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The effect of a 24-hour photoperiod on the survival, growth and swim bladder inflation of pre-flexion yellowfin tuna (*Thunnus albacares*) larvae.

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Abstract

The effects of two different continuous photoperiod regimes on survival, growth and swim bladder inflation of pre-flexion yellowfin tuna (*Thunnus albacares*) larvae were investigated. Each photoperiod regime was tested twice with a different larval cohort to confirm the observed results. Trials 1 and 2 tested the effect of a reduced night-time light intensity (10 $\mu moles m^{-2} sec^{-1}$ = 30% of the daytime intensity) and found that those larvae reared for 8 days under the 24 hour lighting (24-L) photoperiod exhibited a slight improvement in survival compared to those reared under the control photoperiod of 12 hours light (12-L); however these improvements were not significant. In addition, those larvae reared under this photoperiod regime were equal in length to those in the control. Trials 3 and 4 compared the same variables in larvae reared under a continuous photoperiod (24-L) with a constant light intensity of 30 $\mu moles m^{-2} sec^{-1}$, against those reared under the aforementioned 12-L photoperiod. Survival of larvae under the continuous photoperiods were 9 ± 1% (n = 2) and 10 ± 2% (n = 3) for Trials 3 & 4, respectively, compared to less than 1% in both control treatments; differences that in both cases were highly significant. In addition, in both trials larvae cultured under the 24-L photoperiod were significantly larger and exhibited more advanced development than those reared under the 12-L photoperiod, however swim bladder inflation was significantly lower. We suggest that the improved survival and growth achieved under a continuous photoperiod is due to the extended foraging time combined with the prevention of mortality caused by night-time sinking.

Keywords: early larval rearing; early mortality; hatchery; buoyancy; swim bladder inflation.

1. Introduction

The global demand and subsequent production of tuna has increased exponentially over the last 50 years (Miyake et al., 2004). Approximately 32,000 MT of tuna marketed worldwide are now ranched; a process that involves the capture and fattening of wild caught juveniles or sub-adults (Lioka et al., 2000; Farwell, 2001). The consequent pressure on wild stocks is now threatening tuna fisheries worldwide, with some populations considered to be at the limits of sustainability, or overfished (Williams, 2007; Safina and Klinger, 2008; FAO, 2009). The total yield of tuna able to be produced via ranching is also limited via the quota restrictions placed on wild caught fish. In order to alleviate the pressures on wild tuna stocks; to overcome the constraints associated with ranching and to satisfy the growing demand for tuna, hatchery techniques for tuna must be developed. Major progress has recently been reported regarding reproduction, larval rearing and fingerling production of Pacific bluefin tuna (*Thunnus orientalis*) in Japan (Normile, 2009). In addition, progress has been reported regarding spawning of wild bluefin tuna broodstock as well as the rearing of their larvae in Australia and Europe (Mylonas et al., 2007; Hutchinson, 2009). Despite research efforts on tuna hatchery production for three decades in many countries, commercial scale production is yet to become reality, with survival rates of <0.5% to weaned juveniles typical for most tuna species (Sawada et al., 2005; Margulies et al., 2007).

The first feeding stage of all marine fish larvae is one of the most critical and high mortality is often experienced during this stage. In their review of the constraints to tuna larval rearing, Sawada et al. (2005) listed mortality during the first 10 days as one of the major limiting factors to mass culture and recommended focused research on this issue as a high priority.

Mortality of first feeding marine fish larvae has been attributed to factors including a poor transition from lecithotrophy to exotrophy (Fyhn, 1989); an inappropriate size and/or nutritional composition of feeds (Koven et al., 1992) or inappropriate environmental conditions (Takashi et al., 2006). Key environmental parameters to be considered for effective larval rearing include temperature and salinity (Fielder et al., 2005), the microbial environment (Harboe et al., 1994) and factors including turbulence, light intensity, light quality and photoperiod (Boeuf and Le Bail, 1999; Utne-Palm and Stiansen, 2002). Optimising photoperiod, for example, has been demonstrated to improve the performance of many species of marine fish larvae (Tandler and Helps, 1985; Duray and Kohno, 1988; Trotter et al., 2003); however, there appear to be no such published studies on tuna larvae. Lighting has, however, been shown to be of major importance in preventing high night-time mortalities caused by poor scotopic vision in early juvenile (30 DPH, 5 cm total length) Pacific bluefin tuna (Ishibashi et al., 2009).

