



Facile methods for reusing laboratory plastic in developmental biology experiments

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ABSTRACT

Plastic pollution negatively affects ecosystems and human health globally, with single-use plastic representing the majority of marine litter in some areas. Life science laboratories prefer pristine conditions for experimental reliability and therefore make use of factory standardized single-use plastic products. This contributes to overall plastic waste in the United States and globally. Here, we investigate the potential of reusing plastic culture dishes and subsequently propose methods to mitigate single-use plastic waste in developmental biology research laboratories. We tested the efficacy of bleach and ethyl alcohol in sterilizing used dishes. We then tested the feasibility of washing and reusing plastic to culture *Xenopus laevis* embryos subjected to various manipulations. Cleaning and reusing laboratory plastic did not affect the development or survival of *X. laevis*, indicating that these cleaning methods do not adversely affect experimental outcome and can be used to sterilize plastic before reuse or recycling. Lastly, we performed a survey of various life science laboratories to estimate both waste reduction and savings associated with recycling single-use plastics. Standardization of these procedures would allow research laboratories to benefit economically while practicing environmentally conscious consumption.

1. Introduction

Since the development of the first plastic in 1907, 8.3 billion metric tonnes of plastic have been produced, with 79% of that plastic ending up as waste in landfills or the environment (Geyer et al., 2017). Large-scale single-use plastic production, use, and disposal creates planetary scale problems for human health and ecosystems as plastics degrade and transition into micro-plastics (Sana et al., 2020). Once plastic debris enters an ecosystem, it decomposes and disintegrates into smaller pieces, making it possible for micro-plastics to enter the food web and water cycle (Huerta Lwanga et al., 2017). The increasing production and consumption of plastics have become a threat to human and environmental health. Global plastic waste increases by 5% annually (Huerta Lwanga et al., 2017) and the United States disproportionately contributes to global plastic pollution, exacerbated by single-use plastics (Moll, 2015).

Research laboratories are contributors to this plastic problem. Many laboratories employ single-use plastics due to the guarantee of sterility and confidence in reproducibility. The sensitive nature of many experiments requires pristine conditions and therefore, labs rely on factory sterilized and treated plasticware. Most biological research labs now employ single-use plastics (Urbina et al., 2015), usually in the form of sterile pipettes, pipette tips, centrifuge tubes, and culture dishes. As such, molecular and cell biology labs are reliable consumers of

single-use plastic products despite the high costs and generation of non-degradable waste. Yet, the challenges associated global production of plastic waste will require efforts to reduce plastic consumption even in research settings. The ability to reuse laboratory plastics remains mostly untested due to the sensitive nature of many experiments. Here, we test the feasibility of reusing plastic in experiments using a common model system.

The frog *Xenopus laevis* has been a powerful model system for developmental and neurobiological laboratories for nearly a century (Wallingford, 2022; De Robertis and Gurdon, 2021; Gurdon and Hopwood, 2000). Their readily available eggs and large embryos have made this frog a choice organism for the study of vertebrate development and disease modeling (Corkins et al., 2021; Niehrs, 2022; Walentek, 2021). Researchers have used *Xenopus* for overexpression and knockdown studies to elucidate gene function (Blum et al., 2015). Further, explants and transplants have been used to determine fate maps and tissue competency (Harland and Gerhart, 1997; Nieuwkoop, 1952; Nieuwkoop and Others, 1952; Wylie et al., 1996). These experiments are delicate and require optimal conditions to keep manipulated embryos and tissues alive. Easily available culture dishes from many vendors along with the use of antibiotics have markedly increased the success of these experiments.

Yet, *Xenopus* is a hardy frog and is found in a variety of habitats with variable cleanliness. Therefore, it is able to reproduce in waters with

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high loads of bacteria and other contaminants (TinsleyKobel, 1996). Embryonic survival in the wild is not well-studied, however amphibian embryos are generally encased in a jelly coat which serves to protect the developing embryo from desiccation, predation, and other potential harms (Hansen et al., 2002; Turani et al., 2018). Jelly coat removal is required for most embryonic manipulations of amphibian embryos and likely sensitizes them to their culturing conditions (Sive et al., 2010). Accordingly, most laboratories interested in using *Xenopus* have adopted sterile, single-use plastic dishes.

