Formalin treatments before eyeing and hand-picking of Arctic charr (*Salvelinus alpinus*) eggs; re-evaluating the timing of antifungal treatments

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Summary

Arctic charr (*Salvelinus alpinus*) eggs in hatcheries are treated for fungal infections using formalin before, and hand-picking during the eyed stage. The relative effectiveness of these two treatments was evaluated using a factorial design with the application of weekly hand-picking, and formalin treatments at 380 ppm for 13 minutes three times weekly as factors. The effects of the treatments on total mortality and hatching success were compared using a two-way ANOVA. Both treatments exhibited significant, positive effects on egg survival. Hand-picking during the eyed stage (+ 0.5 to 2.5 % survival to hatch) was found to be more effective than formalin treatments before (+ 0.0004 to 0.8 % survival to hatch). This is likely due to differences in the timing of administration of the treatments. The probability of fungal infections varied with time, and a peak seemed to occur during hatching.

Sammendrag

Formalinbehandling i forkant av øyerognsstadiet og plukking av røyeegg (Salvelinus alpinus); re-evaluering av tidsrammen for soppbehandling. Egg av røye (*Salvelinus alpinus*) blir behandlet for soppinfeksjoner ved å bruke formalin før øyerognsstadiet, og ved å plukke død rogn i øyerognsstadiet. Den relative effektiviteten for disse to behandlingsformene ble bedømt i et faktorialt eksperiment, med ukentlig plukking av egg og formalin behandlinger med 380 ppm formalin i 13 minutter og tre ganger i uken som faktorer. Effekten på dødeligheten av egg ble sammenlignet i en to-veis ANOVA. Begge behandlingene hadde en signifikant, positiv effekt på eggoverlevelse. Plukking i øyerognsstadiet (+ 0.5 til 2.5 % overlevelse til klekking) var mer effektivt enn formalinbehandlingen i forkant (+ 0.0004 til 0.8 % overlevelse til klekking). Dette skyldes sannsynligvis tidsmessige forskjeller i eggutviklingen når behandlingene ble utført. Sannsynligheten for utbrudd av soppinfeksjoner er indikert til å variere over tid, og den synes å være høyest under klekking.

Introduction

Commercial freshwater fish farming in Norway is a small industry despite abundant freshwater resources and its potential to generate wealth in sparsely populated areas. The slow growth of Norwegian freshwater fish farming is largely a result of restrictive policies, which are anchored in environmental concerns connected to eutrophication, escaped fish and pathogens (Haug et

al. 2006). One of the most commonly farmed species in Norwegian freshwater is Arctic charr (*Salvelinus alpinus*), due to its low temperature requirements (Brännäs & Linnér 2000; Siikavuopio et al. 2009; Siikavuopio et al. 2010), its tolerance to high density conditions, and its amenability to niche markets (Johnston 2002; Summerfelt et al. 2004; Skybakmoen et al. 2009; Jobling et al. 2010). Arctic charr farmers have few objections to the strict regulations, as an environmentally friendly image suits the market (Sæther et al. 2013). However, some environmental impact is unavoidable to maintain the entire production cycle of Arctic charr. Disinfection and disease control for instance require some use of hazardous chemicals.

Fungal infections of eggs are a common problem in salmonid hatcheries (e.g. Barnes et al. 1997). They are also abundant at our study site, Telemarkrøye AS (an Arctic charr hatchery in Fyresdal, southern Norway). Formalin is still widely used in aquaculture (Boyd & McNevin 2015; Leal et al. 2016). Formalin is effective at treating fungal infection of salmonid eggs (Burrows 1949; Marking et al. 1994; Waterstrat & Marking 1995; Schreier et al. 1996; Barnes et al. 1997; Barnes et al. 2000; Arndt et al. 2001; Barnes et al. 2001). However, formalin is toxic to aquatic organisms, such as fish, amphibians, invertebrates and microorganisms (Kitchens et al. 1976), if the effluent is insufficiently treated (Katz 1989; Marking et al. 1994; GESAMP 1997; Leal et al. 2016). Formalin is also carcinogenic in humans (reviewed by Swenberg et al. 2013), thus poses a risk to hatchery workers if not properly handled. As an alternative to formalin treatments, dead eggs can be removed by hand-picking. It can be performed safely during the eyed stage (Barnes et al. 1997). Chemical fungal infection treatments are necessary before the eyed stage (Piper et al. 1982; Post 1987).

