A low-cost method of rearing multiple batches of fish

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Abstract

Experimental studies based on inferential statistics typically require the rearing of many batches of eggs or fish separately. If this is done with conventional fish rearing methods, the need for laboratory space and equipment are normally very high. This may prevent many researchers from experimentally approaching problems in fish genetics or ecology, especially if treatment differences are expected to be small. We have developed and successfully tested a new procedure with *Coregonus* sp. fry. Eggs were hatched in Petri dishes kept at 6°C. Yolk-sac fry were reared in a hanging bag system at 15°C with continuous water exchange. We estimate that our new fry-rearing method reduces space needs, infrastructure and material costs by a factor of 10 or more, while being comparable to previously described methods with respect to animal welfare requirements and the experimenter's working time. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

A typical problem in genetic or ecological studies based on inferential statistics is that many independent replicates are needed (e.g., Hurlbert, 1984), while laboratory space and resources are limited. This is especially a problem when the expected magnitude of the effect of the biological phenomenon that is investigated is not very large. Small treatment differences require relatively large sample sizes if the investigator wants to have sufficient statistical power (Cohen, 1988). Moreover, multi-factorial ANOVA designs that allow testing for interactions among the factors, in addition to testing the main effects, typically increase the number of independent replicates by a factor of two or more, compared to sequentially performed studies on main effects only. Space and resource limitations may be a reason why many interesting research questions have only been examined with invertebrates as model species. A new method that allows the rearing of multiple batches of young fish while reducing space needs, infrastructure and material costs is presented. The method is comparable to previously used methods with respect to animal welfare requirements and experimenter's working time. To test the utility of this new method, the system was challenged by using a fish genus that is difficult to rear in captivity, the whitefish (*Coregonus* sp.) (reviews in Flüchter, 1980; Rösch, 1995).

Before fry can be reared for genetic studies, multiple groups of eggs must be reared separately. Rojas-Beltran and Gillet (1995) tried to rear whitefish eggs in Petri dishes but concluded that this method had severe limitations in Coregonidae because of low dissolved oxygen. Here, a successful attempt to rear whitefish eggs in Petri dishes with a slightly different method than the one used by Rojas-Beltran and Gillet (1995) is presented.

2. Material and methods

2.1.. Rearing of separate batches of eggs

Whitefish (*Coregonus* sp.) from Lake Hallwil in Switzerland were used. The taxonomy of this population is not yet solved (for a recent discussion of this problem, see Douglas et al., 1999). Ten males and ten females were stripped to produce 100 different sib groups for another study on heritability of developmental problems (Wedekind et al., submitted). The eggs of each sib group were distributed among four 9-cm diameter, uncovered Petri dishes. Egg in Petri dishes were incubated at 6°C in a climate chamber on a rack with three shelves. The mean number of eggs per Petri dish was 140 and ranged from 45 to 294 (S.E. = 2.6). Care was taken to randomize the position of these 400 Petri dishes within the climate chamber with respect to parental origin and number of eggs per Petri dish. Water was exchanged once every 2 weeks during the first 30 days, and subsequently once per week until hatching. Water was exchanged by emptying the Petri dish over a stiff piece of 1000-μm nylon net and immediately adding new water to the eggs. Exchange water was sand-filtered, lake water brought to incubation temperature in an aerated aquarium in the climate chamber for at least 1 day. No effort was made to keep the water exchange procedure aseptic or treated for infections.

Dead, deformed, or diseased eggs were removed at least three times per week with a pipette and their number recorded. On day 58, shortly before hatching, water oxygen saturation was measured on a sample of eggs, 1 mm from egg surfaces and directly at their surface. A "MasCom" oxygen-microelectrode with a tip diameter of less than 100 μm, fixed on a "Newport" micro-manipulator that allowed for exact positioning, was used. The measurement system was controlled by computer. To test whether egg size correlates with egg mortality, the diameter of 10 randomly selected eggs per female were measured under a dissecting scope.