As stated above, these single-use plastics likely end up in landfills and do not readily degrade. Given the popularity of *Xenopus* as a model for developmental biology, culturing of their embryos alone likely contributes to a considerable amount of plastic waste in the form of plastic dishes. We therefore, set out to determine whether these dishes can be reused without compromising experimental integrity. Next, we test different methods of cleaning and compare them in the survival of embryos following common manipulations. Finally, we survey various laboratories on their plastic dish use to determine both the cost and waste savings associated with reusing plastic dishes for developmental biology experiments.

2. Methods

2.1. Microbiological culture

Microbiological cultures were grown in 5 mLs of LB broth for 18hrs at 37 °C in a shaking incubator. Microbial growth was quantified by measuring OD 600 on a UV/VIS spectrophotometer (Beckman Coulter).

2.2. Cleaning protocols

Used culture dishes were rinsed three times in distilled water and then coated in either 70% EtOH, 10% bleach, or bleached diluted below 1% for at least 10 min (Fig. 1A). Dishes were then rinsed three times in distilled water. All dishes were then air-dried on the bench prior to use in experiments.

2.3. Embryo culture, microinjection, and manipulation

Xenopus laevis embryos were obtained via *in vitro* fertilization. Female frogs were induced to ovulate by injection of 500 units of human chorionic gonadotropin (Chorulon). Male frogs were sacrificed and testes were harvested. Macerated testes were added to freshly ovulated

eggs to produce zygotes. Embryos were then cultured in 1/3 MR supplemented with gentamicin unless otherwise noted.

Embryos were injected with 200 pg of *mCherry* RNA and 200 pg of *LacZ* RNA. Vitelline membranes were removed using fine watch-maker's forceps and microsurgery was performed using hairloops and eyebrow knives. Nieuwkoop recombinants were made in 1X MR and transferred to 1/3X MR after 1 h. Anterior and posterior halves of embryos were separated at stage 15 in 1/3X MR and allowed to heal.

2.4. Survey of plastic use

11 laboratories across the United States were surveyed to determine research focus and plastic culture dish purchasing practices. Laboratories were asked for their yearly plastic dish purchases and their preferred manufacturer. The respondents were then categorized according to their size (number of individuals) and field of study.

3. Results

We wanted to determine whether it is possible to reuse plastic dishes for experiments using *Xenopus* embryos without sacrificing experimental integrity. To that end, we designed our experiments to reflect typical conditions of used culture dishes. When cultured, if dead and dying embryos are not removed then the healthy embryos within the dish will begin to die. Therefore, we seeded the dishes that were to be tested for reuse in all of our experiments with 1/3 MR that contained dead embryos and eggs left to fester for over 24 h (Fig. 1A). Following seeding, we tested how effective our cleaning protocols were at sterilizing used dishes. To that end, we inoculated LB culture media with 100 µL of water from dishes both before and after cleaning, then incubated the cultures for 18 h. We first compared the cultures of those inoculated with water from a new culture dish to those that weren't cleaned (Fig. 1B). As expected, there was no bacterial growth in cultures from the new dishes but there was substantial growth in the culture from the uncleared dishes. All of the reused dishes showed bacterial growth before cleaning but growth was limited after employing any of the cleaning methods. The lack of bacterial growth following cleaning with either 70% EtOH (Figs. 1C), 10% bleach (Fig. 1D), or dilute bleach (Fig. 1E) suggests that each of the different cleaning protocols was effective in removing excess bacterial contamination and limiting bacterial growth.

