SUMMARY

STUDY ON PREVENTION OF SINKING DEATH IN THE FINGERLING PRODUCTION OF PACIFIC BLUEFIN TUNA

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General introduction

Bluefin tuna aquaculture has been developed an economically important industry. Consequently, excessive demand of wild-caught tuna for seedling fish of the tuna aquaculture has resulted in over-fishing. Therefore, development of full-cycle bluefin tuna aquaculture methodology, which does not rely on natural resources, is necessary for sustainable tuna aquaculture.

Recent attempts at developing Bluefin tuna fingerling production technology have been made in many countries. In Pacific bluefin tuna (PBT); however, the survival in fingerling production is still low. Although, the poor PBT survival is due to various cause, mass mortality by both larval surface death and sinking death during the early larval stage have been considered to be seriously affects PBT aquaculture operations. Especially, larval sinking death has been identified as a particularly serious problem causing mass mortality of PBT larviculture.

Swimbladder inflation plays an important role in controlling larval body density and their buoyancy. On the other hand, PBT larval body density is greater than that of rearing sea water, even when larvae possess an inflated swimbladder, and is believed to be the primary cause of sinking death particularly during the night-time on ceasing swimming (Takashi et al. 2006). Therefore, larval swimbladder inflation and flow control of rearing water during the nighttime to suspend larvae within the rearing water column have the possibility to improve larval sinking death in PBT larviculture. However, the definite relationships between swimbladder inflation failure and survival have not yet been investigated in PBT not to mention the effective promotional method of initial swimbladder inflation (ISI). In addition, the flow control in rearing tanks has also not yet been examined in detail its enhancement effect of survival in larviculture. Furthermore, regarding the swimbladder, its inflation failure induces poor growth and vertebral deformity, and it often negatively affects the production efficiency in other aquaculture fish species. However, the definite relationships between swimbladder inflation failure and growth, vertebral deformity have not yet been elucidated in PBT.

The purpose of this study is to improve PBT fingerling production technology. Firstly, in Chapter 1, the effects of different air supply rate during the nighttime on larval survival and water circulation of rearing tank were evaluated, and larval sinking velocity was also determined to improve larval mortality due to sinking death.In Chapter 2, the influence of ISI failure on larval vertical distribution and survival were investigated. In Chapter 3, the promotional and inhibitory conditions of water surface for ISI and the optimal timing for ISI promotion were investigated to develop the suitable ISI promotion method; additionally, the relationship between ISI promotion and occurrence of surface death was also investigated. In Chapter 4, influence of swimbladder inflation failure on mortality, growth and lordotic deformity in postflexion larvae and juveniles were investigated to obtain the information for the improvement of fingerling production technology in PBT.

Chapter 1: Flow control by aeration to prevent sinking death in PBT larvae

This study evaluated the effect of flow control by aeration in the nighttime on larval survival, and examined sinking velocity of PBT larvae. The experiment was held in 500 *l* tanks, in which larval survival was compared among air supply rate of 0, 300 and 900 *ml*/min during the night. Larval sinking velocity was also measured in the larvae with (WIS) and without inflated swimbladder (WOIS) at night. In addition, the flow field in a 500 *l* tank was measured at air supply rate of 300 and 900 *ml*/min, and estimated danger zone for larval sinking (EDZ), where the upward flow velocity was less than the larval sinking velocity, was calculated as the area within the cross-section of the tank to assess the correlation between larval survival and water circulation patterns in the tank.

Larval survival increased with increasing air supply rate, and it showed highest at air supply rates >900 *ml*/min. Moreover, larval sinking velocities were significantly higher in WOIS than in WIS from 5 days-post-hatch (dph). Water circulation speed increased, and the size of the EDZ reduced at higher air supply rates. These results suggest that higher air supply rate increase larval survival by generation of counteracting upward flow to larval sinking.

Chapter 2: Influence of ISI failure on survival and vertical distribution in PBT larvae

This study investigated the influence of ISI failure on survival and vertical distribution in PBT larvae to prevent mass mortality due to sinking death.

