

## Supplementary Table S3

### Ethanol used in each facility

<b>EPFL</b>	Ethanol Absolute	Fisher Chemical E/0650DF/17, Fisher Scientific, UK
<b>FCI</b>	Ethanol 96%	Fisher Chemical E/0555DF/17, Fisher Scientific, UK
<b>IP</b>	Ethanol Absolute	32205-M, SIGMA-ALDRICH, USA
<b>KU</b>	Ethanol Absolute	1009831011, SIGMA-ALDRICH, USA
<b>IIM</b>	Ethanol 96%	Alcoholes Galicia S. L., Spain
<b>UAB</b>	Ethanol 96%	Not available

### Blinded experiments

<b>EPFL</b>	No
<b>FCI</b>	Not blinded for anaesthesia overdose. Blinded for electrical stunning.
<b>IP</b>	No
<b>KU</b>	No
<b>IIM</b>	Yes
<b>UAB</b>	Yes

### Tables presenting data per fish

Data are presented per treatment, time point, facility, and pool date. Each row represents a dish (or a group of 3 dishes for IP). Each pool is identified by the date at which the pool of eggs was fertilised.

- In the third column, the number of embryos or larvae undergoing treatment is indicated.
- In the fourth column, the number of embryos or larvae that survived 24 hours post treatment is indicated.
- The fifth column indicates the percentage of embryos or larvae that survived in each dish.
- The pH of the anaesthetic solution was measured before and after anaesthetics, buffer, and solvent were added, and these data are indicated for each day of treatment.

<u>Treatment</u>	<u>Pages</u>
Lidocaine HCl with EtOH	2-4
Lidocaine HCl	5-7
Tricaine	8-10
Clove oil	11-12
Benzocaine	13-14
2-phenoxyethanol	15-16
Electrical stunning	17
Hypothermic shock	18

## Lidocaine HCl with EtOH

Lidocaine hydrochloride 1 g/L + sodium bicarbonate 2 g/L + 50 mL/L ethanol

### < 12 hpf Lidocaine HCl with EtOH

Facility	Pool	<12hpf	<36hpf	% Survival	pH before	pH after
EPFL	22JUN20	14	0	0%	7.7	7.7
		15	0	0%		
		15	0	0%		
	01JUL20	15	0	0%	7.6	7.8
		15	0	0%		
		15	0	0%		
	07JUL20	15	0	0%	7.5	7.7
		15	0	0%		
		15	0	0%		
IP	16NOV20	45	0	0%	7.3	7.7
	23NOV20	45	0	0%	7.3	7.7
	30NOV20	45	0	0%	7.3	7.7
KU	25FEB20	10	0	0%	7.6	8.2
		10	0	0%		
		10	0	0%		
IIM	12JAN21	15	0	0%	Not measured	Not measured
		15	0	0%		
		15	0	0%		
		15	0	0%		
		15	0	0%		
UAB	27OCT20	10	0	0%	7.6	7.8
		10	0	0%		
		10	0	0%		

## 24 hpf Lidocaine HCl with EtOH

Facility	Pool	24hpf	48hpf	% Survival	pH before	pH after
EPFL	23JUN20	15	0	0%	7.7	7.8
		14	0	0%		
		13	0	0%		
	30JUN20	15	0	0%	7.6	7.7
		15	0	0%		
		15	0	0%		
	06JUL20	15	0	0%	7.5	7.7
		15	0	0%		
		15	0	0%		
IP	16NOV20	45	0	0%	7.3	7.7
	23NOV20	45	0	0%	7.3	7.7
	30NOV20	45	0	0%	7.3	7.7
KU	25FEB20	10	0	0%	7.6	8.2
		10	4	40%		
		10	0	0%		
	16JUN21	10	0	0%	7.5	7.9
		10	0	0%		
		10	0	0%		
IIM	12JAN21	15	0	0%	Not measured	Not measured
		15	0	0%		
		15	0	0%		
		15	0	0%		
		15	0	0%		
UAB	27OCT20	10	0	0%	7.5	7.7
		10	0	0%		
		10	0	0%		

## 48 hpf Lidocaine HCl with EtOH

Facility	Pool	48hpf	72hpf	% Survival	pH before	pH after
KU	16JUN21	10	0	0%	7.5	8.0
		10	0	0%		
		10	0	0%		