This result showed that used dishes can be restored with respect to microbial contamination. However, *Xenopus* frogs naturally live in water and ponds that likely have high levels of micro-organisms and therefore,

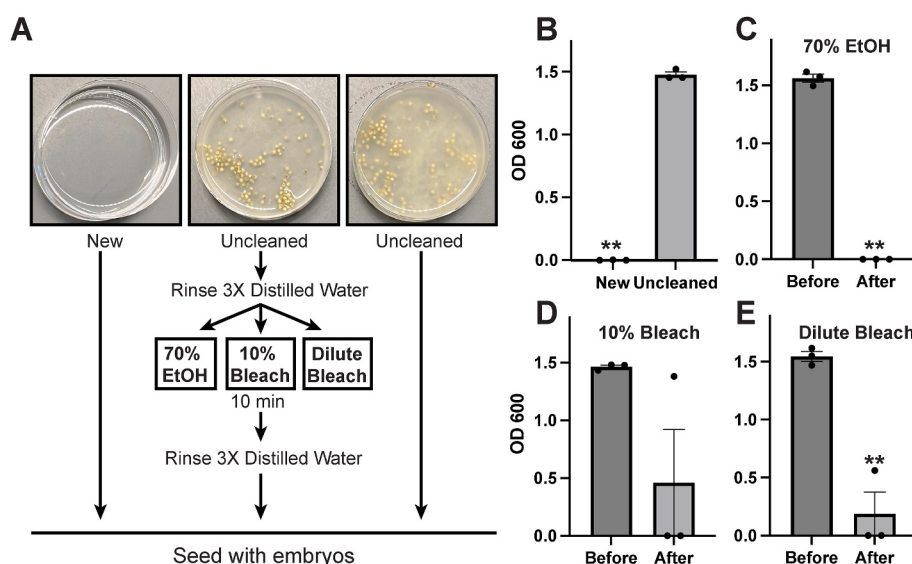


Fig. 1. Cleaning methods for reusing plastic culture dishes. **A.** Schematic illustrating the experimental design and different methods used to clean dishes. **B.** Means of OD 600 readings ($\pm$ S.E.M.) of microbial cultures from indicated dishes. **C.** Means of OD 600 readings ($\pm$ S.E.M.) of microbial cultures from dishes before and after cleaning with 70% EtOH. **D.** Means of OD 600 readings ($\pm$ S.E.M.) of microbial cultures from dishes before and after cleaning with 10% bleach. **E.** Means of OD 600 readings ($\pm$ S.E.M.) of microbial cultures from dishes before and after cleaning with dilute bleach. Error bars show $\pm$ S.E.M. Data points show individual replicates. ** indicates $p < 0.001$ (Pairwise T-test).

microbial growth is unlikely to be a major factor affecting embryo survival. We next sought to test our cleaning methods in different assays of embryo generation and culture. First, we compared fertilization rates between the treatments. We carried out each of these experiments by collecting eggs from a single female into different dishes and fertilizing them using sperm from a single preparation. This was done to limit variation that might be due to different egg or sperm quality. *Xenopus laevis* is amenable to *in vitro* fertilization with usually high success (Sive et al., 2010). We found an average fertilization rate of 85.2% in new dishes compared to 53.8% when dishes were left uncleaned. However, average fertilization rates rose in the cleaned dishes and ranged from 75.2% in the dilute bleach condition to 79.2% in the 10% bleach condition (Table 1, Fig. 2A). Though there was a decrease in fertilization rates in cleaned dishes, the variances between experiments were too large to statistically distinguish between treatments. This may reflect batch-to-batch differences in embryo quality.

Given the trend towards a lower fertilization rate in uncleaned dishes, we decided to follow the survival rate of the embryos from the fertilization tests. These embryos were kept at room temperature without water changes and their survival measured daily over seven days. Survival in the new dishes averaged about 90% after seven days, whereas in uncleaned dishes average survival dropped to 25%. Survival in the cleaned dishes was over 90% in all treatments after seven days. (Fig. 2B). These results demonstrate that all three cleaning protocols restored survivability in used dishes to equal or better than that of new factory dishes. Further, all surviving embryos were indistinguishable from one another based on their treatment (Fig. 2C). This suggests that neither the cleaning methods, nor rearing in uncleaned dishes, caused obvious defects in embryonic development beyond that of survival.

To confirm these results, we followed this experiment with several additional survivorship assays comparing total percent survival in new, uncleaned, and cleaned dishes. Similar to what we found in the post-fertilization survival rates, there was no difference between the cleaned dishes and the new dishes with survival percentages in the mid to high nineties. There was a significant difference, however, in the survival of embryos reared in uncleaned dishes with an average survival rate of 24.7% (Fig. 2D, Table 1).