To balance the negative environmental and health effects from formalin treatment with its benefits of reducing the amount of eggs lost to fungal infections, the current disinfection protocol at our study site combines formalin treatment before the eyed stage with hand-picking during the eyed stage. This protocol is generally recommended for Arctic charr (Johnston 2002). The combination of the different approaches, however, obscures their relative effectiveness. In this study, a factorial design under high exposure to fungal infections is used to determine the relative effectiveness of the factors formalin treatment and hand-picking in this particular protocol. In addition, mortality and the presence of fungal infections over time are monitored to generate further insight to the dynamics of fungal infections and egg mortality. In light of the findings, possible adaptions to the protocol are proposed.

Material and Methods

Ethical Statement

No ethical consent was required for this study. Adult fish and hatched larvae were handled by trained personnel of Telemarkrøye AS, following their standard procedures for production. Eggs of Arctic charr were used in the experiment, and handled by the researchers after fertilization until hatch. This life stage is not covered by the Norwegian legislation on the use of animals in research (Lovdata 2018). According to §2, larvae of vertebrates are first included in the regulation when they feed independently. Persistent harm or pain in later life stages was not expected as a result of this study. All experimental procedures were usual husbandry practice.

Fish

All gametes used in this study derive from the hatchery brood-stock at Telemarkrøye AS. The brood-stock fish primarily originate from the nearby Lake Fyresvatn, and were caught in 2011. Their offspring hatched in captivity are included in the brood-stock without any direct artificial selection. Two half-sibling (half-sib) families and one full-sib family were used in our study. The first two families derived from one five-year-old female each, and were fertilized by two males. The females were hatched in captivity. The two males used in these two families were wild fish. In the third family, only one female

and one male were used, both hatchery reared five-year-old individuals.

Fertilization protocol

All brood-stock individuals used in the experiment were checked weekly for ripeness. Ripe fish were sedated by clove oil (Scan Aqua AS, Årnes, NO), using a bathing treatment at 0.32 g L^{-1}. The genital papilla was wiped dry, and gametes were removed by gentile abdominal massage (stripping), carefully avoiding contamination with urine, faeces or mucus. Unfertilized eggs were rinsed using physiological saline solution. Milt was stripped into a beaker and transferred to the eggs by a syringe. Fertilization occurred in plastic buckets containing physiological saline solution for 2 minutes in darkness. The fertilized eggs were rinsed in physiological saline solution, and disinfected in a buffered iodophore solution (PHARMAQ Ltd, Fordingbridge, Hampshire, UK) following the manufacturers protocol.

Incubation environment

All eggs were incubated in a vertical flow incubator (Alvestad Marin, Oslo, NO) in two compartments (drawers), which were divided into 30 rectangular plots of ≈ 100 cm^2 each using 6 cm high PVC-plates (Fishtech, Vestby, NO) with circular perforations with a diameter of 2 mm (Figur 1). The plates were attached on the 20.10.2017 using a silyl-modified polyether glue (Relekta, Oslo, NO), certified as toxicant free. The compartments were disinfected using buffered iodophores (PHARMAQ Ltd, Fordingbridge, Hampshire, UK) and rinsed with hatchery water before transfer to the incubator on the 26.10.2017. The water flow rate in the incubator was 1.5 L min^{-1}. Incubation occurred at temperatures between 6.2 and 7.7 °C, recorded once daily (Supplement 3). The accumulation of degree days (dd) were calculated as the sum of all daily temperature measurements up to, and including, the corresponding day. Major water chemistry was analyzed weekly during this study to indicate growth conditions of the water moulds identified (Table 1).

Figur 1 Experimental plot for one chemical treatment group. Photographer: Tom Robin Olk.