In Experiment 1, swimbladder inflation frequency and survival within an ISI promoted (PS) group, for which surface film of rearing water was removed, and a group without ISI promotion (NPS) were compared in 20 and 30 *kl* tanks. The PS group demonstrated significantly higher swimbladder inflation frequency and increased survival than the NPS group within 20 *kl* tanks at 9 dph. Similar tendencies were observed within 30 *kl* tanks.

In Experiment 2, larval vertical distribution and swimbladder inflation frequency in the nighttime were examined through larval sampling within the upper and middle layers of the water column and tank bottom within 1.6 and 30 *kl* tanks at 5 dph. Larvae at tank bottom had higher distributional density and significantly lower swimbladder inflation frequency than those distributed in the upper and middle layers within 1.6 *kl* tanks. Similar tendencies were observed within 30 *kl* tanks.

Results of this study indicate larval ISI promotion improve larval survival via prevention of sinking death in mass-scale PBT larviculture.

Chapter 3: Promotion of ISI in PBT larvae

3.1 Conditions of water surface and optimal period to promote ISI in PBT larvae

In this study, ISI promotion of PBT larvae was studied by following three experiments to improve the larval survival.

Experiment 1 was conducted to explore promotion and inhibition of ISI under different water surface conditions; including the use of surface skimmer to remove autogenous surface film (SS), covering the water surface with liquid-paraffin-layer (LP) and oil film (OF), and a control (non-treatment, NT). Significantly higher swimbladder inflation frequency was observed in SS (62.2%) than NT (11.9%), LP (2.7%) and OF

(3.9%). This indicates that ISI in PBT larvae can be promoted by removal of surface substances on rearing water (surface film) which inhibit larval air gulping at water surface.

Experiment 2 aimed to elucidate proper day of larval age (days-post-hatch: dph) to start skimming to promote ISI with the following four periods differing by the commencement dphs of removing the artificially formed surface oil film using surface skimmer : from 3 to 8 (SF3D), 4 to 8 (SF4D),5 to 8 (SF5D), 6 to 8 (SF6D) dph. Significant improvement in swimbladder inflation frequency was observed in SF3D (80.2%); however, the frequency was very poor in SF4D, SF5D, and SF6D (17.8–7.5%) at the end of experiment (8 dph).

Experiment 3 aimed to elucidate the optimal period to promote ISI with the following four different periods of removing the artificially formed surface oil film using surface skimmer: 1 day of 3 dph (SF3 group), from 3 to 4 dph (SF3–4 group), from 3 to 5 dph (SF3–5 group) and from 3 to 8 dph (SF3–8 group). Skimming in 1 day of 3 dph (SF3: 57.0 ± 14.6%) gave the similar promotion effect of ISI to skimming on 3 dph and later (SF3–4: 62.0 ± 9.1%, SF3–5: 61.8 ± 4.9%, SF3–8: 71.0 ± 13.5%) without significant difference in swimbladder inflation frequency at the end of experiment (8 dph). These results indicate the need of surface film removal (SFR) without missing a narrow window, 1 day of 3 dph, to promote ISI effectively in practical PBT larviculture.

On the other hand, the removing surface film caused highest incidences of surface death on 3 dph, and this dph with highest incidence of surface death corresponded with the optimal period to promote ISI on 3 dph in Experiment 2. This result indicates that the promotion of ISI and the prevention of surface death conflict with each other on 3 dph.

3.2 Optimal timing in the day to promote ISI in PBT larvae

This study investigated the optimal timing of day to promote ISI for improved PBT larval survival.

Larval swimbladder inflation frequency was compared based on three experiments using different time schemes of SFR. SFR was conducted from 05:00 to 19:00 (light period: S.5–19), 19:00 to 05:00 (dark period: S.19–5), 08:00 to 19:00 (S.8–19) and the entire day (S.24) in Experiment 1; from 08:00 to19:00 (S.8–19-E2), 08:00 to 13:00 (S.8–13), 13:00 to 19:00 (S.13–19) in Experiment 2; and from 13:00 to 16:00 (S.13–16), 16:00 to 19:00 (S.16–19), 18:00–19:00 (S.18–19) in Experiment 3.

