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# Evaluating the success of large-scale whitefish stocking at Lake Constance

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with 2 figures and 1 table

**Abstract:** Artificial incubation of whitefish eggs at Lake Constance (L.C.) started in 1887/88. Today there are six hatcheries at Upper L.C. which all use refrigerated water to delay hatching. They stock around 350 million recently hatched and 3–5 million prefed whitefish larvae annually. Despite this long history of artificial incubation at L.C., it remains unknown whether stocking indeed fulfils its aims, to dampen yearly yield fluctuations and/or to increase yields. We used a newly developed method to label the otoliths of whitefish embryos during incubation with Alizarin Red S (ARS) to evaluate the contribution of unfed hatchery larvae to cohort size. In winter 2003, 600 L of whitefish eggs were treated with ARS and by early April around 40 million larvae were released. In autumn 2003, the otoliths of age-0 whitefish sampled from the lake were examined. We found that 18 out of a total of 290 fish originated from the marking experiment. Since only 10% of stocked larvae had been marked, the contribution of hatchery-reared fish to the cohort 2003 was estimated as 62% with binomial confidence intervals of 42 and 79%. It should, however, not be concluded that stocking is necessary to maintain the current level of commercial yields, since it still remains unknown whether hatchery fish are added to the naturally recruited stock leading to stronger cohorts, or whether stocking only increases intraspecific competition without enhancing cohort size.

## Introduction

Releasing hatchery-produced coregonids into natural water bodies is an old and widespread practice throughout their native distribution range. The first reports of coregonid larvae stocking date back e.g. to 1867 in Finland (SALOJÄRVI 1992), to 1887 in Germany (RÖSCH 1993), and to 1868 in Canada and the U.S. (TODD 1986). Since hatching of both naturally and artificially reproduced larvae mostly occurred at times when temperature and food conditions were considered suboptimal for larval growth and hence survival, the notion became general-

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ly accepted that later stocking of larvae would favour their survival. To delay hatching accordingly, the use of cold or artificially cooled water became standard practice in many hatcheries where larvae are released soon after hatching.

As another means to foster larval survival, prefeeding of larvae with zooplankton was adopted early in the 20th century. The labour-intensive cropping of zooplankton during times when ambient concentrations were still low promoted studies on the rearing of coregonid larvae based entirely on the use of formulated feeds (DABROWSKI et al. 1984, BERGOT et al. 1986, LUCZYNSKI et al. 1986, RÖSCH 1995). Only at the end of the 20th century did suitable feeds become available that allowed the large-scale rearing of coregonids without any supplementation by natural food.

In addition to stocking larvae directly after hatching or after a few weeks of prefeeding, stocking of juveniles during summer or autumn of their first year of life became increasingly popular. The first attempts to rear coregonid juveniles in Finland date back to the beginning of the 20th century (SALOJÄRVI 1986), but it was not before the 2nd half of the century that large numbers of juveniles were successfully produced in different countries for stocking into inland and coastal waters. As with coregonid larvae, juveniles were reared on natural food at the beginning, but nowadays they can be reared to the yearling stage on a diet of formulated feeds alone.

From the beginning of coregonid stocking, fisheries scientists and managers were keen to assess the efficiency and eventually optimize their particular stocking practices. The most straightforward approach has been taken by CHRISTIE (1963) by stocking whitefish into the Bay of Quinte, Lake Ontario, every other year. As commercial catches did not follow the pattern of stocking, he concluded that climatic conditions had a dominant influence on year-class strength and subsequent yields. Several studies on the efficiency of coregonid stocking used statistical analyses (GERDEAUX & DEWAELE 1986, ECKMANN et al. 1988, LESKELA et al. 1995, WOLOS et al. 1995) but could at best provide evidence that stocking may influence yields or year-class strength. Reliable quantitative estimates on the influence of stocking on the fishery were hardly ever obtained (but cf. GERDEAUX 2004).

In the case of stocking juveniles, the situation is different. These fish can be marked in several ways (spray marking, CWT, burning the adipose fin) so that their fate after release and their contribution to the natural stock can be quantitatively assessed (MENG et al. 1986, CHAMPIGNEULLE & GERDEAUX 1992, LESKELA et al. 2004). Due to the generally good success of juvenile stocking, statistical evaluations were more successful and more reliable results were obtained compared to coregonid larvae stocking programs.