Taken together, our results suggest that used dishes are suitable for culturing embryos. However, most experiments with *Xenopus* involve manipulations beyond simple culturing. We therefore tested whether sensitization of the embryos via experimental manipulation made them vulnerable to culturing in cleaned dishes. *Xenopus* embryos are large and relatively easy to inject. Accordingly, microinjection of nucleotides is frequently done. We selected embryos at the one-cell stage and injected them with 200 pg of *mCherry* and *LacZ* RNA. Following injection, embryos were randomly distributed into either new, uncleaned, or dishes cleaned using one of the three cleaning conditions. Embryos were screened for injection success via visualization of *mCherry* and then cultured until stage 40. Surviving embryos were then stained for the presence of β -Gal. Overall, there was lower survival in uncleaned dishes when compared to that of new or cleaned dishes (Fig. 3A, Table 1). We found an average survival rate of 70.5% in the new dishes. Uncleaned dishes had a markedly lower average survival rate of 39.5% for injected embryos though the variation amongst independent experiments was particularly high. Post-injection survival in the cleaned dishes ranged from 68.3% in the dilute bleach condition to 72.9% in dishes cleaned with 10% bleach. Dishes cleaned with 70% EtOH had an average survival rate of 71.3%. The embryos that did survive were indistinguishable across the various treatments (Fig. 3B–F) suggesting that any effect dish quality has on survival occurs shortly following culturing in the dish and does not adversely affect the development of surviving embryos.

Another advantage to *Xenopus* embryos is that their large size makes them amenable to *Xenopus* embryos is that their large size makes them amenable to transplanting and explanting tissues. These experiments are commonplace and routine, however they do cause considerably more stress on the embryo than a simple injection. To test transplant survival in our recycled dishes, we bisected stage 15 embryos

Table 1
Summary of all results testing cleaning methods in culture dishes for *Xenopus* embryos. Averages are calculated from the independent replicates indicated. Combined total survival is calculated by summing all observations across replicates. ** indicates $p < 0.001$ (Kruskal-Wallis H-test).

Condition	Fertilization			Overall Survival			Post-Injection Survival			Anterior-Anterior Transplants			Nieuwkoop Recombination		
	Fertilization Success (± S.D.)	Replicates (n)	Combined Fertile/Total Eggs (%)	Average Survival (± S.D.)	Replicates (n)	Combined Survival/Total (%)	Average Survival (± S.D.)	Replicates (n)	Combined Survival/Total (%)	Average Survival (± S.D.)	Replicates (n)	Combined Survival/Total (%)	Average Survival (± S.D.)	Replicates (n)	Combined Survival/Total (%)
New Dish	85.2 (11.1)	7	572/695 (82.3)	94.5 (6.6)	12	244/255 (95.7)	70.5 (23.7)	6	168/220 (76.4)	100 (0)	9	90/90 (100)	97.6 (5.3)	5	29/29 (100)
70% Ethanol	77.1 (19.5)	7	587/786 (74.7)	98.3 (4.1)	6	192/195 (98.5)	71.3 (26.1)	5	160/208 (76.9)	98 (5.3)	7	78/80 (97.5)	93.6 (9.2)	5	27/29 (93.1)
10% Bleach	79.2 (13.7)	7	390/471 (82.8)	93.8 (7)	12	246/255 (96.5)	72.9 (27.7)	6	160/220 (72.7)	100 (0)	9	90/90 (100)	95 (8.9)	5	28/30 (93)
Dilute Bleach	75.2 (17.4)	7	410/568 (72.2)	94.2 (5.1)	12	242/255 (94.9)	68.3 (27.4)	6	154/220 (70)	100 (0)	9	91/91 (100)	80 (28.3)	5	25/30 (83.3)
Uncleaned	53.8 (27.8)	7	280/502 (55.8)	24.7 (28.1)**	12	88/255 (34.5)	39.5 (43.4)	6	126/220 (57.3)	48.6 (50.2)**	9	45/90 (50)	37.6 (51.7)	5	11/29 (37.9)**