Experimental setup

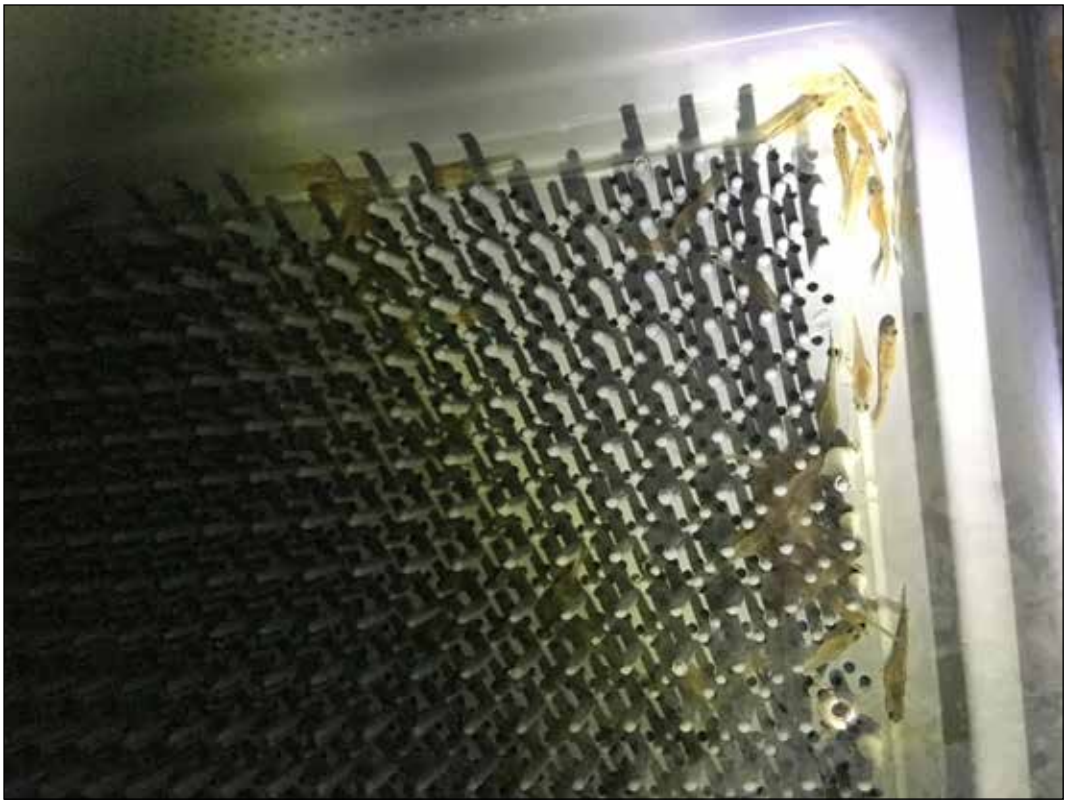
The disinfected eggs were submerged in physiological saline solution and transferred to counting plates for 100 eggs (originally designed for rainbow trout (*Oncorhynchus mykiss*)) (Fiap Gmbh, Ursensollen, GER). The plates were rinsed in physiological saline solution until each depression contained eggs with no additional eggs on the plate. As eggs of Arctic charr are relatively small compared to eggs of rainbow trout, there were either one or two eggs in each depression. Two counting plates, i.e. between 200 and 400 eggs, were transferred to each plot. The eggs were allowed to swell, using the municipal freshwater source in the hatchery (Table 1). Both fertilization and stocking of the incubator were conducted on the 26.10.2017. Three treatments were assigned to 15 plots each, formalin treatment only, formalin treatment and hand-picking, and hand-picking only. One untreated control group was assigned to 15 plots. One of the incubator compartments was treated with 20 mL formalin solution (formaldehyde: 380 mg mL^{-1} with 10.8 – 13.2 Vol-% methanol; Cenavisa S. L., Reus, ESP) added at the upstream end of the compartment. The formalin concentration was 380 ppm (mg L^{-1}). The formalin added was

Table 1: Water chemistry data for hatchery water during autumn.

Date	pH	H^+ (µeq L^{-1})	Alkalinity (µeq L^{-1})	Conductivity (µS cm^{-1})	Turbidity NTU	Ca^{2+} (mg L^{-1})	Mg^{2+} (mg L^{-1})	Na^+ (mg L^{-1})
26.10.2017	7.53	0.03	999	NA	NA	NA	NA	NA
01.11.2017	7.72	0.02	1016	95.9	0.14	17.84	0.45	1.56
09.11.2017	7.57	0.03	979	94.0	0.32	17.82	0.44	1.49
16.11.2017	7.62	0.02	1077	110.7	0.33	20.70	0.51	1.88
23.11.2017	7.73	0.02	1036	100.9	0.24	18.94	0.49	1.87
30.11.2017	7.72	0.02	1017	97.6	0.37	18.38	0.45	1.77
07.12.2017	7.60	0.03	1002	103.5	0.25	18.94	0.47	1.69
14.12.2017	7.76	0.02	967	98.5	0.26	18.26	0.46	1.65
21.12.2017	7.71	0.02	1035	100.0	0.23	18.80	0.45	1.53