Thus, the ambiguities that still exist as to the efficiency of larval stocking could possibly be overcome by treating these fish in a way similar to juveniles, i.e. marking these fish and assessing their contribution to the fishery. Most marking techniques that have been used with juveniles are not applicable to larvae due to their size at stocking (10–20 mm). The most applicable, cost-effective and safe method to mark fish of this size is by chemical labelling of otoliths. Appropriate methods of labelling otoliths with fluorescent dyes had been available for some time (DABROWSKI & TSUKAMOTO 1986, TSUKAMOTO 1988, RUHLÉ & WINECKI-KUEHN 1992, BLOM et al. 1994, NAGATA et al. 1995, ROJAS-BELTRAN et al. 1995, 1998). No test has, however, been reported so far on the use of chemical labelling in a large-scale coregonid larvae stocking program (but cf. ECKMANN et al. 1998). A first attempt at Lake Constance was made in the 1990s using tetracycline. But, since hatchery managers have declined to use tetracycline in large-scale studies, a different technique using Alizarin Red S has been

established recently (ECKMANN 2003). The otoliths of whitefish embryos are labelled during incubation, thereby avoiding the need to label hatched larvae en masse, which is practically impossible under the hatchery conditions at Lake Constance.

The aims of our study were (i) to scale-up the method of ARS labelling of embryos to hatchery dimensions and (ii) to test the feasibility of this approach of evaluating stocking success under field conditions.

## Materials and methods

The experiment was carried out at Lake Constance in the hatchery at Rorschach, Switzerland, in March 2003. Lake Constance is the second largest prealpine lake in Europe. After a period of anthropogenic eutrophication (cf. BAUERLE & GAEDKE 1998) the lake returned to oligotrophic conditions by the end of the 20th century (total $PO_4$-P during spring turnover was 9 µg $L^{-1}$ in 2004). European whitefish (*Coregonus lavaretus* (L.)) is the dominant fish species accounting for >80% of commercial harvests (ECKMANN & ROSCH 1998). Six hatcheries with a total incubation volume of 12000 L have incubated on average 7000 L of whitefish eggs during the last ten years. With an average hatching success of 70%, approximately 350 million recently-hatched larvae are stocked between mid-March and the end of April, plus an additional three to five million prefed larvae which are stocked in early summer. We used a recirculation system of two circular tanks (1500 L each) and six zug jars (60 L each) to incubate 200 L batches of eggs. An immersion pump (800 L $min^{-1}$) in one tank supplied the jars with a constant flow of labelling solution. The flow rate could be adjusted separately for each jar. The second tank received the solution from all jars, and an immersion pump (1500 L $min^{-1}$), which was controlled by a float switch, recirculated the solution back into the first tank. Since embryos are incubated at around 1°C in this hatchery, a cooling system maintained the temperature between 1 and 5°C during incubation.

The labelling solution was prepared from deionized lake water for the following reason. ARS forms insoluble complexes with Ca ions (the reaction that leads to the irreversible deposition of ARS on the otolith surface), but this reaction will also occur in the solution, if Ca ions are present. Through this reaction, the amount of dissolved ARS may decrease by up to 90% (KOCHLIN 1997), leading most probably to lower marking success. The recirculation system was filled with 2000 L of deionized water and cooled to the desired temperature. Tris buffer was added at 1 g $L^{-1}$, and the necessary amount (1 g $L^{-1}$) of ARS (Alizarin Red S monohydrate, Sigma-Aldrich) was stirred in and dissolved by operating both pumps for about one hour. With the pumps still operating, pH was adjusted to a value between 8 and 8.5 with hydrochloric acid (HCl 25%, diluted at 1:10). When pH remained stable during one hour, the treatment was started.

Since the volume ratio of whitefish eggs (settling volume) and labelling solution should not surpass 1:10 (ECKMANN 2003), 200 L of eggs were treated at once. Both pumps were switched off, the jars were drained to about 10 L, and between 30 and 35 L of whitefish eggs were poured into each jar. The eggs were in a developmental stage where the otolith nucleus had just started to form by fusion of the primordia. The pumps were started again and the flow rate was adjusted for each jar. During the 24 hours incubation, pH and temperature were checked regularly and adjusted when necessary. After 20–22 hours, water from the hatchery's cold incubation system was slowly added to the system, so that the ARS was washed out after some hours. For one or two additional days, the eggs were flushed with cooled water to remove any eventually remaining ARS from the chorion. They were then reincorporated into the hatchery's standard incubation system. The ARS solution was drained to the local wastewater treatment plant, where the concentration was estimated to be well below 1 mg $L^{-1}$. This was admissible since a test by the Swiss Federal Laboratories for Materials Testing and Research (EMPA) had shown that the respiration rate of activated sludge was not influenced by ARS, even at a concentration of 100 mg $L^{-1}$.

