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Evaluating the effectiveness of stocking vendace (*Coregonus albula* (L.)) eleutheroembryos by alizarin marking of otoliths

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with 2 tables

Abstract: Lake Werbellin (804 ha, 18 m mean depth), a mesotrophic lake in north-eastern Germany yielded between <1 and 14 tons (1.2 and 17.4 kg/ha) of vendace during the 1970's and 1980's. For the last 20 years, the lake has been stocked annually with 0.5 – 3.3 million eleutheroembryos (620 – 4100 per ha) in early spring, but the population is presumably self-sustaining. The contribution of stocking to commercial yields has never been assessed. In spring 1994, 1.5 million embryos were marked by immersion in Alizarin Red S (150 mg/l for 3 h) prior to stocking. Survival rate was better than 90 % after 48 h (76 % in one tank where the concentration had been higher initially) and ranged from 74 to 83 % after 24 d (21 % in one tank). Mark quality was excellent, as was mark retention after almost two years. In autumn 1995, more than one thousand specimens of the 1994 year-class were sampled from the lake. Lapilli and sagittae were removed, embedded in epoxy resin, ground and polished, and examined under ultraviolet light. Only 1.3 % of fish (14 specimens) were marked. We conclude that natural reproduction in Lake Werbellin was successful in 1994. The cost/benefit ratio of stocking was above unity. Evaluation of stocking programs in which eleutheroembryos are added to self-sustaining coregonid populations is strongly recommended.

Introduction

Coregonid eggs have been incubated in hatcheries and freshly hatched embryos have been stocked into natural water bodies for more than a century (DABROWSKI & CHAMPIGNEULLE 1986). While originally freshly hatched embryos were stocked only, feeding of larvae with zooplankton for several days up to some weeks became popular after World War II. During the 1970's and early 1980's, techniques for large-scale rearing of coregonids were developed, and today young-of-the-year fish and fingerlings are routinely stocked in different countries.

While there were some attempts to evaluate stocking success in the past, rigorous assessment of stocking effectiveness is a rather new line of research. When the purpose of stocking is to introduce coregonids into a body of water that has never held coregonids or where coregonids have gone extinct, evaluation of stocking success is an easy task. When conditions for natural reproduction are deteriorating, but there is still some chance for natural recruitment, stocking success is more difficult to evaluate. The most difficult task for fisheries managers and scientists, however, is to quantify the contribution of stocking to commercial harvests when there is natural reproduction, and stocking is done in the hope of promoting yields.

There are indirect and direct methods to quantify the contribution of stocking to the size of a certain year-class. Indirect methods comprise stocking in alternate years (CHRISTIE 1963), surveying larval abundance before and after stocking (KLEIN 1988), comparing yield with stocking intensity among lakes or during various years (SALOJÄRVI 1988, WOLOS et al. 1995), and multivariate statistical analyses of year-class strength (ECKMANN et al. 1988, LESKELÄ et al. 1995). Direct methods are based on marking all or part of the stocked individuals. This is practicable in the case of young-of-the-year fish or fingerlings. They can be marked with coded wire tags (MENG et al. 1986) or adipose fin burning (CHAMPIGNEULLE & GERDEAUX 1992).

Freshly hatched embryos or prefed larvae, which are still used predominantly in most stocking programs, could not be marked at large scale until recently. This is the main reason why good data about the effectiveness of stocking embryos or larvae is virtually lacking. During the last years, marking fish at different ontogenetic stages with fluorochromes has become a standard tool in fisheries research and for the evaluation of stocking programs (NAGIEC et al. 1995, RUHLÉ & WINECKI-KUEHN 1992, SECOR et al. 1995). The application of this technique to coregonid stocking programs, however, has just begun. To our knowledge, fluorochrome marking of the entire stocking material has not yet been applied successfully in any program where coregonid embryos or larvae are stocked.

It is the purpose of this paper to test the applicability of fluorochrome marking of recently hatched vendace (*Coregonus albula* (L.)) embryos with Alizarin Red S under field conditions, and to evaluate the contribution of stocking to the presumably self-sustaining vendace population in Lake Werbellin.