K^+ (mg L^{-1})	NH_4^+ (µg L^{-1})	SO_4^{2-} (mg L^{-1})	NH_3 (mg L^{-1})	TOC (mg L^{-1})	TotP (µg L^{-1})	TotN (µg L^{-1})
NA	< 50	NA	NA	NA	NA	NA
0.47	< 50	2.38	1.56	0.8	3.05	167.5
0.43	< 50	2.34	1.49	0.7	0.99	160.5
0.49	< 50	3.23	1.88	0.9	4.21	178.5
0.49	< 50	3.33	1.87	0.9	0.19	109.5
0.39	< 50	2.84	1.77	1.1	0.59	123.0
0.44	< 50	2.84	1.69	0.9	3.33	150.0
0.44	< 50	2.66	1.65	1.1	2.64	142.0
0.44	< 50	2.40	1.53	1.3	0.39	108.5

transported out of the compartment by the ordinary effluent with a water residence time of about 13 minutes. The formalin treatment was conducted three times every week before the eyed stage. The treatments were implemented at 13, 15, 18, 20, 22, 25, 27, 29, 32 and 34 days post fertilization (dpf). Both formalin treated groups were kept in one compartment. The other compartment was not treated chemically. Hand-picking, by using forceps, was conducted weekly, beginning at the onset of the eyed stage at 35, 42, 49, 56, 63, 70, 77 and 84 dpf. During hand-picking, dead eggs, empty egg shells, and larvae were counted. The larger number of either hatched larvae or empty egg shells present was registered as a measurement for the number of hatched larvae. Subsequently, empty eggshells and dead larvae were removed. Living larvae escaped the plots through slits in the bottom of the tray into the hatching substrate (Figur 2), and were only counted once. In the plots, which were not hand-picked, dead eggs, larvae and empty egg shells were counted at 84 dpf. The presence of fungal infections was recorded for each plot, defined as visible hyphal infection on at least one egg, larvae or empty eggshell (Figur 3). All eggs were incubated in darkness, and hand-picking was conducted with a headlamp (Black Diamond, Salt Lake City, UT, USA). At the end of the experiment, infected eggs were collected, stored in either hatchery water or 70 Vol-% ethanol, and sent to the Norwegian Veterinary Institute (Veterinærinstituttet) for taxonomical identification of the water mould by morphological traits and genetic markers, respectively. Water moulds from eight eggs, that were infected by hyphae visible to the unaided eye, were cultivated using glucose yeast extract



Figur 2. Hatched larvae that have escaped into the hatching substrate beneath the experimental plots were collected at the end of the experiment (18.01.2018). Photographer: Tom Robin Olk.

(GY) agar, which is especially suitable for cultivation of Saprolegnia. The medium also contained antibiotics. Four eggs were analyzed by direct polymerase chain reaction (PCR) and gene sequencing, using specific ITS-primers for oomycetes.

Data analysis

The total number of eggs, dead eggs, and hatched larvae were calculated for each hand-picked plot by the following equations:

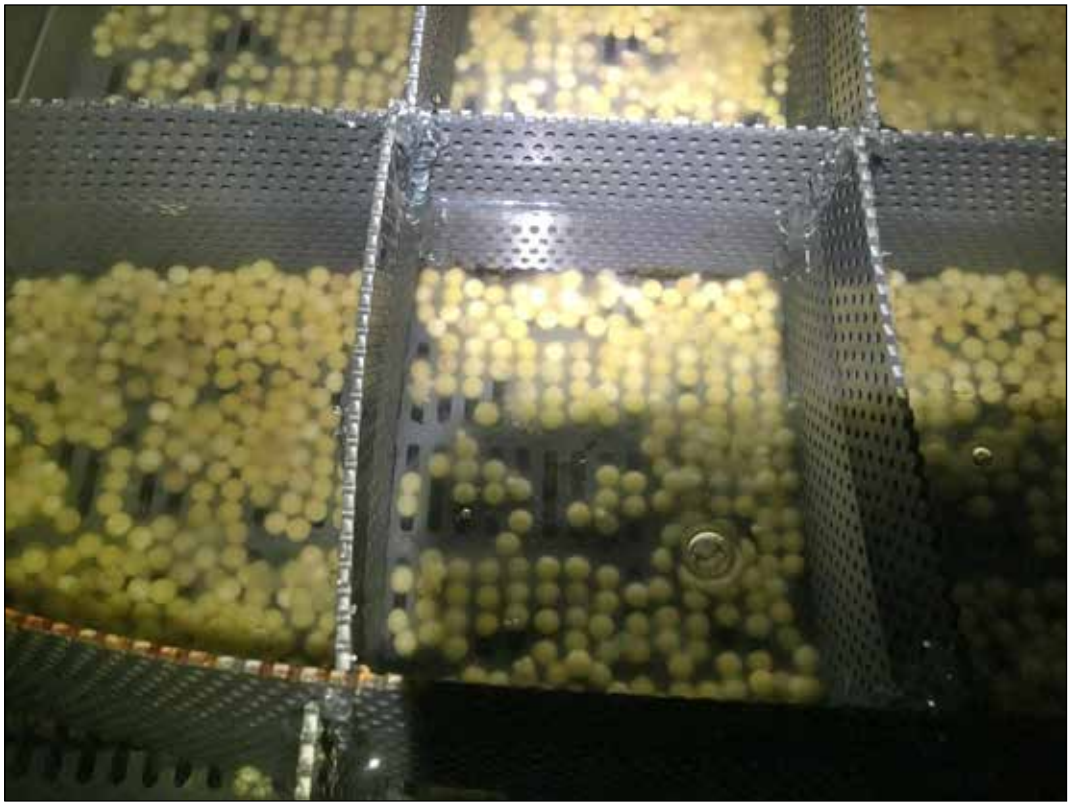
$$N_{TotalDead} = \sum_{i=1}^{8} N_{Deadi} \quad \text{Equation 1}$$