These trials were conducted to determine if a continuous photoperiod would benefit the survival and growth of early stage yellowfin tuna (*Thunnus albacares*) larvae. In order to ensure the results of the experiment were not confounded by the physiologically

stressful event of flexion, the trial was conducted on pre-flexion larvae. Two sets of trials were conducted. The first investigated a continuous photoperiod, but with a reduced intensity during the 12 hour night phase, whilst the second set investigated a continuous photoperiod with equal light intensity during both night and day phases. To confirm the observed results, each trial was conducted twice with separate larval cohorts.

2. Materials and Methods

Larvae for each trial were sourced from a single spawning of eggs from naturally spawning yellowfin tuna broodstock at the Inter-American Tropical Tuna Commission's (IATTC) Achotines Laboratory in Los Santos Province, Republic of Panama. For details on the broodstock system and the management of these fish, refer to Wexler et al. (2003). Harvested eggs were incubated in 280 litre conical bottom tanks and hatching occurred after ca. 24 hours. Consistent with other published data on yellowfin tuna development, the day of hatching in this paper is referred to as Day 1. On Day 2, pre-feeding yolk-sac larvae were randomly stocked into 1000-litre, cylindrical, flat-bottom tanks (1 m diameter and 1 meter deep) with a number of replicated tanks per treatment (see Table 1). Larvae (2.47 ± 0.05 mm notochord length; NL) were stocked at a density of 15 larvae litre^{-1} and reared using the standard larval rearing protocol employed by the IATTC (Margulies et al., 2007). The photoperiod regimes tested in each trial are outlined in Table 1. All control tanks operated under the standard photoperiod of 12 hours per day (6 am to 6 pm), while those in the treatment tanks received light for 24 hours per day. Treatment tanks were screened from control tanks to

ensure no incident light fell on the control tanks during their dark phase. Each tank was illuminated with 4 x 40 W fluorescent lamps (General Electric, Daylight F40D-EX), suspended 300 mm from the water surface. Surface light intensity was 30 $\mu moles m^{-2} sec^{-1}$ (Li-Cor, LI250) (ca. 2,300 lux). The reduced light intensities employed during the night phase in the treatment tanks of Trials 1 & 2 were achieved by covering the lights with shade cloth, which reduced the light intensity to 10 $\mu moles m^{-2} sec^{-1}$ (ca. 760 lux). Aeration in all tanks was provided by four, fine-bubble diffusers equally spaced across the tank base. The rate of aeration was set to achieve the optimum microturbulence for early feeding yellowfin tuna larvae ($1 - 2.4 \times 10^{-8}$ $m^{-2} sec^{-3}$ as an energy dissipation rate) by setting the mean horizontal velocity of a surface drogue to 5-7 cm sec^{-1} (Kimura et al., 2004). Water temperature, dissolved oxygen, pH and salinity were measured in each tank three times per day.

Rotifers enriched on Algamac 3050 (Aquafauna Bio-Marine, Hawthorne, California) were maintained at a density of 5 mL^{-1} in all tanks throughout the trials. All tanks (including controls) were sampled every 6 hours and enriched rotifers added as necessary to maintain this density. Water flowed through the tanks via a 200 µm screen at the turn-over rate of 200% day^{-1}. At this larval stocking density and flow rate, the removal of rotifers from the tank was dominated by flushing rather than ingestion and residual rotifer numbers were subsequently similar in all tanks at 1-2 mL^{-1} prior to the next feed. Sufficient *Nannochloropsis oculata* was added by continuous siphon to maintain a cell density between 0.5 and 1.0×10^6 cells mL^{-1}.