2.2.. Rearing separate batches of fish

Fig. 1A shows a construct drawing of the units built to rear small fish groups. Standard 300×600 mm sterilization bags made of transparent, 50-μm thick polypropylene were used as rearing containers. The lower seam was strengthened with strong adhesive tape ("duct tape," see Fig. 1B). The tops of the bags were triple folded and fixed with plastic cable clamps to a piece of wood. Water was supplied through flexible 9-mm diameter PVC tubes attached via T- or L-connectors to a valve (poly-ethylene/poly-propylene) (Fig. 1). A plastic pipette tip at the end of the water control valve acted as nozzle to create some water circulation in the bags. The drain was made from a stiff PVC pipe fixed in place by placing one end against the inside surface of the sac and jamming both sac and pipe into a 3-cm piece of flexible tubing. This created a tight junction around the drain which prevented leakage when the sac membrane between pipe and tube was cut to allow drainage. This small flexible tube was connected to a longer flexible tube via an open T-piece to prevent suction of water out of the sac (see Fig. 1A). To hinder fish from entering the drainpipe, a small piece of 500-μm nylon net was melted with an industrial hot air blower to form a sac and fixed to the opening of the pipe with a plastic cable clamp.

Two one-sac units were supported by a 50-cm piece of wood and two such pieces of wood were hung one above the other to a support girder using packing string. The supporting structure was strong enough to hold the weight of 25 such four-sac units (see Fig. 1C). Each four-sac unit was hung in a slanted position, fixed by string with a simple loop to a screw in the wooden piece.

The water supply of the two-sac units, as indicated in Fig. 1A, was attached in series via T- and L-connectors in such a way that 25 units were supplied by one of four exits from a tap manifold located in the middle of the system. When all the valves were fully open, 200 l/h were fed into the system with bags on the lower row receiving 1.7 l/h and bags on the upper row receiving 2.3 l/h. If necessary, difference in water flow could, of course, be corrected with the valves. With a mean sac volume of 6 l, the water was theoretically exchanged about 7–9 times a day. Organic fallout, which consisted of dead food items, detritus and dead larvae, had a tendency to accumulate in the corners of the bags where it was easily removed by siphon.