$$N_{TotalHatched} = \sum_{i=1}^{8} N_{Hatchedi} \quad \text{Equation 2}$$

and,

$$N_{Total} = N_{TotalDead} + N_{TotalHatched} \quad \text{Equation 3}$$

The calculations were performed for dead, hatched and total number, respectively. Observation *i* refers to the individual observation on the corresponding sampling date. There were eight observations per plot. The proportions of accumulated hatch at the end of the experiment (84 dpf) were arcsine-transformed to stabilise their variances (Ott & Longnecker 1984), and compared using a two-way ANOVA in R (R Core Team 2017). As explanatory factors, the use of formalin, hand-picking and an interaction between these two factors were included. Individual between-group differences were evaluated with Tukey's multiple comparison test in R. Results at a significance level of $\alpha = 0.05$ were accepted as significant. Results are reported as percentages, where the total number of eggs incubated in the respective plot(s) represents 100 %.



Figur 3. Arctic charr eggs in the unpicked experimental plots towards the end of the experiment (04.01.2018). There are some eyed eggs in the lower middle plot. Large accumulations of water mould hyphae make egg masses appear blurry, especially in the upper middle plot. Photographer: Tom Robin Olk.

Results

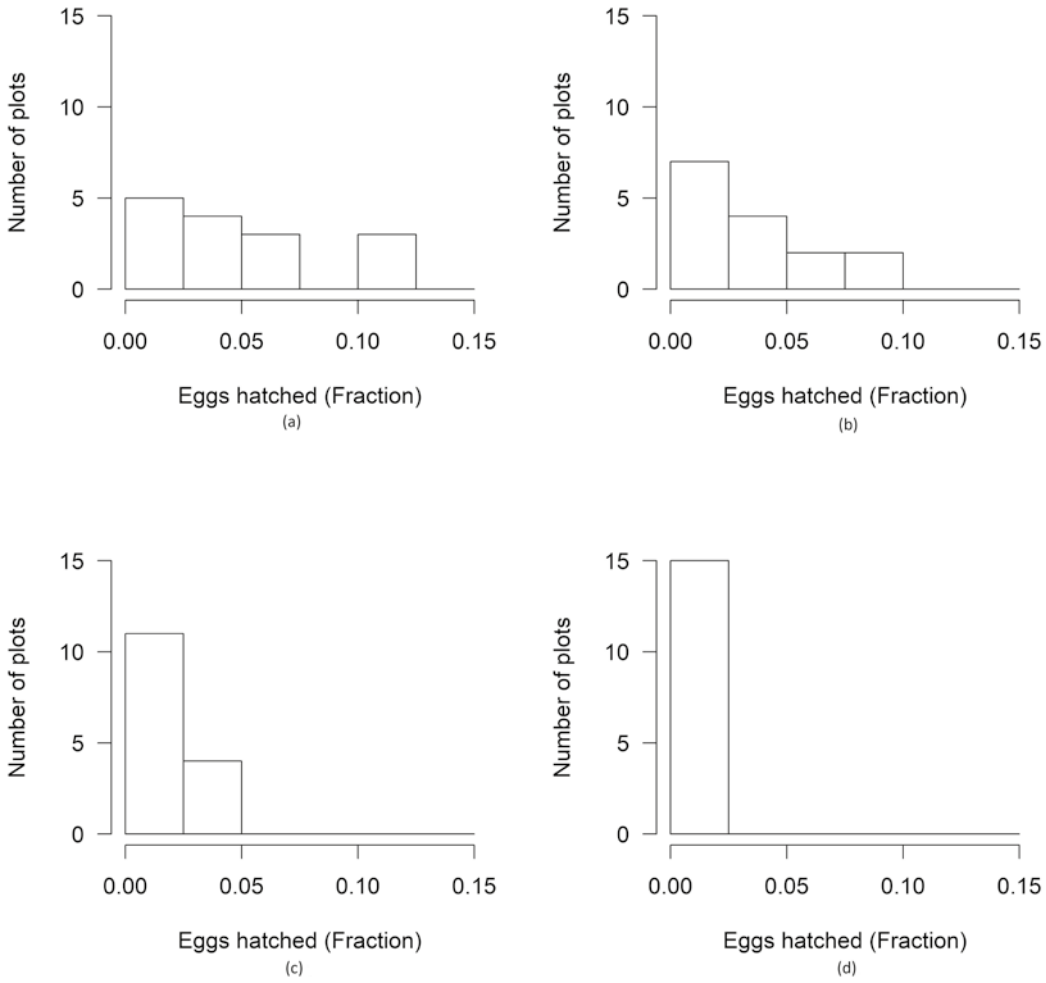
Total percentage hatched

The average proportion of hatched larvae for the entire incubation was low, and exhibited values of 5 ± 4, 3 ± 3, 1 ± 1, and 0 ± 0 % for formalin treated and picked, picked untreated, formalin treated not picked, and control groups, respectively (Figur 4).

The proportion of hatched larvae for the entire incubation differed significantly between the hand-picked and non-hand-picked groups (ANOVA, df = 1, F = 27.19, $p < 0.001$), and between groups treated with formalin and non-treated groups (ANOVA, df = 1, F = 4.43, p = 0.040). Both treated groups and hand-picked groups exhibited higher hatching rates than groups that were not treated with formalin or hand-picked. No significant interaction between hand-picking and formalin treatment was found (ANOVA, df = 1, F = 0.983, $p > 0.100$). Group differences in hatching proportion between formalin treated and non-treated groups were estimated to 0.2 % (Tukey's multiple comparison test: 95 % Confidence interval: 0.0004 – 0.8 %). Group differences in hatching proportion between picked and non-picked groups were estimated to 1.3 % (Tukey's multiple comparison test: 95 % Confidence interval: 0.5 – 2.5 %) (Tab. 2).