Trials 1 and 2 were terminated on Day 8 and Trials 3 and 4 on Day 9. At the conclusion of each trial, all tanks were drained and all remaining larvae counted. Twenty larvae from each tank were sampled. Notochord length was measured to 0.05 mm under a stereo microscope using a calibrated eye piece graticule and the presence or absence of a swim bladder was noted. Flexion was deemed to be commencing if signs of a developing caudal fin were evident under the notochord.

Survival, notochord length, percentage incidence of swim bladder inflation, percentage incidence of the commencement of flexion and the average daily values of each measured water quality parameter were compared between treatments with one-way analyses of variance. Percentage data (survival, swim bladder inflation and pre-flexion rates) were arcsine transformed prior to analysis. All reported errors are standard errors around the mean values and statements of significance refer to the 0.05 level, unless otherwise stated.

3. Results

In each trial, there were no significant differences in any of the measured water quality parameters between the control and photoperiod treatments (Table 2).

In Trials 1 and 2, larvae reared under continuous photoperiods displayed survival rates that were slightly higher than those of their respective controls (Table 3) , however in both cases these improvements were not significant ($P>0.05$). Those reared in Trial 1 achieved $2.3 \pm 0.6\%$ survival in the treatments and $1.0 \pm 0.6\%$ in the controls, whilst in

Trial 2 survival was 0.2 ± 0.1% and 0.1 ± 0.1% in the treatments and controls, respectively. Final notochord length of larvae in both experiments was equal between treatments and controls (4.59 ± 0.05 and 4.59 ± 0.16 mm, respectively for Trial 1 and 4.00 ± 0.27 and 3.97 ± 0.07 mm, respectively for Trial 2) (Table 3). No measurements of the onset of flexion or swim bladder inflation were made during these two trials.

Increasing the night-time light intensity to match that of the daytime intensity in Trials 3 and 4, resulted in significant improvements in larval survival. Larvae in the treatment tanks of Trial 3 had a survival of 8.9 ± 0.8% compared with 1.0 ± 0.3% in the control (Table 3); a difference that was highly significant (P = 0.01). Similarly, survival of larvae reared under the continuous photoperiod in Trial 4 was 10.4 ± 1.9%, whilst the mean survival in the control treatment of this experiment averaged 0.2 ± 0.1%; a difference that was again highly significant (P =0.005)(Table 3). In contrast to Trials 1 and 2, larvae reared under the continuous photoperiod of Trials 3 and 4 also experienced significantly faster growth. Larvae cultured in the 24-L treatment from Trial 3 had a mean final notochord length of 5.1 ± 0.1 mm compared with 4.2 ± 0.1 mm in the control (P = 0.03) (Table 3). In Trial 4, treatment larvae averaged 4.5 ± 0.2 mm, compared with 3.9 ± 0.1 mm in the control (P = 0.03)(Table 3). Coincident with improved growth, larvae reared under continuous light were developmentally more advanced. In Trial 3, 90 ± 0% of those larvae reared under continuous light had commenced flexion by Day 9, compared with only 5 ± 5% in the control tanks (P=0.0006). Similarly, in Trial 4, 90 ± 3% of larvae in the treatment tanks had commenced flexion by Day 9 compared with 52 ± 7% in the controls (P=0.006).

The continuous photoperiod employed in Trial 4 had a significant detrimental impact on swim bladder inflation (P=0.001). On Day 9, 10 ± 0% of larvae in the treatment tanks had inflated swim bladders, compared with 50 ± 5% in the controls.

4. Discussion

The results of this study demonstrate that providing continuous light with an intensity of 30 µmoles$m^{-2}sec^{-1}$ significantly improves both survival and growth of yellowfin tuna larvae. During food selectivity studies, Margulies et al. (2007) reported that larval yellowfin tuna feed only during daylight hours, confirming that like most species they are visual predators (Blaxter, 1986). We therefore attribute the improved growth and advanced development of larvae reared under continuous light in Trials 3 and 4 to a longer foraging time and subsequently greater food intake. Larvae reared under the continuous photoperiod regimes of Trials 1 & 2 showed no improvement in growth, suggesting that either the reduced night-time light of 10 µmoles$m^{-2}sec^{-1}$ was below the threshold of light required for yellowfin tuna larvae to see and capture their prey or that the level of ingestion of prey at this light level was insufficient to offset any prolonged activity of the larvae at this light intensity.