In total, 600 L of eggs were labelled in three batches of 200 L each. From each batch, several subsamples of around 500 mL were incubated separately to assess marking mortality during incubation. When larvae had hatched, one batch of each treatment and a control were further kept in the hatchery to compare survival and growth between labelled and control fish.

One or two days after hatching, the larvae were widely distributed in the main basin of Upper Lake Constance (Fig. 1), in the same way as larvae are normally stocked by hatchery personnel. In late summer/autumn of 2003, young-of-the-year whitefish were sampled from the lake at four areas (Fig. 1) with a pelagic beam trawl of 4x4 m opening. Sagittae and lapilli were prepared from all fish sampled and embedded in epoxy resin. Lapilli were examined *in toto* independently be two readers with an epifluorescent microscope (Zeiss filter set no. 487915, 546 nm excitation wavelength). When results were doubtful, sagittae were ground close to the nucleus, polished, and examined as above.

## Results

Labelling success was 100% in all three batches (27, 30 and 40 larvae examined per batch). Mark quality was scored 3 (cf. ECKMANN 2003), i.e. marks were shining brightly at 100x magnification. Mortality losses from the time of labelling until hatching were similar in treatment and control batches, amounting to around 14%. Thus, in early April 2003, 40 million labelled whitefish eleutheroembryos were stocked into Upper Lake Constance. As mass hatching of unmarked embryos occurred around early April in all hatcheries at Upper Lake Constance, the release time of labelled and unmarked larvae coincided well. Naturally spawned whitefish, however, hatch about two months earlier.

Trawl samples in autumn 2003 yielded large numbers of juvenile perch, commercial-sized whitefish, and 290 young-of-the-year whitefish. The latter were more abundant towards the north-eastern as compared to the south-western shore (Table 1), which corresponds to the general pattern of whitefish distribution during both the growth season (APPENZELLER 1998) and winter (ECKMANN 1995). In 18 fish, i.e. 6.2% of sampled fish, ARS marks were easily detected upon inspection of the lapilli (mark quality 3). Additional inspection of the sagittae confirmed this result. It was necessary to check the sagittae in only two fish to confirm the

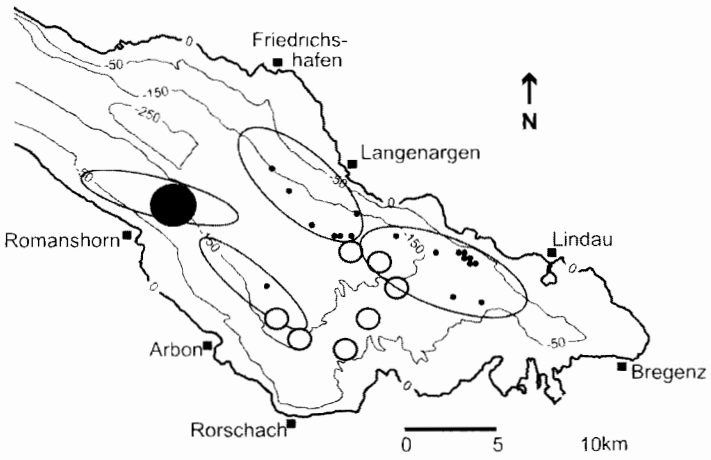


**Fig. 1.** Release sites for ARS-labelled whitefish in Upper Lake Constance (open circles: $26 \cdot 10^6$ larvae, filled circle: $14 \cdot 10^6$ larvae released in April 2003). Ellipses indicate regions where trawl samples were taken in late summer/autumn 2003 (clockwise from top: Langenargen west, Langenargen east, Arbon, Romanshorn). Dots: recapture sites of labelled whitefish (n=18).

**Table 1.** Results of trawling young-of-the-year whitefish in the central basin of Upper Lake Constance during autumn 2003. For location of trawling regions, refer to Fig. 1.

| Region | No. of hauls | Total no. of whitefish | Whitefish/haul | No. of whitefish per labelled whitefish |
|---|---|---|---|---|
| Langenargen west | 20 | 135 | 6.8 | 19.3 |
| Langenargen east | 14 | 123 | 8.8 | 12.3 |
| Arbon | 6 | 17 | 2.8 | 17.0 |
| Romanshorn | 5 | 15 | 3.0 | - |

non-existence of an ARS mark. One labelled whitefish was found among 12 to 19 fish (no labelled fish were found at the trawling region near Romanshorn), indicating random distribution of labelled fish within the cohort (cf. Fig. 1). The lengths of labelled fish fell into the length range of the entire sample (Fig. 2), indicating that ARS labelling did not affect growth in length. This conclusion is further supported by observations in the hatchery, where mortality and growth of labelled and control fish did not differ during three months.