Identification of water moulds

No axenic culture of a single water mould species could be separated, and identified by morphological features. None of the water moulds that grew in culture resembled any species of the genus *Saprolegnia*. Instead, unidentified mould and bacteria grew. The analysis using PCR and gene sequencing revealed several different PCR



Figur 4. Number of plots of the different treatments exhibiting various hatching success measured as fraction of the total number of eggs. (a) Hand-picked and formalin treated plots; (b) Hand-picked not formalin treated plots; (c) Not hand-picked formalin treated plots; (d) Not hand-picked and not formalin treated plots.

Table 2: Differences in the proportions of hatched eggs between plots that were either not treated with formalin and not hand-picked, not treated with formalin and hand-picked, treated with formalin and not hand-picked, or treated with formalin and hand-picked.

Formalin Group 1	Picking Group 1	Formalin Group 2	Picking Group 2	Difference Hatch Proportion (%)	Lower Limit (%)	Upper Limit (%)	P
Yes	No	No	No	0.5	0.0	2.2	> 0.100
No	Yes	No	No	1.8	0.3	4.7	< 0.001
Yes	Yes	No	No	2.6	0.6	5.8	< 0.001
No	Yes	Yes	No	0.5	0.0	2.3	> 0.100
Yes	Yes	Yes	No	0.9	0.0	3.0	0.021
Yes	Yes	No	Yes	0.1	-0.3	1.1	> 0.100

bands for two of the samples, meaning that several unidentified species of water moulds were present. The two remaining samples could be identified, and exhibited 99 % correspondence to *Pythium monospermum* and 96 % correspondence to *Aphanomyces stellatus* as the closest matches in the gene bank, respectively.

Discussion

Total percentage hatched

Hatching success of all egg batches in this study is extremely low, with an average egg survival of 5 ± 4 % in the treatment group, and 12.2 % in the plot with the highest performance, respectively. The underlying reasons for the low survival are likely linked to egg quality and the fertilization process. In addition, some additional mortality is caused by fungal infections, as the effort invested in antifungal treatments was low compared to other studies (Barnes et al. 1997; Barnes et al. 2000; Barnes et al. 2003). Plots of all treatments were stocked with eggs of similar origin, meaning that egg quality and the fertilization process influenced total egg survival, but not the relative differences between treatments. The incubation environment of the eggs was also consistent between treatments regarding the type of incubator, stocking density, temperature, and water chemistry.

