

**VIABILITY OF MILKFISH EGGS AND LARVAE AFTER SIMULATED
AND ACTUAL TRANSPORT**

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Abstract

The viability of milkfish eggs and larvae after simulated and actual transport was investigated. Naturally-spawned milkfish eggs were collected and subjected to simulated or actual transport at early cleavage stage (stage 1), blastula (stage 2), gastrula (stage 3), "eyed" (stage 4), or newly-hatched larvae (stage 5). Replicate samples in aerated plastic jars served as controls. Mean hatching and survival rates and the percentage of newly-hatched larvae were significantly affected by the modes of transport and by the stage of embryonic development at transport. Eggs transported at the 'eyed' stage had higher viability compared to those transported at cleavage, blastula, or gastrula stages. There was no significant difference in the mean survival rate of the larvae after 26 days of rearing. However, the percentage of 45 day old larvae with apparent morphological abnormalities was lower in groups transported at stages 4 and 5. These observations indicate that milkfish eggs should be handled and transported during the late embryonic stages to minimize mortalities and the incidence of abnormalities in larvae.

Introduction

Milkfish, *Chanos chanos* Forsskal, is one of the important cultured fish in Southeast Asia. Since milkfish was reported to spontaneously spawn in cages (Marte and Lacanilao, 1986), ponds (Lin, 1985), and tanks (Pjorono, 1988; Emata and Marte., 1993), hatchery-bred fry were made available to fish farmers. In the Philippines, there are anecdotal reports about the high incidence of osteological abnormalities in hatchery-reared milkfish fry. Hilomen-Garcia (in press) recently characterized the morphological abnormalities in hatchery produced milkfish juveniles.

Available literature suggest that osteological abnormalities in hatchery-reared fish may be induced during embryonic and post-embryonic stages by some environmental factors (Houde, 1973; Barahona-Fernandes, 1982; Longwell et al., 1992). Deformities in the embryos and skeleton in some fish has been attributed to aquatic pollution such as in winter flounder (Perry et al., 1991) and perch (Lindesjoo et al., 1994), to mechanical stress during routine hatchery operations (Daoulas et al., 1991; Kitajima et al., 1994), or to nutritional deficiencies (Kanazawa, 1985).

In the Philippines, naturally spawned eggs are presently transported from the broodstock floating net cages to land based hatcheries for rearing to fry or fingerling stage. Fertilized eggs are collected within an hour after spawning and eggs are routinely packed in oxygenated plastic bags during blastula to neurula stage for transport (Garcia and Toledo, 1988; Emata and Marte, 1993).

Milkfish fish farmers often complain about the large numbers of malformed marketable size milkfish they produce from hatchery seeds.

This study was conducted to investigate the viability of milkfish eggs and larvae after simulated and actual transport. Viability was determined based on survival after transport, hatching rate, occurrence of lordosis in newly-hatched larvae, survival of larvae after metamorphosis, and the prevalence of gross morphological abnormalities in fry. Based on the results, recommendations to improve viability of eggs after handling and transport were made.

Materials and Methods

Egg Collection and Handling

Spontaneously spawned milkfish eggs from a floating net cage were collected within 30 min to 1 hr after spawning. Broodstock conditions were as described by Emata and Marte (1993). Spawning eggs were collected following Garcia et al. (1988). Collected eggs were temporarily stocked in a 2x2 1.5 m hapa net cage prior to their use. Moderate aeration was provided to prevent the eggs from clumping.

Simulated and Actual Transport

Viability of milkfish eggs and larvae after handling and transport at different developmental stages was examined. Milkfish eggs were subjected to simulated or actual transport conditions at early cleavage (stage 1), blastula (stage 2), neurula (stage 3), "eyed" stage (stage 4), or at hatching (stage 5).

Eggs or larvae were transferred from the hapa net cage into three replicate plastic bags (10x20 cm) containing 100 ml of ambient sea water at a stocking density of 1,000 eggs/l. Plastic bags were inflated with oxygen at a ratio of 1:2 (water:oxygen). To simulate actual transport conditions, packed plastic bags were placed in a tray secured on top of a laboratory shaker and shaken at 50 rpm for 2 hours (treatment A).