The swimbladder inflation frequency at the end of experiment (9 dph) was significantly higher ($P < 0.001$) in S.24 (91.1 ± 5.7%), S.5–19 (92.2 ± 5.1%) and S.8–19 (93.3 ± 3.4%) than in S.19–5 (11.1 ± 5.1%) in Experiment1, and remarkably higher in S.8–19-E2 (81.7%) and S.13–19 (88.3%) than in S.8–13 (0.0%) in Experiment 2, and significantly higher ($P < 0.001$) in S.16–19 (84.4 ± 5.1%) and S.18–19 (70.0 ± 12.0%) than in S.13–16 (7.8 ± 3.9%) in Experiment 3. These results indicate the SFR during light period is effective; while that during the dark period had no effect to promote larval ISI, moreover, indicate that the optimal timing of the day to promote ISI by SFR is a few hours before the end of light period (16:00–19:00).

Additionally, in S.24 of Experiment 1, incidence of surface death was highest at 18:00 during 24 hours on 3 dph. This time with highest incidence of surface death also corresponded to the optimal timing of the day to promote ISI as well as the corresponding between the dph with highest incidence of surface death and the optimal period to promote ISI on 3 dph observed in Section 3.1 in Chapter 3.

Chapter 4: Influence of swimbladder inflation failure on mortality, growth and development of lordotic deformity in PBT postflexion larvae and juveniles

This study examined the influence of swimbladder inflation failure on mortality (Experiment 1), lordotic deformity (Experiment 2), and growth (Experiment 1, 2) in PBT postflexion larvae and juveniles by generation the cohort failed swimbladder inflation.

In Experiment 1, rearing experiment was conducted for postflexion larvae to juveniles (from 18 to 30 dph). Mortality was not significantly different between the fish with (WIS) and without (WOIS) inflated swimbladders. Standard length (SL) and body weight (BW) were significantly smaller in WOIS than WIS. Moreover, the swimbladder inflation was found in WOIS after postflexion stage. This inflation considered to be due to the so-called late or secondary inflation of the swimbladder independent of air gulping for ISI.

In Experiment 2, two examination trials were conducted on swimbladder inflation, vertebral deformity and growth for juvenile stage. In both trials, lordotic deformities were found neither in WOIS nor in WIS. Although SL and BW were significantly smaller in WOIS than WIS at 22 dph, no significant differences in SL and BW were found between them after 37 dph.

The results of Experiment 1 and 2 indicate that swimbladder inflation failure in PBT causes growth retardation until juveniles of 30 dph as well as other aquaculture fish species; however, it cause neither significant levels of mortality after the transitional period from postflexion to the juvenile stage nor growth retardation in juveniles after 37 dph and lordotic deformities in juvenile differently from various other aquaculture fish species.

General discussion

1. Achievements in this study

Chapter 1 demonstrated that increased air supply rate to rearing water during the nighttime mitigate larval mortality due to sinking death in PBT larviculture.Chapter 2 demonstrated that larval ISI failure reduces survival via larval sinking to tank bottom in PBT larviculture in mass-scale tanks. Therefore, larval mortality due to sinking death should be improved by mitigation of larval sinking via both promotion of larval ISI and flow control of rearing water during the nighttime in combination. Moreover, Chapter 3 elucidated that PBT larval ISI can effectively promote by SFR, and that the optimal period and timing of the day to effectively promote ISI is extremely finite term of twilight just before the end of light period on 3 dph.Therefore, to promote ISI effectively, SFR should be done without missing extremely finite term of a few hours before the end of light period on 3 dph in PBT larviculture. Additionally, Chapter 4 elucidated that swimbladder inflation failure cause neither significant levels of mortality after the transitional period from postflexion to the juvenile stage nor growth retardation in juveniles after 37 dph and lordotic deformities in juvenile differently from various other aquaculture fish species reported, although, swimbladder inflation failure causes growth retardation from early larval to early juvenile stage in PBT.