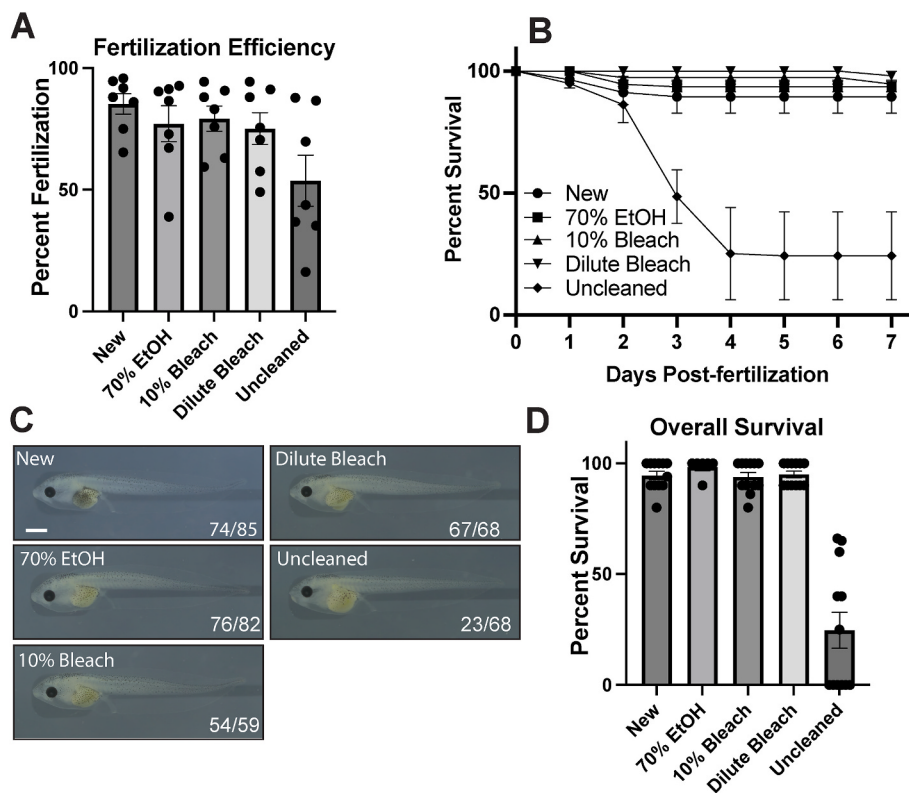


Fig. 2. Post-fertilization survival in cleaned culture dishes. **A.** Plot of average fertilization rates in dishes subjected to the indicated treatments. **B.** Survival curve in percentages of embryos fertilized and cultured in dishes subjected to the indicated treatments. Error bars show $\pm$ S.E.M. **C.** Representative embryos from the experiments quantified in **B.** Anterior to the left and dorsal to the top. Scale bar: 1000 μ m. Numbers indicate prevalence of the observed phenotype. **D.** Plot of overall survival in dishes subjected to the indicated treatments. Error bars show $\pm$ S.E.M. Data points show individual replicates.

and joined the anterior portions by aligning the cut surfaces. We then allowed them to heal for 30 min. The recombined embryos were transferred randomly to dishes as described above and cultured until stage 45, just before they would begin feeding. All transplanted embryos cultured in cleaned and new dishes had average survival percentages at 97% or higher, whereas the average survival significantly dropped to below 50% in uncleaned dishes (Fig. 3G, Table 1). In these experiments, we found that the surviving transplanted embryos cultured in the uncleaned dish showed some developmental delay as determined by overall cranial size when compared to those cultured in other conditions. Conversely, culturing in cleaned dishes did not noticeably affect embryonic development (Fig. 3H–L).