Materials and methods

Lake Werbellin is a mesotrophic lake in the north-eastern lowland in Germany, situated about 100 km north of Berlin. Average epilimnetic concentrations of total-P and chlorophyll a during summer stagnation 1993 were 40 µg/l and 4.7 µg/l, respectively (ECKMANN 1995). The lake is 804 ha large, mean depth is 18 m, and maximum depth is 50 m. Vendace are fished with anchored gill nets, and yields ranged from <1 to 14 tons per year (<1.2 to 17.4 kg/ha) between 1970 and 1989 (BURCKHARDT 1990).

The lake has been stocked annually with 0.5 to 3.3 million eleutheroembryos (620 – 4100 per ha) during the last two decades. Managers and fishermen always suspected that natural reproduction was successful and that the contribution of stocking to harvests might be of minor importance.

The stocking material originated from a hatchery at Lake Arendsee, situated at about 150 km north-west of Lake Werbellin. There, vendace eggs are regularly incubated in Zug jars with lake water, and embryos usually hatch during March. In March 1994, we conducted preliminary tests at this hatchery. Groups of 100 one-day-old eleutheroembryos were immersed in small plastic aquaria in solutions of Alizarin Red S (ARS) (0, 50, 100, 150, 200, 300 mg/l) for 3 to 9 hours. Mortality was assessed after immersion was completed, and 24 and 48 hours later. Water temperature and pH were checked at the start and at the end of the immersion period. Water from Lake Arendsee and from Lake Werbellin was used as well as 1:1 and 1:3 mixtures of Lake Arendsee and Lake Werbellin water. Samples of some of these test groups were reared with *Artemia* nauplii for 10 days to assess mark quality.

On March 28, 1.5 million vendace eleutheroembryos were transported from the hatchery to Lake Werbellin. Upon arrival, they were distributed among three tanks of 200 l and one tank of 600 l. One tank of 50 l served as the control. Stock solutions of ARS were added to obtain final concentrations of 150 mg/l. The initial concentration in tank 1 was 200 mg/l. Tanks were aerated during immersion treatment, and temperature and pH were checked frequently. After three hours of immersion, samples were secured from all containers, and embryos were released at the centre of the lake.

Samples were transported to the laboratory and reared at 10 ° C with *Artemia* nauplii to estimate mortality and to assess mark quality index (0: no mark; 1: poor; 2: good; 3: very good). Mortality was recorded during 24 days after immersion treatment. Embryos that were still alive after 2 days, but rested on the bottom were counted as dead and removed. An additional sample of 40 marked fish was reared in an aquarium with *Artemia* nauplii, pond zooplankton, and chironomid larvae during 620 days to check for mark retention.

From September to December 1995, vendace were sampled in the lake with gill nets of 14, 15 , 16, and 20 mm bar mesh size. They were measured (total length to 0.5 cm), weighed (wet weight to 1 g), and aged from scales (50 specimens from the 20 mm gill net, 193 specimens from the smaller mesh sizes). Sagittae and lapilli of fish sampled with 14 – 16 mm mesh size were secured, embedded in epoxy resin, ground from one side close to the nucleus, and polished. They were observed independently by two persons in an epifluorescence microscope with 545 nm excitation and >590 nm emission filters. In general, one otolith per specimen was ground and analysed. If the presence of a fluorescent mark was doubtful, a second otolith was ground and inspected.

Results

Fluorescent marks of good quality were obtained by immersion of one-day-old vendace eleutheroembryos in ARS solutions of 100 and 150 mg/l for 3 hours (Table 1). Mortality was generally below 10 % in solutions up to 100 mg/l and immersion times up to 6 hours. At concentrations of 150 mg/l and higher, mortality was variable, ranging from <10 to 100 %. Preliminary tests suggested that pH was an important parameter that influenced embryo survival, irrespective of ARS concentration. At pH values of 7.5 and higher, mortality after 24 hours was below 10 % (Table 1). Since a 1:3 mixture of Arendsee and Werbellin water retained a pH value above 7.5 even at 200 mg/l ARS, it was decided to do the mass marking at this concentration. During mass marking, however, pH dropped below 7.5 in the first tank (Table 2). ARS concentration was therefore lowered to 150 mg/l, and the same concentration was used in the remaining tanks.

Although pH values had dropped below the critical level of 7.5 in all 4 tanks, mortality after 48 hours was unexpectedly low in three tanks. Except for embryos from immersion tank 1, mortality after 24 days was comparable to, or slightly above, that of the control. Growth in terms of length and biomass did not differ among samples. Taking percentage mortality after 24 days as a conservative estimate, a total of 1.046 million marked embryos (1300 embryos/ha) have been stocked.