Eggs or larvae were transported to SEAFDEC's Tigbauan hatchery which is about 2-3 hours travel by land and sea (treatment B) following routine procedures as described by Gapasin and Marte (1990). Briefly, eggs were packed in double lined plastic bags containing 5 liters of ambient sea water at a density of 10,000 eggs per liter. For each stage, three replicate samples were separately incubated in a moderately aerated 1 liter plastic jar at a density of 200-300 pcs/l and served as controls (treatment C).

Larval Rearing

To examine the effects of transport at different embryonic stages on the incidence of abnormalities in hatchery bred milkfish fingerlings, larvae hatched from eggs at actual transport conditions were reared separately in three replicate 250-l conical tanks following Gapasin and Marte (1990), with some modifications. Initial stocking density was 30 ind/l. Larvae were initially fed lipid-enriched rotifer at a density of 15 ind/ml from Day 2 to Day 15. Lipid-enriched artemia metanauplii was given to satiation from Day 15 to Day 25. Total harvest was done on Day 26. Samples (25-30) were taken from each tank for individual growth measurements. The remaining larvae from each stage were pooled and further reared to Day 45 in a 1 ton fiberglass tank. The larvae were totally harvested on Day 46 and preserved in 5% buffered formalin for examination of morphological abnormalities.

Telega et al.

Determination of Viability

Twenty ml aliquot samples of transport water (200-300 pcs/sample) were taken from each replicate bags immediately after simulated or actual transport and transferred to 1 liter plastic jars containing ambient sea water and provided with moderate aeration. To determine the survival rate after transport, aeration was stopped 3 hours later for at least 5 minutes and dead eggs were pipetted out individually. Dead eggs of milkfish are opaque and sink at the bottom (Juario et al., 1984). Hatching rate was examined by counting the total number of unhatched eggs and hatched larvae in a jar. The number of normal, dead and moribund larvae, and larvae with lordosis were noted. The prevalence of external morphological abnormalities were also investigated in Day 46 old fry by randomly taking three teaspoonful of aliquot samples from preserved specimens.

Statistical Analysis

A 3x5 factorial in a completely randomized design was used in the experiment. Data were analyzed by SAS statistical program (1988). Means were arc transformed to correct the unequal distribution of sample sizes. Three-way or one-way ANOVA was used to determine the effects of types of transport and stages of embryonic development on the viability of eggs followed by DMRT to compare significant differences between means at $P > 0.01$.

Results

Figures 1, 2, and 3 show the viability of milkfish eggs or larvae after simulated or actual transport at different embryonic stages. There was a significant interaction between the modes of transport and the stage of embryonic development during transport. Mean survival rates, hatching rates, and the percentage of larvae with lordosis at different stages varied significantly with the modes of transport used. Viability of eggs in terms of survival after transport, hatching rate, and prevalence of newly hatched larvae with lordosis in the control group was higher than those subjected to simulated or actual transport. No significant difference in the mean survival and hatching rates of eggs was observed between stages 1-4 in the control group. Milkfish eggs transported at stage 4 had significantly higher survival and hatching rates than stages 1, 2, and 3 in both simulated and actual transport conditions. Survival after transport was relatively lower in newly-hatched larvae (Stage 5) than in eggs (Stages 1, 2, 3 and 4). Newly-hatched larvae with lordosis varied significantly from 10.3% in the 'eyed' stage of control (treatment C) to 60.1% in those transported at gastrula (treatment A).

The percentage of dead or moribund larvae among lordotic newly-hatched larvae progressively decreased in eggs subjected to actual transport (Fig. 4) at stage 1 (65.6%) to stage 4 (30.9%). Gross morphological abnormalities in 45 day old fry were significantly lower in groups transported at stage 4 (19.9%) and stage 5 (9.7%) than other stages tested (Fig. 5).

There was no significant difference in the mean survival rates (6.7-17.2%) of larvae transported at stages 1-5 after 26 days of rearing. However, mean total length of 26 day old larvae was significantly higher in those transported at stages 4 (12.19 mm) and 5 (12.73mm) than stages 1 (11.39 mm), 2 (11.36 mm), and 3 (11.57 mm).

