ° C

2) Special chamber for environmental stimulation to induce gametogenesis

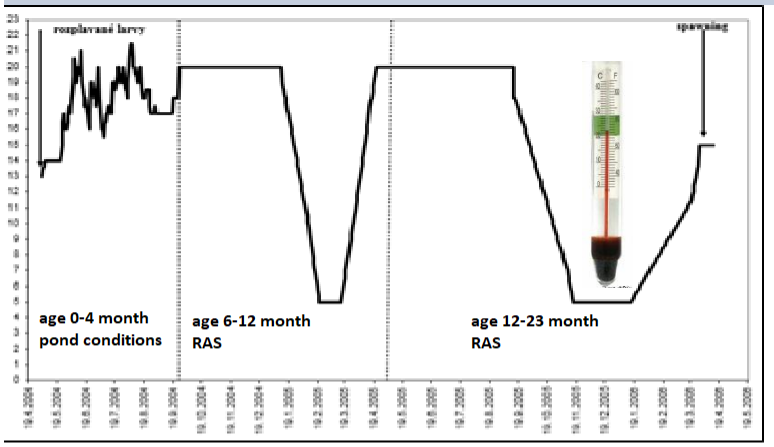
3) Breeding equipment for final fish stimulation ending with spawning

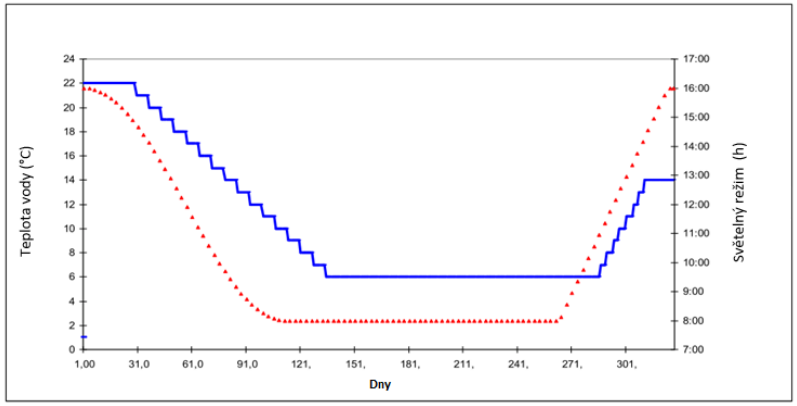
Out-of-seasonal spawning of Percid fishes

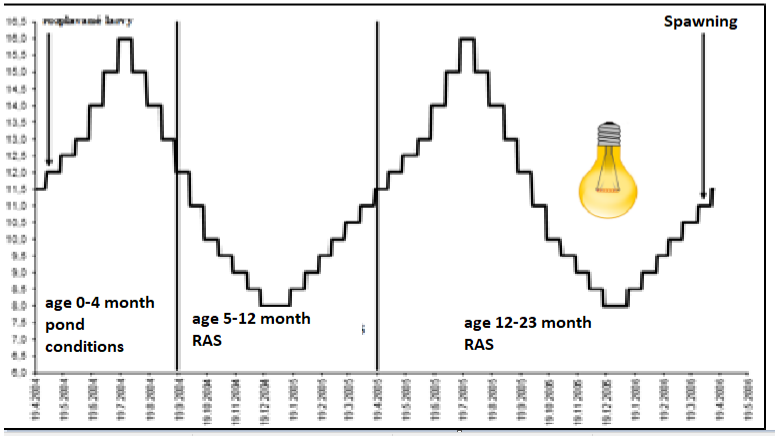
Percids fish - they need to develop their final gamete maturation and subsequent spawning its own special temperature and light regime including:



- Reducing the water temperature to 4-6 ° C and shortening the light day to 7-8 hours,
- 1.5-3 month period of reduced water temperature around 5 ° C at short light photoperiod 8 hours,
- Gradual increase of water temperature (to 12 - 15 ° C) and extension of light mode to 14 h.

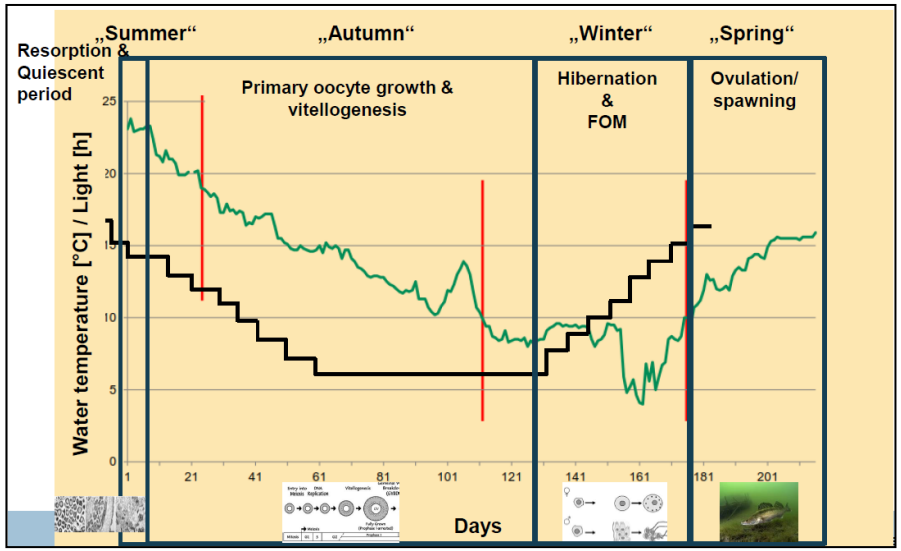






Out of season spawning – protocol used in Hohen-Wangeling (Germany)

	Temperature [°C]	Light regime	Feed intensity [%/d]	Duration [w/d]
„Adaption“	23 – 21	• 16 : 8 (L/D) • 50 lx	0.5	2 / 14
„Autumn“	21 – 10	• 8 : 16 • 10 lx	0.5 – 0.15	8 / 56
„Winter“	< 10	• 6 : 18 • < 8 lx	0.1	8 / 56
„Spring“	8 – 15	• 14 : 10 • 20 lx	0.15 – 0.5	8 / 56
„Spawning“	10 – 16	• 16 : 8 • 30 lx	no feed	4 / 28
„Complete cycle“				30 / 210



Broodstock mortality after spawning

Female's death	
During spawning	8 %
10 days after spawning (stocking to the pond)	65 %
90 days after spawning	90 %

Male's death	
During spawning	5 %
10 days after spawning (stocking to the pond)	50 %
90 days after spawning	95 %

Main problems:

- **Skin damages, injury, bacterial infections**
- **Fungal diseases**

Decreasing of broodstock mortality after spawning

- Application of long-term salt baths in RAS (5-10 g NaCl per liter) for 6 days,
- Intensive feeding of broodstock with forage fish (5 fishes of 2 grams),
- Clean, filtered water,
- Water temperature 16 - 18 ° C, light 10 - 12 hours,
- Fish density 30 - 40 kg.m-3,
- After the bath for an additional 8 days in the RAS, subsequent stocking into ponds

Female's death		
During spawning	8 %	
10 days after spawning (stocking to the pond)	65 %	-45%
90 days after spawning	90 %	-55%

Male's death		
During spawning	5 %	
10 days after spawning (stocking to the pond)	50 %	-32%
90 days after spawning	95 %	-60%