#### 4 dpf Lidocaine HCl with EtOH

Facility	Pool	4dpf	5dpf	% Survival	pH before	pH after
EPFL	27JUN20	15	0	0%	7.6	7.7
		15	0	0%		
		15	0	0%		
	03JUL20	15	0	0%	7.5	7.8
		15	0	0%		
		15	0	0%		
	26AUG20	15	0	0%	7.6	7.9
		15	0	0%		
		15	0	0%		
IP	26NOV20	41	0	0%	7.3	7.7
	23NOV20	45	0	0%	7.2	7.7
	30NOV20	45	22	49%	7.2	7.3
IIM	14JAN21	15	0	0%	Not measured	Not measured
		15	0	0%		
		15	0	0%		
		15	0	0%		
		15	0	0%		
UAB	27OCT20	10	0	0%	7.6	7.8
		10	0	0%		
		10	0	0%		

## Lidocaine HCl

Lidocaine hydrochloride 1 g/L + sodium bicarbonate 2 g/L

### < 12 hpf Lidocaine HCl

Facility	Pool	<12hpf	<36hpf	% Survival	pH before	pH after
EPFL	22JUN20	15	0	0%	7.7	7.7
		15	0	0%		
		15	0	0%		
	01JUL20	15	0	0%	7.6	7.8
		15	0	0%		
		15	0	0%		
	07JUL20	15	0	0%	7.5	7.7
		15	1	7%		
		15	2	13%		
IP	16NOV20	45	0	0%	7.3	7.6
	23NOV20	45	0	0%	7.3	7.6
	30NOV20	45	17	38%	7.3	7.6
KU	25FEB20	10	0	0%	7.6	8.2
		10	0	0%		
		10	0	0%		
UAB	27OCT20	10	0	0%	7.7	7.9
		10	0	0%		
		10	0	0%		

### 24 hpf Lidocaine HCl

Facility	Pool	24hpf	48hpf	% Survival	pH before	pH after
EPFL	23JUN20	15	0	0%	7.7	7.7
		15	0	0%		
		15	0	0%		
	30JUN20	14	0	0%	7.6	7.7
		15	0	0%		
		15	0	0%		
	06JUL20	15	0	0%	7.5	7.7
		15	0	0%		
		15	0	0%		
IP	16NOV20	45	5	11%	7.3	7.6
	23NOV20	45	0	0%	7.3	7.6
	30NOV20	45	0	0%	7.3	7.6
KU	25FEB20	10	3	30%	7.6	8.2
		10	3	30%		
		10	2	20%		
	16JUN21	10	0	0%	7.5	8.0
		10	2	20%		
		10	4	40%		
UAB	27OCT20	10	0	0%	7.6	7.8
		10	0	0%		
		10	0	0%		

### 48 hpf Lidocaine HCl

Facility	Pool	48hpf	72hpf	% Survival	pH before	pH after
KU	16JUN21	10	0	0%	7.6	8.0
		10	4	40%		
		10	0	0%		

#### 4 dpf Lidocaine HCl

Facility	Pool	4dpf	5dpf	% Survival	pH before	pH after
EPFL	27JUN20	15	5	33%	7.6	7.7
		15	6	40%		
		15	2	13%		
	03JUL20	14	3	21%	7.5	7.8
		15	2	13%		
		15	0	0%		
	26AUG20	15	3	20%	7.6	7.9
		15	0	0%		
		14	0	0%		
IP	26NOV20	43	35	81%	7.3	7.6
	23NOV20	45	39	87%	7.2	7.6
	30NOV20	45	43	96%	7.2	7.6
UAB	27OCT20	10	0	0%	7.6	7.8
		10	0	0%		
		10	0	0%		

## Tricaine

### Tricaine 1 g/L with 2 g/L Sodium bicarbonate

**EPFL and IP:** Tricaine, Ethyl 3-aminobenzoate methanesulfonate salt, Sigma-aldrich, A5040, USA

**UAB:** Tricaine, Ethyl 3-aminobenzoate methanesulfonate, Sigma-aldrich, E10521, USA

#### < 12 hpf Tricaine

Facility	Pool	<12hpf	<36hpf	% Survival	pH before	pH after
EPFL	22JUN20	15	14	93%	7.7	7.7
		15	13	87%		
		15	15	100%		
	01JUL20	15	15	100%	7.6	7.8
		15	15	100%		
		15	15	100%		
	07JUL20	15	15	100%	7.5	7.7
		15	15	100%		
		15	15	100%		
IP	16NOV20	45	38	84%	7.3	7.1
	23NOV20	45	40	89%	7.3	7.1
	30NOV20	45	44	98%	7.3	7.2
UAB	27OCT20	10	0	0%	7.6	7.6
		10	0	0%		
		10	2	20%		