Ambient air temperature and salinity during the handling and transport tests varied from 28.2 C to 29.8 C and 32 ppt, respectively. Dissolved oxygen of water in all plastic bags was above 5.6 ppm after transport.

Tolentino et al.

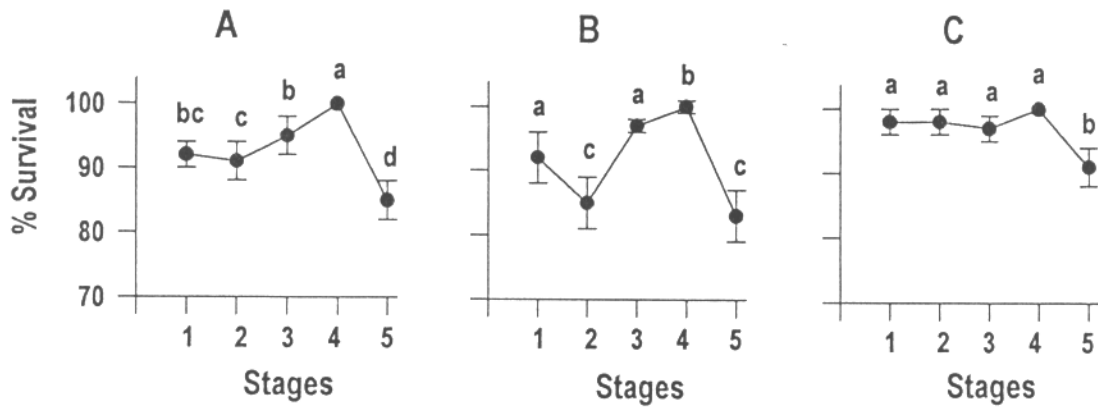


Figure 1. Percentage survival of milkfish eggs or larvae 3 hrs after actual (A) or simulated transport (B). C, control; stage 1, early cleavage; stage 2, blastula; stage 3, gastrula; stage 4, 'eyed'; and stage 5, newly-hatched larvae. Points are means \pm standard deviation of 3 replicates. For each figure, means with the same superscript are not significantly different ($P>0.01$).

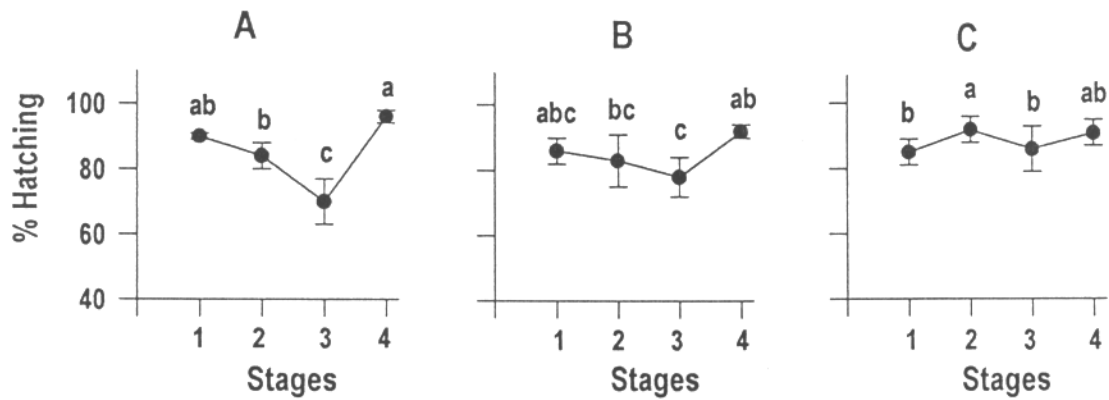


Figure 2. Percentage hatching of milkfish eggs after actual (A) or simulated (B) transport. C, control; stage 1, early cleavage; stage 2, blastula; stage 3, gastrula; and stage 4, 'eyed'. Points are means \pm standard deviation of 3 replicates. For each figure, points with the same superscript are not significantly different ($P>0.01$).