Mark retention in fish from the mass marking was excellent up to 620 days after immersion. All fish that were examined showed a mark, and mark quality was good or very good. The last sample of fish from the mass marking that had been reared in an aquarium was analysed in December 1995. Average length was 156±8 (S.D.) mm and average weight was 30.2±6.8 (S.D.) g. Marks were clearly discernible in unground sagittae of fish up to 60 mm total length. Sagittae of larger specimens needed to be ground.

During autumn 1995, more than 1500 vendace were sampled from the lake. Age analyses revealed that all fish from the 14, 15, and 16 mm gill nets (1082 specimens) belonged to the year-class 1994. Only 5 out of approximately 450 specimens caught in the 20 mm gill net belonged to this year-class. Therefore, only fish from the three smaller mesh sizes were analysed for fluorescent marks. These fish ranged from 14 to 18 cm total length (mean length: 16.2 cm) and from 21 to 47 g wet weight. The ratio of males to females was 0.83. Otoliths from 1072 specimens (99 %) could be evaluated for marks. In 936 specimens (87 %), one otolith was inspected, and in 136, two or more otoliths were checked for marks. Fourteen fish (1.3 %) had marked otoliths.

Discussion

Mass marking of vendace eleutheroembryos by immersion in Alizarin Red S is a reliable tool for evaluating the contribution of stocking to year-class strength and to commercial harvests. During immersion treatment, special care should be taken to avoid low pH values. Otherwise, mortality during or after immersion might be considerable. Although the results of our preliminary

Table 1. Experimental parameters and results of preliminary tests at the Lake Arendsee hatchery for marking freshly hatched vendace eleutheroembryos with Alizarin Red. S. S.A.: Lake Arendsee; W: Lake Werbellin; mark quality index was judged with a scale from 0 to 3. n is the number of fish analysed.

Water	A	A	A	A	A	A	A	A	A	A	W	W	A:W 1:1	A:W 1:1	A:W 1:1	A:W 1:3	A:W 1:3	A:W 1:3
Alizarin Red S [mg/l]	0	50	50	100	100	100	100	150	200	300	100	150	0	100	150	0	150	200
Immersion time [h]	3	6	9	3	4	5	6	3	3	3	3	3	3	3	3	3	3	3
pH start	7.9	7.6	7.7	7.6	7.5	7.5	7.5	7.2	7.3	6.9	7.5	7.5	8.1	7.7	7.5	8.0	7.6	7.5
pH end	7.8	7.8	7.8	7.7	–	7.8	7.7	–	7.4	7.1	7.8	7.7	8.1	7.9	7.8	8.1	7.9	7.9
T start [°C]	3.4	4.2	3.7	3.4	3.3	3.3	3.4	3.2	3.4	3.4	2.8	2.0	5.4	4.9	4.8	4.7	4.7	4.5
T end [°C]	4.3	4.3	4.4	4.3	–	–	4.1	–	4.3	4.3	2.6	2.5	3.0	3.0	3.0	3.9	3.7	3.7
Mortality post-imm. [%]	0	0	0	0	0	0	–	100	90	100	0	0	0	0	0	–	–	–
Mortality 24 hours [%]	3	0	0	7	0	0	2	–	92	–	4	3	0	4	2	2	1	12
Mark quality index 4d	0 (n=3)	–	–	–	–	–	–	–	–	–	–	1.4 (n=5)	0 (n=3)	–	2.2 (n=6)	–	–	–
Mark quality index 8–10 d	–	–	–	1.8 (n=5)	–	–	–	–	–	–	–	2.0 (n=5)	–	–	1.5 (n=4)	–	–	–

Table 2. Parameters and results of mass marking vendace eleutheroembryos at Lake Werbellin in March 1994. Number of fish stocked is estimated from number of fish treated and mortality after 24 days. Samples from all tanks were reared in duplicate, but length and weight data were not determined in all samples.