According to the comparison between all four treatments, both the application of formalin treatment three times weekly before the onset of the eyed stage, and hand-picking of dead eggs during the eyed stage, increased the number of hatched eggs. Formalin treatments at various stages, times of exposure and concentrations, have previously been shown to enhance survival of eggs of rainbow trout (Marking et al. 1994; Schreier et al. 1996; Barnes et al. 2000; Arndt et al. 2001), and fall Chinook salmon (*Oncorhynchus tshawytscha*) (Waterstrat & Marking 1995; Barnes et al. 1997; Barnes et al. 2003). Contrary, studies on rainbow trout and blueback salmon (*Oncorhynchus nerka*) (Burrows 1949), and brown trout (*Salmo trutta*) (Barnes et al. 2001) did not exhibit any clear improvement in survival caused by the use of formalin. However, the authors concluded in both cases, that this was due to absence of extensive fungal infections. Despite the agreement between the results of our study with previous studies on the effectiveness of formalin treatments *per se*, the benefit of hand-picking appeared to be much greater than the effect of formalin treatment in our study. In addition, the effect of hand-picking on egg survival in Arctic charr is likely underestimated in our study, as hand-picked and unpicked plots were kept in the same compartments, and spores may have spread from unpicked to picked plots. Contrary, formalin treated and untreated plots were kept in different compartments with separate in- and outflows, rendering cross-contamination unlikely. This is inconsistent with the results of previous studies comparing the effectiveness of the daily administration of formalin to hand-picking, which resulted in an increase in hatched eggs by 3 – 5 % in the formalin treated groups (Barnes et al. 1997; Barnes et al. 2000; Barnes et al. 2003).

Theoretically, formalin treatments should be more effective, as microscopic zoospores and hyphae, that would not be removed by hand-picking (Smith et al. 1985; Rand & Munden 1993), could weaken additional egg membranes, and ultimately result in an increased number of failed hatches in the picked groups (Burrows 1949). Fungicides, such as formalin, would likely remove large proportions of these microorganisms (Willoughby & Roberts 1992). However, this theory could not be confirmed in later investigations on fungal infections in hatcheries on eggs of rainbow trout (Kitancharoen & Hatai 1996) and Atlantic salmon (*Salmo salar*) (Thoen et al. 2011). Effects of egg mortality caused directly by the treatment are expected to be larger for hand-picking than for formalin treatments, as hand-picking may cause physical harm to living fish eggs as hypothesized for brown trout and Atlantic salmon (Sutela et al. 2007). Formalin did not produce toxic effects to rainbow trout eggs at an exposure of 5000 ppm for 15 to 30 minutes (Marking et al. 1994). Consequently, such effects are expected to bias the results in favour of more effective formalin treat-

ments, thus they are not the underlying reason for more effective hand-picking found in our study.

However, several underlying reasons for the discrepancy between the results of our study and previous studies can be outlined. In our study, formalin treatments were administered before the onset of the eyed stage, while hand-picking was conducted at weekly intervals throughout the eyed stage until hatch. This may strongly influence the results, as the extent of fungal infections likely varied throughout development. Barnes et al. (1997) observed higher mortalities during the eyed stage in groups of fall Chinook salmon eggs that were only hand-picked, indicating that fungal infections may cause lower mortality rates before. One underlying reason might be the accumulation of fungal spores and hyphae throughout the incubation period, also observed in all not hand-picked plots in this study. The accumulation of spores of *Saprolegnia ferax* throughout the breeding season has previously been demonstrated in field experiments on egg masses of amphibians (Kiesecker & Blaustein 1997). In our experiment, the plots were only separated by perforated PVC-walls. Spores and freely floating hyphae may have contaminated other plots, while infected eggs were too large to cross the barriers. Contamination between plots by vegetative growth of hyphae was not observed. Consequently, hand-picking was likely more effective in this particular protocol, as it was administered when infections were most intense.