2. Proposal to improve PBT fingerling production efficiency and research for the future

2-1. Prevention of larval sinking death in PBT larviculture

Flow control of rearing water by higher air supply rate during the nighttime improved larval survival due to sinking death via generation the counteracting upward flow to larval sinking in PBT larviculture. Moreover, promotion of larval ISI improved the survival via mitigation of larval sinking to rearing tank bottom in mass production tanks, even when flow field was controlled by aeration to prevent larval sinking. Therefore, the author proposes that larval survival due to sinking death should be improved by mitigation of larval sinking using measure of both flow control of rearing water and promotion of larval ISI in combination in mass-scale PBT larviculture.

2-2. Promotion of ISI in PBT larviculture

The ISI in PBT larvae can be promoted effectively by SFR using cleaning devise such as surface skimmer. Moreover, in PBT larviculture, the ‘window’, when effective promotion of ISI is possible, was extremely narrow 1 day of 3 dph compared with other reported fish species. Furthermore, the optimal timing of the day to promote ISI was extremely finite term of a few hours before the end of light period, and SFR during the dark period and the time from morning to early afternoon produced no effect of ISI promotion. Therefore, SFR should be done without missing this extremely finite term of a few hours before the end of light period on 3 dph to promote ISI effectively in PBT larviculture.

2-3. Future Research on influence of swimbladder inflation failure, flow control and tank design to improve larval survival

Although, larval sinking and the prevention measure by flow control have been reported in other aquaculture fish species with sinking death, the relationship between larval swimbladder inflation and larval survival, vertical distribution within rearing tanks has not yet been investigated. Therefore, study on these topics should be performed to improve larval survival in these fish species other than PBT.

It is suggested that tanks with high AR possess a greater capacity to prevent sinking death (Sumida et al. 2011). Moreover, in this study, PBT larval survival in small experimental tanks with high AR tended to be higher than that in mass-scale tanks with low AR. Therefore, suitable rearing tank design on the AR and the shape to mitigate larval sinking should be investigated to improve mortality due to sinking death. Moreover, the suitable flow control method for the rearing tanks seems to be different with tank AR and shape; therefore, it might have to be examined in each tank AR and shape. The outcomes of these researches would contribute to improve the mass-production technique of other aquaculture fish species with larval sinking death in hatchery.

2-4. The relationship between the promotion of ISI and occurrence of surface death in PBT larviculture

In this study, both the optimal period and timing of the day to promote ISI corresponded with the time of highest incidence of surface death on the extremely finite term of a few hours before the end of light period on 3 dph in PBT larviculture. Moreover, PBT larval swim up behavior was observed more frequently within a few hours before the end of light period on 3 dph than other times and dphs. These behaviors are considered for air gulping at water surface for ISI, and to trigger their surface death. Consequently, larval surface death will not avoid in ISI promotion by existing SFR using surface skimmer.

Therefore, further study should be performed on the surface condition of rearing water and the method in which both ISI promotion and surface death prevention can be achieved, or on the effective prevention method of surface death other than making oil film, although, the minimized operation of SFR within the optimal timing for ISI promotion during the few hours before the end of light period on 3 dph is only solution to mitigate surface death at present.

2-5. Influence of swimbladder inflation failure on mortality, growth and lordotic deformity in postflexion larvae and juveniles PBT

In this study, swimbladder inflation failure cause neither significant levels of mortality after the transitional period from postflexion larvae to the juvenile stage nor growth retardation in juveniles after 37 dph and lordotic deformity in PBT juveniles differently from other reported fish species. However, swimbladder inflation failure cause mortality due to sinking death in PBT early larval stage and growth retardation from early larval to early juvenile stage, therefore swimbladder inflation should be promoted in PBT fingerling production.