Tank number and volume [l]	1 200	2 200	3 200	4 600	5 50
Alizarin Red S [mg/l]	200/150	150	150	150	0
Immersion time [h]	3	3	3	3	–
pH start pH end	7.2 7.4	7.2 7.3	7.1 7.4	7.1 7.4	–
T start [° C] T end [° C]	4.9 7.0	5.2 6.0	– –	– 6.2	– –
Mortality after 2 days [%]	24.0	8.3	6.0	3.9	1.7
Mortality after 24 days [%]	78.7	26.1	24.2	17.5	15.3
Number of fish treated	250 000	250 000	250 000	750 000	2 500
Number of fish stocked	53 250	184 750	189 500	618 750	–
Mark quality after 8 days	–	2.6 (n=5)	–	2.9 (n=9)	–
Mark quality after 24 days	2.5 (n=6)	–	2.4 (n=5)	2.6 (n=5)	–
Fresh weight after 24 d [mg]	21.8 –	– –	– –	24.2 21.6	23.6 –
Dry weight after 24 d [mg]	3.16 –	– –	– –	3.46 3.17	3.45 –
Total length after 24 d [mm]	17.4 –	17.3 –	17.3 –	18.3 17.8	18.0 17.8

tests and of the mass marking did not match exactly, it is obvious that pH values below 7.5 may be critical and values below 7 are fatal. It is, therefore, recommended to adjust pH in the range of 7.5 – 8.

An immediate drop in pH after ARS was dissolved in water has also been reported by BECKMAN & SCHULZ (1996). These authors observed that pH stabilised after the solutions had been kept overnight with aeration. In our experiments, pH tended to increase during the three hours immersion period with continuous aeration (Tables 1, 2). Whether it is practicable in mass marking operations to keep solutions overnight will mainly depend on the available infrastructure and the amount of embryos to be marked.

Marking with ARS was successful at temperatures as low as 2–5 ° C. Thus, this technique can be applied under normal hatchery conditions. Since the embryos that were used in our experiments had just hatched the day before marking, this technique can also be used in hatcheries without any rearing facilities where embryos are released soon after hatching.

Fluorescent marks were of good or very good quality, i.e. they were easily recognizable even by inexperienced readers. Mark retention was excellent for almost two years. However, marks were not visible in the intact otoliths from fish larger than 6 cm. These otoliths had to be ground from one side. It was not necessary to hit the otolith nucleus precisely. As soon as the nucleus became discernible, grinding was stopped. This procedure reduces the risk of overgrinding, yet the fluorescent mark is easily detected. When fish can be sampled during their first summer, i.e. before they reach 6 cm total length, the time-consuming grinding procedure can be avoided and hence larger samples can be analysed.

Natural reproduction of vendace in Lake Werbellin was successful in winter 1993/94. Results of our test fishing suggest that a strong cohort was produced. The contribution of stocking to this cohort (1.3%) was negligible. This estimate is based on the assumption that survival rates in the laboratory are representative of those in the lake. However, mortality due to marking was slightly higher in three tanks and considerably higher in one tank than in the control (Table 2). If we assume an initial survival rate of 85 % for the stocking of unmarked embryos, 1.275 million embryos would have been stocked instead of 1.046 million. This would, theoretically, raise the contribution of stocked fish to the year-class 1994 to about 1.5 %, still an insignificant number.

From an economic point of view, stocking was unprofitable in 1994, as can be shown by the following gross estimate. The lake yields between 1 and 10 tons of vendace per year. No data on age composition of commercial catches are available. Since fishing intensity, mesh sizes, and trophic state of the lake had not changed during the last two or three decades, it is assumed that age composition remained the same during this time. Annual yields are, therefore, taken as a measure for average yield from single year-classes. Commercial weight of vendace is 80 g, so one year-class yields between 12 500 and 125 000 specimens. If the contribution of stocking is set to 1.3 %, irrespective of year-class strength, then between 162 and 1620 specimens would originate from stocking. This corresponds to an overall mortality rate from stocking until harvest of 99.84 – 99.98 %. At a price of 12 DM per kg of vendace, the benefit from stocking would be 156 – 1560 DM. The cost of 1.5 million embryos was 4170 DM in 1994 and hence, the cost/benefit ratio was well above unity.

For the year 1994, the stocking program for Lake Werbellin was needless and unprofitable. It is perhaps a premature conclusion to consider this stocking program as generally useless. Our results, however, suggest that stocking might be suspended tentatively without risking severe yield reduction. Lake Werbellin is just one example for a stocking program where recently hatched eleutheroembryos are added to a self-sustaining population. For the time being, no general conclusion about the importance and profitability of these stocking programs can be drawn. Nevertheless, we recommend to evaluate any other stocking programs with eleutheroembryos by means of fluorochrome marking of otoliths. After more than 100 years of stocking, it is about time to tackle this problem.

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