Contrary, Johnston (2002) empirically claimed, that problematic fungi mainly occur during the pre-eyed stage. As no experimental evidence was provided, this may also be the result of different protocols or other confounding factors. Differences in the susceptibility to physical damage to eggs during hand-picking or the resistance to formalin between species may also be a cause for the conflicting results. We are not aware of previous studies investigating the use of formalin to disinfect eggs of Arctic charr. In addition, the previous studies documenting increased survival in formalin treated groups compared to hand-picked groups (Barnes et al. 1997; Barnes et al. 2000; Barnes et al. 2003), were based on daily formalin treatments and hand-picking. In our study, treatments were conducted less frequently. The lower total effort invested in our treatments may have had a larger impact on the effectiveness of formalin than on the effectiveness of hand-picking.

Fungal infections in our study were more intensive than in other studies, which may also have influenced the relative effectiveness of hand-picking and formalin treatments. Hand-picking effectively removes infected eggs as soon as the infection becomes visible by the unaided eye. Contrary, a formalin treatment at a concentration of 380 ppm formalin for ca. 13 min was likely only inhibiting fungal growth without eliminating their active presence in water (Marking et al. 1994). This means that it was merely effective as a prophylactic measure prior to the establishment of visible hyphae. However, as the formalin treatment in our study was applied prior to the onset of excessive fungal growth in the treated compartment, higher concentrations of formalin would not have been necessary to prevent fungal growth.

Identification of water moulds

Evidence for the presence of several different species of water moulds in eggs of Arctic charr was found. Two samples exhibited multiple PCR bands, which matched different oomycetes. In addition, unidentified mould could be grown in culture, and hyphae were frequently observed in the trays during incubation of the eggs. In one sample, *Pythium monospermum* was identified using PCR and gene sequencing. The sequenced DNA exhibited a 99 % match to this species. A 96 % match to *Aphanomyces stellatus* was detected in another sample, meaning that either *Aphanomyces stellatus*, or a closely related species, was identified. *P. monospermum* is primarily known as a plant pathogen, also found in river water (Matsiakh et al. 2016), while *Aphanomyces* sp. are found in soil or water as saprotrophs or parasites of roots, algae, aquatic fungi, insects, crayfish or fish (Markovskaja 2007). Species of both genera were previously found on

fish eggs in Polish hatcheries (Czeczuga & Woronowicz 1993). Data on water chemistry in hatcheries with fish eggs infected by the identified species of oomycetes remains insufficient to draw general and detailed conclusions on the conditions promoting fungal growth. However, the pH was similar in our study (7.53 – 7.76) and the hatcheries investigated by Czeczuga & Woronowicz, (1993), where either *Pythium* sp. or *Aphanomyces* sp. were found (7.5 – 8.35).

Possible adaptions to the protocol

This study demonstrates, that both formalin treatment before the eyed stage and hand-picking during the eyed stage increased survival until hatch of hatchery reared eggs of Arctic charr, even under severe pressure of fungal infections. However, the discrepancy between the relative effectiveness of formalin and hand-picking between our study and previous studies (Barnes et al. 1997; Barnes et al. 2000; Barnes et al. 2003) indicates, that the limitation of the use of formalin to the period before the eyed stage allows for additional mortality during the eyed stage. This is especially problematic, as higher mortalities caused by fungal infections were reported during the eyed stage in fall Chinook salmon (Barnes et al. 1997). The results of our study indicated the possibility of a higher risk of fungal infections during hatch. Consequently, the use of formalin during the entire incubation period, eventually combined with hand-picking is one alternative, maximising survival until hatch. It would also be economically beneficial to combine prolonged formalin treatment with reduced hand-picking effort, as hand-picking is a laborious procedure (Leitritz & Lewis 1976) generating high operating expenses at the hatchery. This adaption to the protocol is designed to maximise egg survival without considering the environmental and health concerns regarding formalin. Thus it should only be applied until sufficient information on the dynamics of fungal infections in Arctic charr eggs is available to develop appropriate protocols, which synchronise the application of formalin to stages of higher infection risk.

Conclusions

Combining formalin treatment before the onset of the eyed stage with hand-picking during the eyed stage to treat fungal infections in hatchery reared eggs of Arctic charr does not balance the environmental and health concerns connected to formalin and its efficiency as a fungicide in an adequate way. Antifungal treatment during the eyed stage appears to have the largest total impact on egg survival through hatch, meaning that formalin is inefficiently used in this protocol. Based on our current knowledge on the dynamics of fungal infections, formalin should be administrated during the entire incubation period to maximise hatching success and reduce labour expenses. Further research on the dynamics of fungal infections in hatcheries is encouraged to render chemical treatments more effective by synchronising their administration to peaks in infection risk. Our study indicates that one major infection peak may occur during hatch.

Supplementary material

Supplements, data, software code, and model figures are available at dx.doi.org/10.23642/usn.7334573.

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