## 24 hpf Tricaine

Facility	Pool	24hpf	48hpf	% Survival	pH before	pH after
EPFL	23JUN20	15	15	100%	7.7	7.8
		15	15	100%		
		15	15	100%		
	30JUN20	15	15	100%	7.6	7.7
		15	15	100%		
		15	15	100%		
	06JUL20	15	15	100%	7.5	7.7
		15	15	100%		
		15	15	100%		
IP	16NOV20	45	43	96%	7.3	7.1
	23NOV20	45	45	100%	7.3	7.1
	30NOV20	44	44	100%	7.3	7.2
UAB	27OCT20	10	10	100%	7.6	7.6
		10	10	100%		
		10	10	100%		

#### 4 dpf Tricaine

Facility	Pool	4dpf	5dpf	% Survival	pH before	pH after
EPFL	27JUN20	15	15	100%	7.6	7.7
		15	15	100%		
		15	15	100%		
	03JUL20	15	15	100%	7.5	7.7
		15	15	100%		
		15	14	93%		
	26AUG20	15	15	100%	7.6	7.9
		13	13	100%		
		15	15	100%		
IP	26NOV20	45	45	100%	7.3	7.2
	23NOV20	45	45	100%	7.2	7.1
	30NOV20	44	43	98%	7.2	7.1
UAB	27OCT20	10	10	100%	7.7	7.6
		10	10	100%		
		10	10	100%		

## Clove oil 0.1%

1:10 clove oil in ethanol, then 10 mL/L

**EPFL and FCI:** Clove Oil, Sigma-Aldrich, C8392, USA

**UAB:** Essential clove oil, Sol Natura, 2,215 lab 122735, Spain

### < 12 hpf Clove oil

Facility	Pool	<12hpf	<36hpf	% Survival	pH before	pH after
EPFL	22JUN20	13	0	0%	7.7	7.4
		14	0	0%		
		10	0	0%		
	01JUL20	15	0	0%	7.6	7.4
		15	0	0%		
		15	0	0%		
	07JUL20	15	0	0%	7.5	7.6
		15	0	0%		
		15	0	0%		
UAB	27OCT20	10	0	0%	Not measured	Not measured
		10	0	0%		
		10	0	0%		

## 24 hpf Clove oil

Facility	Pool	24hpf	48hpf	% Survival	pH before	pH after
EPFL	23JUN20	15	0	0%	7.7	7.5
		14	0	0%		
		15	0	0%		
	30JUN20	12	0	0%	7.6	7.6
		15	0	0%		
		13	0	0%		
	06JUL20	15	0	0%	7.5	7.4
		15	0	0%		
		15	0	0%		
FCI	17NOV20	15	0	0%	7.0	7.2
		15	0	0%		
		15	0	0%		
	24NOV20	15	0	0%	6.8	7.1
		15	0	0%		
		15	0	0%		
	01DEC20	10	0	0%	6.7	7.4
		10	0	0%		
		15	0	0%		
UAB	27OCT20	10	0	0%	Not measured	Not measured
		10	0	0%		
		10	0	0%		

## 4 dpf Clove oil

Facility	Pool	4dpf	5dpf	% Survival	pH before	pH after
EPFL	27JUN20	15	0	0%	7.6	7.5
		15	0	0%		
		15	0	0%		
	03JUL20	15	0	0%	7.5	7.4
		15	0	0%		
		15	0	0%		
	26AUG20	15	0	0%	7.6	7.1
		15	0	0%		
		15	0	0%		
UAB	27OCT20	10	0	0%	Not measured	Not measured
		10	0	0%		
		10	0	0%		

## Benzocaine 1g/L

20 g/L in ethanol, then 50 mL/L

### < 12 hpf Benzocaine

Facility	Pool	<12hpf	<36hpf	% Survival	pH before	pH after
EPFL	22JUN20	15	4	27%	7.7	7.6
		14	4	29%		
		15	4	27%		
	01JUL20	15	0	0%	7.6	7.6
		14	0	0%		
		15	0	0%		
	07JUL20	15	0	0%	7.5	7.7
		14	5	36%		
		15	3	20%		