Lastly, we tested the ability for embryos to survive following a more severe manipulation. For this, we used embryos at stage 9 and made Nieuwkoop recombinations by removing the mesoderm and recombining the animal cap with the vegetal portion. This recombination results in mesoderm induction of the animal cap ectoderm and a subsequent elongation of that mesoderm (reviewed in Gerhart, 2004). We used this assay to test for any adverse effect on tissue differentiation following culture in cleaned dishes. 24 h post treatment, we found robust survival in all cleaned dishes though slightly lower than that observed in new dishes (Fig. 3M, Table 1). A majority of the surviving recombinants showed observable elongation (black arrows, Fig. 3N–R). A minority of the living recombinants failed to show extension, likely a result from extensive endoderm removal (gray arrows, Fig. 3N–R). Lastly, we found that a significant reduction in survival of recombinants cultured in uncleaned dishes (Fig. 3M, Table 1). We did observe rare instances where there was survival even in uncleaned dishes (Fig. 3R). Nevertheless, the results across multiple replicates showed cleaning with 70% EtOH or 10% bleach restored dishes to new-like quality.

Ultimately, we wanted to determine potential economic and environmental benefits of employing recycling methods in a research setting. To that end, we surveyed labs of different sizes for their estimated monthly culture dish purchasing. We used the data gathered from this survey to estimate the plastic consumed monthly by weight and cost.

These data showed that the surveyed laboratories generate between 13 and 52 Kg of plastic waste a year, which equates to a cost of approximately \$1300 to \$5200. We then determined the reduction in plastic waste and funds saved if dishes were cleaned and reused only once. We found that smaller labs with less need for dishes can reduce over 10 Kg of plastic waste while also saving over \$1000 in funds (Table 2). Across the 11 surveyed laboratories, plastic waste would be collectively reduced by 60 Kg translating to savings of over \$12,000 annually.

4. Discussion

Here we set out to test the feasibility of using simple cleaning methods to recycle used culture dishes. We conclude that cleaning dishes using any of our three methods restores them to the equivalent of new factory dishes in terms of *Xenopus* embryo survival for both unmanipulated embryos and embryos following various typical treatments. Given the ability to reuse dishes, we found that doing so just once can save as much as \$12,000 and 63 Kg of waste per year collectively in surveyed research labs. Employing these methods will further reduce the carbon footprint of research laboratories by reducing shipping and production. It is worthwhile to note the use of glass dishes in a laboratory setting. While glass dishes are ideal, they cost considerably more than plastic. As a result, laboratories have historically resorted to using plastics, creating the problem we attempt to address here. Thus, this study acts as a method to mitigate plastic use while considering the economic necessity to use plastics.

Our work used three different methods of cleaning and sterilizing used dishes and all three were found to be effective in restoring them to a new-like quality. While we did not see a distinction for any one of the cleaning methods, we would suggest that 70% EtOH is likely to be the most convenient method. Ethanol is highly volatile and readily evaporates without leaving traces on the dish (“Chemical Disinfectants | Disinfection & Sterilization Guidelines | Guidelines Library | Infection Control | CDC,” n. d.). This means there is no need to collect it after it has been used for sterilization. Conversely, bleach leaves a salty residue if