The connections between the two-sac units (i.e., the water supply via the flexible PVC tubes) were long enough to allow each four-sac unit to be separately disconnected from the loop and to hang straight. Hence, it was possible to get convenient access to each sac if necessary.
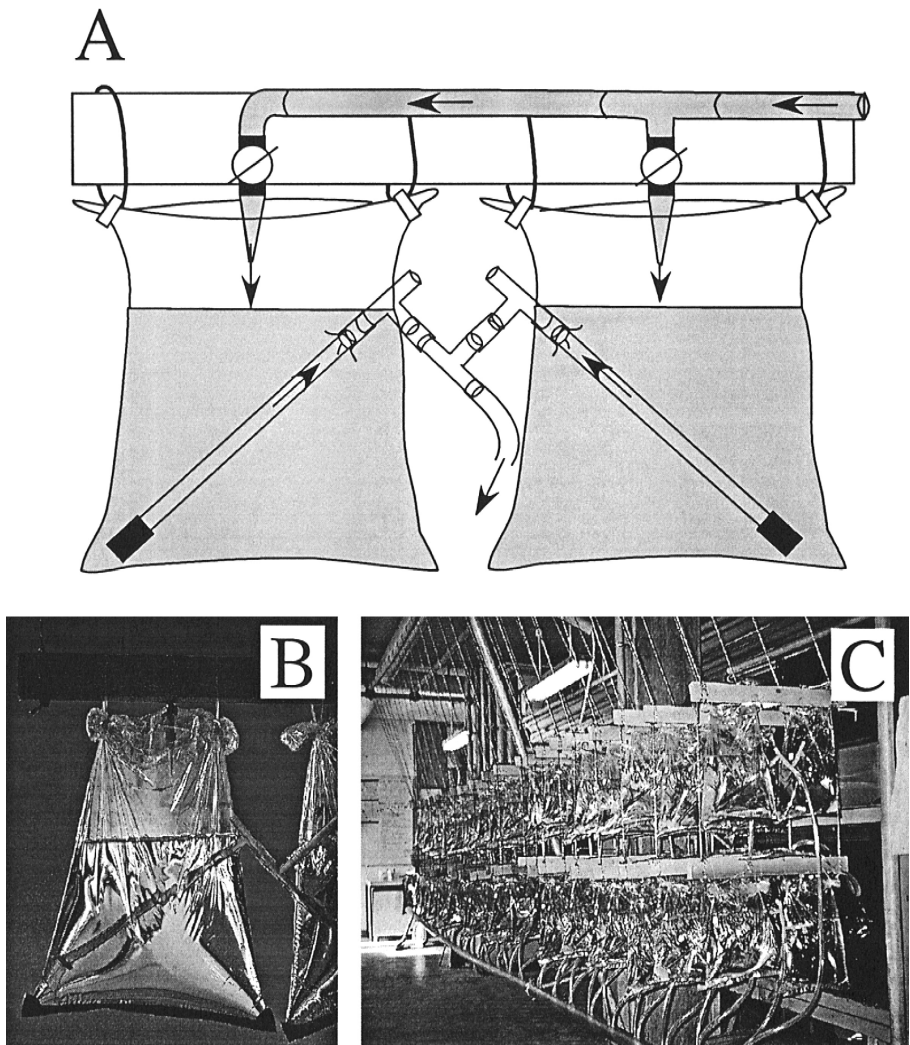
Fig. 1. Construct drawing (A) and photographs (B and C) of the fish fry rearing units. See text for description.

To test the system, yolk-sac larvae of whitefish from Lake Lucerne (*Coregonus* sp., see again Douglas et al., 1999, for a discussion of the taxonomy) were used. Eggs had been incubated in conventional 8-l Zuger jars (Woynarovich and Horváth, 1980, p. 79). After hatching, 240 fish were distributed to 12 sacs (20 fish per sac). The water temperature was adjusted to 15°C with a continuous-flow water heater. The fish were fed with live *Artemia salina* twice a day. We used a 30-cm stiff plastic tube connected via a long flexible tube to a 3-l beaker containing the food items. This beaker was secured above the sac water level so that the food items were automatically siphoned out of the beaker through the tube into the sacs. To feed the fish, the experimenter only

needed to put the stiff end of the tube into the sac and let the flow run for few seconds each morning and each evening.

3. Results

3.1.. Egg hatching and oxygen measurements

Mean egg mortality during the 76 days of incubation was 23.3% (S.E. = 1.0%, range: 0–100%). Mortality did not appear to be influenced by the position of the Petri dish in the climate chamber (one-way ANOVA on effect of shelves: $F = 1.2$, $df = 2$, $P = 0.31$), nor did it significantly correlate with the number of eggs per Petri dish ($r = 0.04$, $n = 400$, $P = 0.37$). Mean egg diameter per female ranged from 2.10 to 2.35 mm (mean = 2.22 mm, S.E. = 0.025) and did not significantly correlate with mean mortality rates ($r = -0.231$, $n = 10$, $P = 0.52$).

At approximately day 35, an uncontrolled and untreated epidemic started that caused a marked increase in egg mortality and continued until hatching. Early mortality before day 30 seemed only correlated with developmental defects of the embryos (as observed under a dissecting scope on a sample). Early mortality rate averaged 9.7% (S.E. = 0.62%, range: 0–64.5%). The late mortality until hatching, i.e., the mortality of the eggs that had survived until day 30, averaged 15.2% (S.E. = 0.95%, range: 0–100%). All the eggs analyzed for bacterial infection contained *Pseudomonas fluorescens* (Wedekind et al., submitted), a bacterial fish disease that probably entered our system via the water supply (Schäperclaus, 1990).