### 24 hpf Benzocaine

Facility	Pool	24hpf	48hpf	% Survival	pH before	pH after
EPFL	23JUN20	15	15	100%	7.7	7.6
		15	15	100%		
		14	14	100%		
	30JUN20	15	15	100%	7.6	7.7
		14	14	100%		
		14	14	100%		
	06JUL20	15	15	100%	7.5	7.6
		15	15	100%		
		15	14	93%		
FCI	17NOV20	15	15	100%	7.0	7.4
		15	15	100%		
		15	15	100%		
	24NOV20	15	15	100%	6.8	7.1
		15	15	100%		
		14	12	86%		
	01DEC20	10	10	100%	6.7	7.5
		10	10	100%		
		10	10	100%		

#### 4 dpf Benzocaine

Facility	Pool	4dpf	5dpf	% Survival	pH before	pH after
EPFL	27JUN20	10	0	0%	7.6	7.7
		14	0	0%		
		13	0	0%		
	03JUL20	15	0	0%	7.5	7.6
		15	0	0%		
		15	5	33%		
	26AUG20	15	0	0%	7.6	7.3
		15	0	0%		
		13	0	0%		

## 2-Phenoxyethanol 3 mL/L

**EPFL:** 2-Phenoxyethanol, Sigma-Aldrich, 77699, USA

**FCI:** Aqua-Sed, VETARK, UK

### < 12 hpf 2-Phenoxyethanol

Facility	Pool	<12hpf	<36hpf	% Survival	pH before	pH after
EPFL	22JUN20	15	1	7%	7.7	7.5
		13	2	15%		
		15	3	20%		
	01JUL20	15	0	0%	7.6	7.4
		13	0	0%		
		15	0	0%		
	07JUL20	15	0	0%	7.5	7.6
		13	0	0%		
		15	0	0%		

### 24 hpf 2-Phenoxyethanol

Facility	Pool	24hpf	48hpf	% Survival	pH before	pH after
EPFL	23JUN20	15	15	100%	7.7	7.5
		15	15	100%		
		15	15	100%		
	30JUN20	15	15	100%	7.6	7.7
		14	14	100%		
		16	16	100%		
	06JUL20	15	15	100%	7.5	7.4
		15	15	100%		
		15	15	100%		
	17NOV20	15	15	100%	7	7.4
		15	15	100%		
		15	15	100%		
FCI	24NOV20	15	15	100%	6.8	7.5
		10	10	100%		
		10	10	100%		
	01DEC20	10	10	100%	6.7	7.5
		10	10	100%		
		10	10	100%		

#### 4 dpf 2-Phenoxyethanol

Facility	Pool	4dpf	5dpf	% Survival	pH before	pH after
EPFL	27JUN20	15	14	93%	7.6	7.5
		10	8	80%		
		13	11	85%		
	03JUL20	15	14	93%	7.5	7.6
		15	15	100%		
		15	15	100%		
	26AUG20	15	14	93%	7.6	7.3
		15	13	87%		
		15	14	93%		



## Electrical Stunning

### 24 hpf Electrical stunning

Facility	Pool	24hpf	48hpf	% Survival
FCI	15DEC20	15	0	0%
		15	0	0%
		15	0	0%
		15	0	0%
		15	0	0%

### 4 dpf Electrical stunning

Facility	Pool	4dpf	5dpf	% Survival
FCI	15DEC20	16	0	0%
		13	0	0%
		14	0	0%
		13	0	0%
		16	0	0%
	08DEC20	15	0	0%
		15	0	0%
		15	0	0%
		15	0	0%
		15	0	0%
		15	0	0%
		15	0	0%
		15	0	0%
		15	0	0%
		22	0	0%

## Hypothermic shock

### < 12 hpf Hypothermic shock

Facility	Pool	<12hpf	<36hpf	% Survival
IP	16NOV20	45	34	76%
	23NOV20	45	40	89%
	30NOV20	45	39	87%
UAB	27OCT20	10	5	50%
		10	2	20%
		10	4	40%

### 24 hpf Hypothermic shock

Facility	Pool	24hpf	48hpf	% Survival
IP	16NOV20	45	39	87%
	23NOV20	45	40	89%
	30NOV20	45	26	58%
UAB	27OCT20	10	10	100%
		10	10	100%
		10	9	90%

### 4 dpf Hypothermic shock

Facility	Pool	4dpf	5dpf	% Survival
IP	26NOV20	44	44	100%
	23NOV20	45	45	100%
	30NOV20	45	45	100%
UAB	27OCT20	10	10	100%
		10	10	100%
		10	10	100%