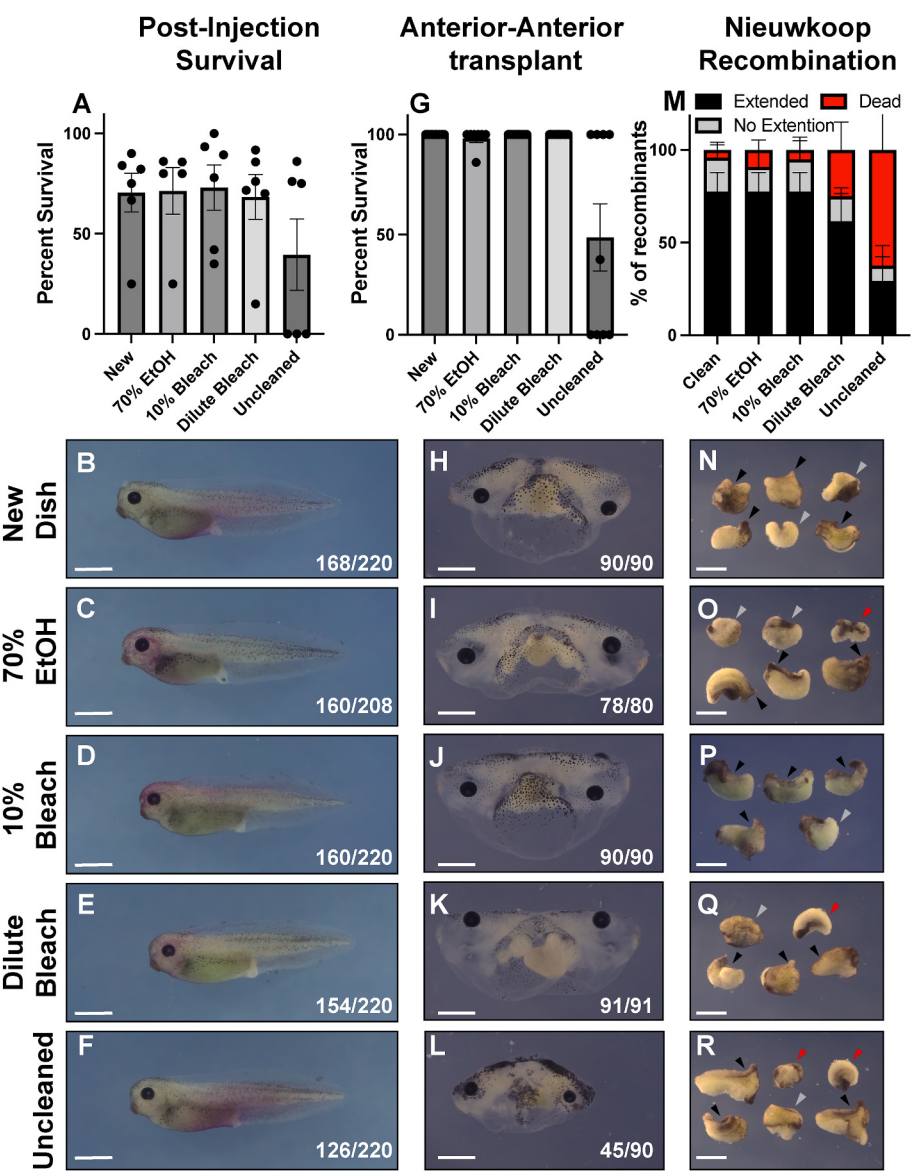


Fig. 3. Post-manipulation culture of embryos in cleaned and reused culture dishes. **A.** Plot of average post-injection survival in dishes subjected to the indicated treatments. **B–F.** Representative embryos injected with 200pg *mCherry* and *LacZ* then stained for β -gal (pink color). **G.** Plot of average anterior-anterior survival rates in dishes subjected to the indicated treatments. **H–L.** Representative images of embryos after joining anteriors and cultured until stage 45. **M.** Plot of average Nieuwkoop recombinants across four independent experiments that either extended (black bars), were alive but not extended (gray bars), or died (red bars) in dishes subjected to the indicated treatments. **N–R.** Results of one Nieuwkoop recombinant experiment with the highest survival across all treatments. Black arrows indicate regions of extension. Gray arrows show living recombinants that didn't extend. Red arrows indicate disintegrating recombinants. **B,H,N.** Embryos cultured in new dishes. **C,I,O.** Embryos cultured in dishes cleaned in 70% EtOH. **D,J,P.** Embryos cultured in dishes cleaned with 10% bleach. **E,K,Q.** Embryos cultured in dishes cleaned with dilute bleach. **F,L,R.** Embryos cultured in uncleaned dishes. Error bars show $\pm$ S.E.M. Data points show individual replicates. All scale bars: 1000 μ m. Anteriors to the left and dorsal to the top in B–F and H–L. Numbers indicate prevalence of the observed phenotype.

Table 2
Summary of survey results and impacts of dish recycling. All values are based on a case of coated Falcon brand 30 mm dishes from general suppliers. The cost is calculated from the case price before any discount from general lab suppliers. The weight is calculated from 25 sleeves of 20 dishes in a case of 500. The annual savings is calculated by determining the plastic and cost savings associated with reusing a dish once.