Measurements on day 58 indicated that oxygen supply was sufficient (Flüchter, 1980). The mean O_2 saturation at 1 mm from the egg was 78% (S.E. = 0.066, $n = 5$) and at the egg surface it was 55% (S.E. = 0.03, $n = 17$), corresponding to 6.5 mg O_2/l.

3.2.. Fry growth and mortality

The initial mean length and mean weight of the yolk-sac fry were 10.05 mm (S.D. = 0.68, $n = 40$) and 2.36 mg (S.D. = 0.57), respectively. Twenty-two days later, the fish had reached a mean length and mean weight of 20.42 mm (S.D. = 1.81, $n = 166$) and 41.63 mg (s.d. = 4.05), respectively. The average mortality rate over 22 days was 30.8% (S.D. = 16.1%, range 5–55%). Oxygen concentration at the end of the experiment ranged from 7.7 to 11.7 mg/l (mean = 8.9 mg/l, s.d. = 1.0 mg/l).

4. Discussion

Mortality of the fish larvae we observed is comparable to that observed in other studies on coregonids (e.g., Rösch, 1995; Koho and Viljanen, 1998) and indicates that our new method can be used successfully for rearing small whitefish. The oxygen measurement at the end of the experiment demonstrated that the water supply through

the pipette tip created enough circulation in the bags, and that organic fallout did not cause an oxygen problem.

Since the sacs are transparent and there are less light absorbent structures, light supply is easy to provide (see Fig. 1C), and observability for the experimenter is good. Space requirements for the hanging units were 5.50×0.70 m/100 units, or 0.154 m^2/four-sac unit. This could easily be reduced by about 20% by hanging the four-sac units closer together. Material costs are small compared to rearing tanks, aquaria, etc., and excessive aging of the material or clogging of the pipes by contamination or algae growth was not observed during a preliminary 2-month test period. We are therefore confident that our rearing method will support large and multi-factorial design ecological and genetic studies on small fish, even if space and resources are severely limited.

The Petri dish method we used here to separately rear different groups of eggs proved to be useful for genetic studies. Total egg mortality observed was low compared to other studies on Coregonidae, although an uncontrolled epidemic by *P. fluorescens* occurred in our study. Dabrowski et al. (1987), for example, incubated eggs of 14 *C. albula* females in small Weiss-type incubators at 8.95°C with constant water flow. They observed a mean mortality of 50.1% (range: 16.8–96.2%, data taken from their Table 5). Ventling-Schwank and Müller (1991) kept eggs of *Coregonus* sp. in sacs made of 500-μm nylon net in aquaria with constant water flow. They observed 30% mortality and described this mortality as comparable to that which is usually observed in Zuger bottles (Woynarovich and Horváth, 1980) used by Swiss fish hatcheries.

Rojas-Beltran and Gillet (1995) kept eggs in batches of about 200 at 5°C in Petri dishes, using methods similar to our procedure. However, they recorded only the early mortality during the first 24 days in two independent runs and found 19.3% and 26.5%, respectively. They stated that this method was limited to studies on early mortality, " ... probably related to increase of oxygen consumption of embryos. For accurate estimation of egg survival, incubation in plastic screens in running water was used" (Rojas-Beltran and Gillet, 1995, p. 310). With these plastic screens, the authors recorded a mortality of 25–30% until hatch (data taken from their Fig. 1). Our results suggest, however, that oxygen supply is sufficient, and that the Petri dish method can be used successfully until fish hatch, even in Coregonidae. Both early and late egg mortality was explained to a large degree by paternal and maternal effects (Wedekind et al., submitted), but not by low dissolved oxygen, egg number per Petri dish, or average egg size as shown here. Mortality due to bacterial infection could probably be prevented by appropriate prophylactics.

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