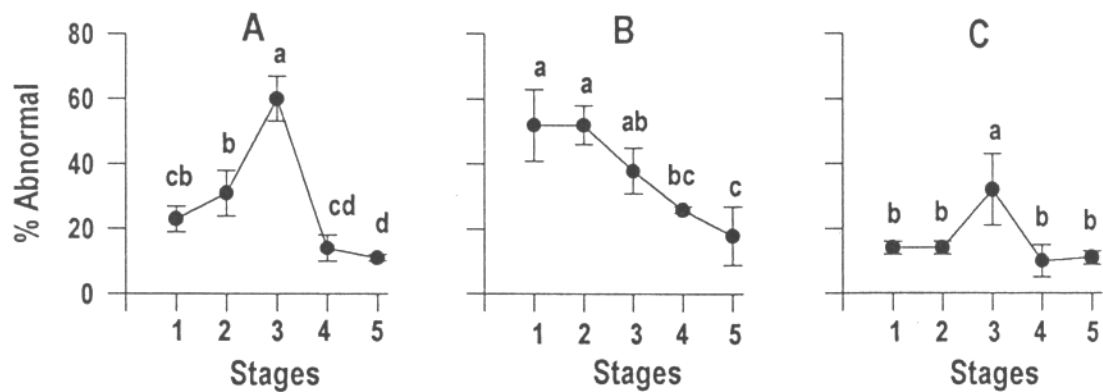


Figure 3. Percentage of newly-hatched larvae with lordosis after exposure of milkfish eggs or larvae to actual (A) or simulated (B) transport. C, control; stage 1, early cleavage; stage 2, blastula; stage 3, gastrula; stage 4, 'eyed'; and stage 5, newly-hatched larvae. Points are means \pm standard deviation of 3 replicates. Means with the same superscript are not significantly different ($P>0.01$).

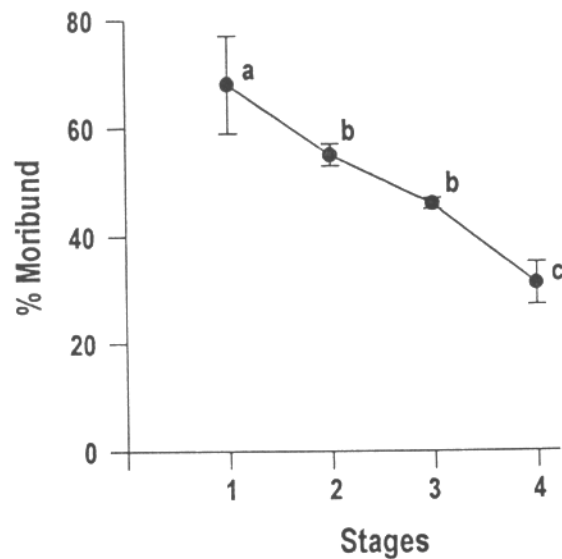


Figure 4. Percentage of moribund and dead larvae among lordotic newly-hatched milkfish larvae after actual transport of eggs. Stage 1, early cleavage; stage 2, blastula, stage 3, gastrula; and stage 4, 'eyed'. Points are means \pm standard deviation of 3 replicates. Means with the same superscript are not significantly different ($P>0.01$).

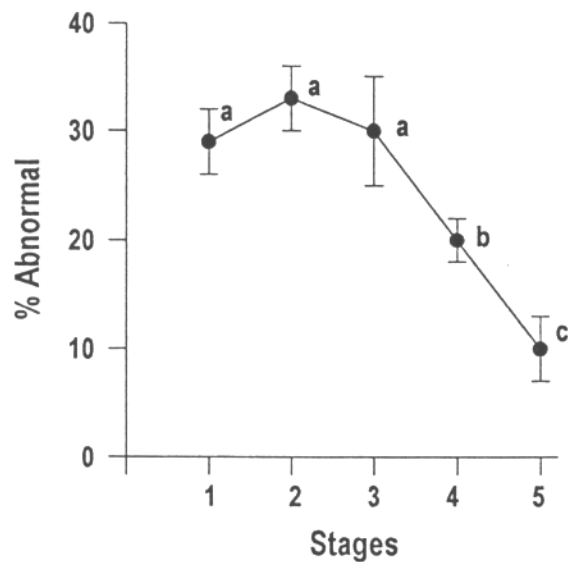


Figure 5. Percentage of 46 day old larvae with gross morphological abnormalities in milkfish eggs or larvae after actual transport. Stage 1, early cleavage; stage 2, blastula; stage 3, gastrula; stage 4, 'eyed'; and stage 4, newly-hatched larvae. Points are means \pm standard deviation of 3 replicates. Means with the same superscript are not significantly different ($P>0.01$).