The positive correlation between larval growth and continuous light and feeding appears to be common amongst many species of marine fish, including sole (*Solea solea*) (Fuchs, 1978), gilthead seabream (*Sparus auratus*) (Tandler and Helps, 1985), rabbitfish (*Siganus guttatus*) (Duray and Kohno, 1988), barramundi (*Lates calcarifer*) (Barlow et al., 1995), greenback flounder (*Rhombosolea tapirina*) (Hart et al., 1996),

Australian snapper (*Pagrus auratus*) (Fielder et al., 2002) and Atlantic cod (*Gadus morhua*) (Puvanendran and Brown, 2002). Downing and Litvak (2000) reported no benefit to haddock (*Melanogrammus aeglefinus)* larvae reared under continuous light, however their control photoperiod of 15L:9D was considerably longer than most other studies. There appears to be only one study in which an extended photoperiod was detrimental to the growth of a marine fish larvae, the sea bream (*Archosargus rhomboidalis*) (Dowd and Houde, 1980).

Despite the consistent, positive relationship between photoperiod and larval growth described above for a wide range of species, the effect on survival is more equivocal. The majority of studies report no difference in survival between natural and extended photoperiods (Fuchs, 1978; Barlow et al., 1995; Hart et al., 1996; Fielder et al., 2002; Moustakas et al., 2004); however, a 24 hour photoperiod had a detrimental effect on the survival of European seabass *Dicentrachus labrax* (Barahona-Fernandes, 1979; Ronzani Cerqueira and Chatain, 1991). Tandler and Helps (1985), Duray and Kohno (1988) and Trotter et al. (2003) all reported significantly higher survival under continuous light for gilthead seabream, rabbitfish and striped trumpeter, respectively, although the gains in survival were not as great as those reported here for yellowfin tuna larvae.

The improved survival obtained in the 24 hour light treatments with continuous intensity in this study may be attributable to a greater number of larvae successfully commencing feeding. Fielder et al. (2002) pointed out that prey capture is a learned response, and extended photoperiods may therefore provide a greater opportunity for first feeding larvae to learn this vital lesson.

Alternatively, continuous lighting may have improved survival by preventing negatively buoyant larvae from sinking to the bottom of the tank at night. Takashi et al. (2006) pointed out that a major cause of mortality of early stage Pacific bluefin tuna larvae is their tendency to sink to the tank bottom at night and showed that the density of larvae increases with increasing age. Between Days 4 and 8 these larvae are denser than seawater, even with an inflated swim bladder, and at night when swimming ceases they sink to the bottom of the tank and die. From Day 9, swim bladder volume of Pacific bluefin tuna larvae greatly increases, which assists in keeping them suspended at night. In addition, once the process of flexion is complete, the larvae become more efficient swimmers and perhaps more adept at swimming away from the tank bottom. Regardless of the mode, mortality due to sinking becomes less problematic from Day 8-9 (Takashi et al., 2006). We believe that yellowfin tuna are similar to Pacific bluefin in this regard, as mortalities are commonly seen on the tank bottom in the early morning when reared under the standard photoperiod.

The results of this study demonstrate that the mode of action for the improved survival of larval tuna from the provision of 24 hour light is different to that for juvenile tuna. Ishibashi et al (2009) reported that the improved survival obtained in juvenile Pacific bluefin tuna in the 23 days post transfer from tanks to seacages was due to the prevention of collisions with the walls of the net pen. Due to the inability of early larvae to burst swim, lethal impact collisions with tank walls is not believed to be a cause of larval mortality.

A continuous photoperiod had a negative impact on swim bladder inflation in the current study. As discussed by Trotter et al. (2003), many physostomic fish larvae inflate their swim bladder at the water surface in darkness and continuous lighting can therefore inhibit this process. This appears to be the case with yellowfin tuna larvae, however swim bladder inflation in the control treatment was also relatively poor. Munday et al. (2003) pointed out that it is common practice to place a layer of oil on the surface of bluefin tuna larval rearing tanks during the first 7 days to prevent larvae getting trapped in the surface tension. This suggests it may be possible for tuna larvae to inflate their swim bladder after this time. It may therefore be necessary to shift from a 24 hour photoperiod, to one with at least some darkness once the larvae have passed the negatively buoyant stage in order to allow swim bladder inflation to occur.