Since only 10% of larvae that had been stocked in 2003 were ARS-labelled, we extrapolate that around 62% of the young-of-the-year whitefish in 2003 were of hatchery origin. The 95% binomial confidence interval for detecting 180 hatchery fish in a sample of 290 fish spans from 42 to 79%, i.e. with an error probability of 5% the contribution of hatchery fish would fall into this range.

## Discussion

ARS labelling of whitefish otoliths during egg incubation is possible at large scale under hatchery conditions. Since embryos are labelled during egg incubation, holding capacity for lar-

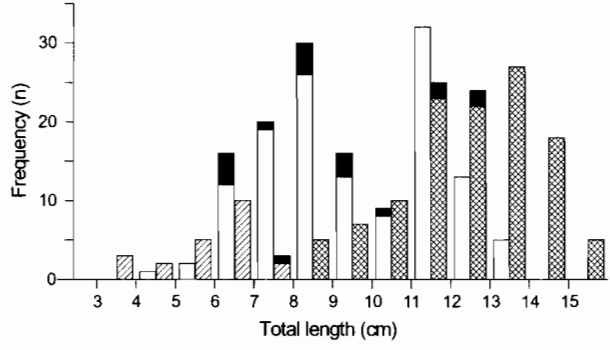


**Fig. 2.** Length-frequency distributions of young-of-the-year whitefish trawled from Upper Lake Constance in 2003. To account for growth in length during the sampling period, data are grouped in three blocks: August samples (slashed bars), September samples (open bars), November samples (cross-hatched bars). Labelled whitefish are highlighted in black for all three periods.

vae is not a bottleneck so that any hatchery can use our procedure. Thus, a method is available for evaluating the success of the massive stocking of recently hatched coregonid larvae into large lakes. This provides the possibility for objectively comparing different stocking practices, e.g. incubation at a natural temperature regime, delayed hatching via cold incubation, or prefeeding of larvae. Evaluating these different practices in addition to the already well-studied stocking of juveniles would allow selecting the practice, which is most appropriate for a particular management goal.

The scaling-up from laboratory to hatchery dimensions required some adjustments of the technical procedures. Care had to be taken to dissolve all the ARS before the pH was finally adjusted. Undissolved ARS floating at the surface was repeatedly stirred into the solution, while the strong currents caused by the immersion pumps prevented settling of the chemical. When adjusting the pH, a certain time-lag had to be allowed for the solution to get thoroughly mixed after the addition of hydrochloric acid. The use of deionized water effectively prevented the precipitation of Ca-ARS complexes in the recirculation system. Since the concentration of dissolved ARS was probably much higher under these conditions as compared to the earlier laboratory trials, a reduced amount of ARS might be sufficient for successful labelling. Alternatively, the ratio eggs:solution could be increased. Small-scale trials are, therefore, recommended to find out how much ARS can be saved by preparing the labelling solution with deionized water.

Our first, unreplicated estimate of 62% hatchery origin of the 2003 young-of-the-year cohort does by no means prove that stocking supports or increases commercial yields. Firstly, the confidence interval for this estimate is rather broad (42...79%) and the test has not been replicated so far. Secondly and most importantly, even if the contribution of hatchery larvae to a cohort amounted to around 60% it would not follow that without any stocking the cohort would be smaller. This would only be true if recruitment to the young-of-the-year stage was entirely density-independent. To our knowledge, this has never been shown for coregonids. Instead, it is reasonable to assume at least some density-dependence of survival during the first months of life. This has been suggested as a general principle of population regulation in fish (e.g. ROTSCHILD 1986, HOUDE 1987), and there is evidence for density-dependent processes in coregonids from Finnish lakes (SALOJÄRVI 1992), where the magnitude of density-dependence seems to increase during ontogeny. Under the scenario of strictly density-dependent survival to the young-of-the-year stage, cohort size could remain largely unchanged even by massive stocking, through replacement of naturally-reproduced fish by those originating from the hatcheries. Alternatively, if the carrying capacity of the system was not fully used by naturally recruited fish, then stocked fish might contribute significantly to year-class strength.

Further insight into, and a quantification of, the effects of larvae stocking on whitefish population dynamics and finally on commercial yields, can only be obtained by assessing survival rates of larvae from both sources. These assessments should preferably be undertaken under conditions of different ratios between, and absolute amounts of, wild and hatchery larvae, i.e. replicates of our experimental approach are absolutely necessary. The notion that is recently becoming popular at Lake Constance, that our first result already demonstrates the effectiveness of large-scale stocking of larvae into Lake Constance must, therefore, be rejected.

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