Discussion

This study demonstrates that handling and transport at different stages of embryonic development affect the viability of milkfish eggs or larvae. Mean survival and hatching rates as well as the percentage of newly-hatched larvae with lordosis were significantly affected by the modes of transport and the stages of development during transport.

Viability was significantly higher in milkfish eggs transported at late embryonic development than those at earlier stages (Figs. 1, 2 and 3). Our present results agree well with those reported in other fish species (Jensen and Alderdice, 1983; von Westernhagen, 1988). Increasing levels of sensitivity to mechanical stress from fertilization to the gastrulation stage was observed in coho salmon by Jensen and Alderdice (1983). Sensitivity declined at the beginning of completion of epiboly until the early eyed stage. Early embryonic stages (before gastrulation) of a variety of freshwater eggs are more vulnerable to aquatic pollutants than those that have completed epiboly (von Westernhagen, 1988). In this study, mean survival and hatching rates were significantly lower in eggs transported at the cleavage, blastula, and gastrula stages compared to those at the eyed stage. A significantly higher incidence of newly-hatched larvae with lordosis and higher number of dead or moribund hatchlings was similarly observed in stages 1, 2, and 3 than stages 4 and 5.

Lordosis in newly-hatched larvae were also observed in the control group. Fertilized eggs in this study were collected from the floating net cage during two to four cell stages which were considered to be sensitive to mechanical stress in coho salmon (Jensen and Alderdice, 1983). It is not clear in our present results whether the presence of aberrant newly-hatched larvae in the control groups was caused by mechanical stress during egg collection or by other factors.

In most cases, survival of milkfish eggs was significantly higher than newly-hatched larvae. Fine mesh scoop nets were used in this study during handling and transfer of eggs or larvae. While newly-hatched larvae were left naked during abrupt dehydration during handling and packing, milkfish embryos were protected with an outside covering (chorion) and a small perivitelline membrane that covers the embryo (Juario et al., 1984). Fertilized milkfish eggs exposed in air for about 3 min in a wet fine scoop net were observed to develop normally until hatching (JD Toledo, personal observations).

Although no significant difference in survival was observed in larvae transported at different embryonic stages after 26 days of rearing, total length of the larvae transported at stages 1, 2, and 3 was significantly shorter than those transported at stages 4 and 5. The incidence of gross morphological abnormalities in Day 46 larvae was also significantly higher in groups transported before the eyed stage (Fig. 5). Almost all the abnormalities were observed in the head region with high prevalence in the operculum. Hilomen-Garcia (in press) similarly observed that milkfish with opercular and branchiostegal abnormalities had slow growth and had high mortality rate after handling and transfer. Cell damage due to transport stress may have been lethal to embryos at earlier stages as reflected by the significantly high incidence of dead and moribund larvae (Fig. 4). Sublethal damage incurred at these stages may have been repaired as the larvae developed and was discernable only at the juvenile stage.

Based on the results, fertilized milkfish eggs should be transported at least past the gastrulation phase, preferably at more advanced developmental stages to minimize mortalities and lower the incidence of abnormal larvae in the hatchery. At ambient temperature and salinity, it takes about 14-16 hours after fertilization to reach the C-shaped embryo with formed optic vesicles which was shown in this study to be the best stage for transport resulting in highest viability. Fertilized milkfish eggs are sometimes required by hatcheries several hours away and eggs transported at late embryonic stage may hatch along the way if transport water temperature is not lowered. In this case, newly-hatched larvae should be transported at far distances instead of eggs.

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Toledo et al.

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Trich et al.

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57

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Tolack et al.