Although Takashi et al. (2006) pointed out that photoperiod manipulation may be effective in preventing mortality of tuna larvae due to sinking, this appears to be the first study to have investigated the effects of a continuous photoperiod on the larvae of any tuna species. Previous trials conducted by the IATTC at the Achotines Laboratory have indicated variable patterns of larval growth and survival in response to extended photoperiods (D. Margulies, pers. comm.). Given the highly significant benefits to growth and survival reported here using a 24 hour period, further detailed investigations into the use of extended photoperiods for tuna hatchery production are clearly warranted. Additional research should confirm the mode of action of the improved survival and whether continuing a 24 photoperiod beyond the pre-flexion stage remains beneficial. More data are required to determine the window of opportunity for swim

bladder inflation and to optimise photoperiod strategies to ensure the process of swim bladder inflation is not compromised.

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Table 1. Photoperiod and light intensity investigated in each trial.

Trial	Photoperiod (hours)	Day Phase Intensity (μmoles $m^{-2}sec^{-1}$)	Night Phase Intensity (μmoles $m^{-2}sec^{-1}$)	*n*
1	24-L	30	10	3
	12-L	30	0	
2	24-L	30	10	3
	12-L	30	0	
3	24-L	30	30	2
	12-L	30	0	
4	24-L	30	30	3
	12-L	30	0	

Table 2. Average daily water quality parameters (± S.E.) measured in each trial.

Trial	Photoperiod (hours)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Salinity (g/L)
1	24-L	28.38 ± 0.69	6.25 ± 0.01	8.20 ± 0.05	31.36 ± 0.01
	12-L	27.73 ± 0.02	6.28 ± 0.01	8.25 ± 0.05	31.35 ± 0.01
2	24-L	27.11 ± 0.05	6.58 ± 0.02	8.19 ± 0.05	30.94 ± 0.01
	12-L	27.12 ± 0.02	6.56 ± 0.01	8.20 ± 0.05	30.94 ± 0.01
3	24-L	27.34 ± 0.02	6.36 ± 0.01	7.93 ± 0.01	32.10 ± 0.01
	12-L	27.44 ± 0.01	6.32 ± 0.07	7.93 ± 0.01	32.10 ± 0.01
4	24-L	27.59 ± 0.01	5.99 ± 0.06	8.04 ± 0.02	31.34 ± 0.01
	12-L	27.45 ± 0.01	5.80 ± 0.05	8.07 ± 0.01	31.34 ± 0.01

Table 3. Final notochord length (mm), % survival, % swim bladder inflation and % of yellowfin tuna larvae commencing flexion from Trials 1 to 4. All data are mean ± S.E. Within each trial, parameters sharing the same letter are not significantly different ($P > 0.05$). Refer to Table 1 for light intensities within each photoperiod.

Trial	1		2		3		4	
Photoperiod (hours)	24-L	12-L	24-L	12-L	24-L	12-L	24-L	12-L
Survival (%)	2.3 ± 0.6^{a}	1.0 ± 0.6^{a}	0.2 ± 0.1^{a}	0.1 ± 0.1^{a}	8.9 ± 0.8^{b}	1.0 ± 0.3^{a}	10.4 ± 1.9^{b}	0.2 ± 0.1^{a}
Final notochord length (mm)	4.59 ± 0.05^{a}	4.59 ± 0.16^{a}	4.00 ± 0.27^{a}	3.97 ± 0.07^{a}	5.1 ± 0.1^{b}	4.2 ± 0.1^{a}	4.5 ± 0.2^{b}	3.9 ± 0.1^{a}
Initiating flexion (%)	-	-	-	-	90 ± 0^{b}	5 ± 5^{a}	90 ± 3^{b}	52 ± 7^{a}
Swim bladder inflation (%)	-	-	-	-	-	-	10 ± 0^{b}	50 ± 5^{a}