Laboratory Size	Field of Study	Yearly Culture Dish Consumption			Potential Annual Savings	
		Plastic (Kg)	Cases of 500	U.S. Dollars (\$)	Plastic (Kg)	U.S. Dollars (\$)
<10 Individuals	Genetics	13	6	2569.98	6.5	1284.99
	Immunology	13	6	2569.98	6.5	1284.99
	Cell Biology	52	24	10279.92	26	5139.96
	Developmental Biology	21.7	10	4283.28	10.85	2141.64
>10 Individuals	Immunology	26	12	5139.96	13	2569.98
Totals		125.7	58	24843.12	62.85	12421.56

left to evaporate meaning bleach must be collected and discarded following use according to institutional regulations. That said, the practice of having a standing bucket with diluted bleach to collect used dishes followed by rinsing would be useful in lieu of ethanol treatment as ethanol sterilization tends not to remove debris after used plates are left to dry without cleaning.

We did observe considerable variation across replicates in some of our experiments, most notably in the post-injection survival rates

(Table 1, Fig. 3). We observed instances where none of the injected embryos in uncleaned dishes survived and others where they survived commensurate with cleaned dishes. This suggests that there are batch-to-batch variations in egg quality such that less hardy eggs may be more susceptible to impure culturing. However, we observed that the cleaned dishes always supported survival similar to that of new dishes. This strongly suggests that cleaning dishes remains a viable option for waste reduction without compromising experimental outcomes

independent of embryo quality. It may seem curious that the more severe manipulations (anterior-anterior transplants and Nieuwkoop recombinations) resulted in higher survival rates across all conditions than observed in the overall and post-injection survival. However, the nature of these experiments poses a likely explanation. The “cut and paste” experiments used embryos at blastula and neurula stages and therefore, already selects for embryos that are of sufficient quality to survive. Nevertheless, we observed that both survival and experimental success were restored in cleaned dishes. Taken together, our results demonstrate that cleaning and reusing dishes does not compromise experimentation and presents a viable solution for excessive plastic waste.

It remains to be determined how many times an individual culture dish can be reused. Though it is unlikely that the sterilization methods will become less effective, the dishes themselves do tend to accumulate scratches and other blemishes. Scratches have an effect of rendering the dish less useful for photography or snagging embryos and thus damaging them. Additional work would need to be done to determine the full lifespan of a reused dish, but in our hands, we found them to be continually reusable. We restricted our quantitative analyses to smaller dishes but the methods outlined here are easily adopted for larger and multi-welled dishes. This would result in additional cost savings and waste reduction.

We focused our study on reusing culture dishes for *Xenopus* experiments. However, these protocols are likely directly applicable to experiments using other organisms such as zebrafish, chicks, or mouse embryos where culture dishes are used. We did not investigate the potential for recycling dishes used for cell culture as these usually require specialized coating which is likely compromised in the cleaning process (Kleinman et al., 1987). It remains to be determined if there is a viable option for dish recycling in mammalian cell culture. That aside, basic dish reuse is likely to limit plastic waste in several laboratories but is unlikely to address the major contributor of single-use plastics. Most molecular biology labs rely on single-use pipette tips that are stored in plastic boxes, plastic serological pipettes, and centrifuge tubes. There are anecdotal reports of recycling methods for these consumables but standardization of cleaning methods is currently lacking. Some labs use glass alternatives followed by washing. Accordingly, a recent study showed that substituting plastic pipettes with glass was an effective method of reducing laboratory carbon footprints (Kilcoyne et al., 2022). Glass, however, is more fragile and costly. Our results suggest that reusing plastic dishes is an alternative and effective method to reduce single-use plastic waste. Further, our analyses indicate that implementing these practices in more labs will have a scalable reduction in both plastic waste and costs. An expenditure affiliated with plastic reuse is the time it takes to wash dishes. While we did not include this in our analyses, it is inevitable that sustainable initiatives come with costs to their implementation. Thus, individual laboratories can evaluate the costs and benefits associated with plastic reuse relative to their needs to determine its efficiency.

Plastic waste has contributed 4977 million metric tons of waste to landfills (Geyer et al., 2017), a percentage of which is generated by research laboratories. Efforts to limit plastic waste are needed to avoid continued pollution and natural resource depletion. More studies showing the efficacy of recycling laboratory plastics will be needed to fully address this global issue.

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