

HATCHERY PROTOCOL FOR SILVER THERAPON (*LEIOPOTHERAPON PLUMBEUS*, KNER 1864)

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TECHNICAL FIELD OF INVENTION

- 5 The invention relates to the protocol for larval rearing of silver therapon, *Leiopotherapon plumbeus* in outdoor tanks, using improved system for pre-conditioning of rearing water, appropriate stocking density, larval feed preferences and enhanced production of natural food.

BACKGROUND

- 10 Silver therapon is an indigenous species present throughout the year in Laguna Lake, Philippines. However, the natural population of this valuable commodity has been steadily declining over the last few decades. It has characteristics desirable for aquaculture, such as readiness to spawn in captivity. This species can reproduce year round, with peaks during the latter part of the dry season (March to May), and attain sexual maturity even at a total length of
- 15 less than 15 cm. However, hatchery seed production trials have remained tentative due to difficulties encountered during the early larval rearing stages. In other fishes, larval feeding during the early rearing stages is a major constraint in large scale production in captivity. Like some tropical freshwater and marine fish larvae, silver therapon larvae have small mouth size (about 211µm) at first feeding when larvae aged 2 days after hatching (DAH) have grown to
- 20 2.74 mm total length and their yolk reserve depleted. Thus, small-size prey items are needed to fulfill the food requirements of silver therapon larvae at the onset of exogenous feeding.

- Food and feeding studies of silver therapon are few and have dealt mainly with wild juveniles and adult individuals. Zooplankton is considered important in the diet of smaller fish, whereas
- 25 benthic crustacean, fish, aquatic insects, and other allocthonous sources are primarily consumed by the larger ones.

- Rearing of silver therapon larvae has been successful in outdoor concrete tanks using a semi intensive method, following the same approach used in the rearing of grouper larvae (Duray et al., 1997; Toledo et al., 1997, 1999; Russo et al., 2009). In this method, tank water was
- 30 preconditioned 14 days before stocking of larvae (about 2,000 individuals per tank) to allow

natural phytoplankton to bloom in the rearing tanks. No chemical or organic enrichment was done to avoid excessive phytoplankton bloom. However, the survival rate of hatchery reared therapon larvae using this protocol ranged only from 16-26%. This low survival rate is probably linked to low density of food items, particularly before or during the onset of exogenous feeding (Toledo et al., 1999) or due to high stocking density of the larvae.

In some fish species larvae, to enhance the natural food supply in tanks, inorganic (ammonium nitrate) or organic fertilizers (cow or chicken manure) or a combination of both may be used (Qin and Culver 1992). Fertilization of rearing tanks will promote blooms of phytoplankton species which are then grazed on by zooplankton, thus improving the availability of natural food for the larvae (Russo et al., 2009). Success in larval rearing can also be achieved through early weaning of fish larvae from live foods to artificial diets as shown in catfish larvae (Fermin and Bolivar, 1996).

The aquaculture of silver therapon has not yet been successfully conducted, constraints in hatchery protocol, specifically larval rearing, being one of the reasons behind it.

References:

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SUMMARY OF THE INVENTION

The present invention addresses the problem on low survival and low growth rates of silver therapon larvae reared in captivity. It relates to a hatchery protocol for successful rearing of silver therapon larvae, *Leiopotherapon plumbeus*, through established optimum regimes for natural diet composition and feeding preferences, stocking density, natural food enhancement, water conditioning schemes, and choice on the source of rearing water. The stocking density is between 0.1 to 1.0 larvae L⁻¹ using a newly hatched larvae (0 DAH, days after hatching). Fertilizer to enhance the growth of natural food is chicken manure (organic) and 16-20-0 (commercial inorganic fertilizer). Natural food preference of the larvae are rotifers, *Moina* sp., cladocerans, insect larvae and adult copepods and should be chronologically abundant in that order commensurate with the age of larvae. Water type used is either ambient lake water or ambient lake water + *Terminalia catappa* leaves to enhance phytoplankton and zooplankton growth.

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The first objective of this invention is to enhance the production of natural food suitable for the growth and survival of Silver therapon larvae.

Second objective of this invention is to maximize production with less mortality by using optimum stocking density for larval rearing of Silver therapon in tanks.

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Third objective of the invention is to provide natural food preferred by Silver therapon larvae which is an important requirement in establishing a successful hatchery protocol of the species.

5 DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is a protocol to rear silver therapon larvae in outdoor tanks. The methodology developed will increase and improve their survival and growth.

- 10 Silver therapon is an indigenous species present throughout the year in Laguna Lake, Philippines. However, the natural population of this species has been steadily declining over the last decade. Silver therapon has characteristics desirable for aquaculture, such as readiness to spawn in captivity. The hatchery seed production have remained tentative due to difficulties encountered during the early larval stage of rearing. Fertilized eggs were obtained from
- 15 hormone-induced spawning of captive broodstock using human chorionic gonadotropin (hCG) at 50 IU g⁻¹ BW, incubated and allowed to hatch in aerated 10 L round plastic basins.

- In the embodiment of the present invention, newly hatched larvae (0DAH, zero day after hatch) are to be stocked in outdoor concrete tank filled with freshwater up to 50 cm depth. Prior to
- 20 stocking of newly-hatched larvae, tank water was aged for at least 14 days to allow natural plankton growth. Tanks are gently aerated to maintain acceptable levels of water quality during the rearing period. The culture water should be fertilized in order to enhance the growth of natural food. Organic fertilizer like chicken manure at a rate of 14 g/m³/week and commercial inorganic fertilizer, ammonium phosphate (16-20-0) at a rate of 1 g/m³/week are recommended.
- 25 Fertilizers were applied 5 days prior to stocking of larvae and then 4 -5 days thereafter until harvest.

Another embodiment of the present invention is the established appropriate stocking density of the 0 DAH larvae to be between 0.1 to 1.0 larvae L⁻¹.

In another embodiment, the natural food preference of the Silver therapon larvae are rotifers starting from 39 hph, cladocerans at 240 hph, insect larvae and adult copepods at 552 hph.

Another embodiment of the present invention is the rearing water to be used should come from ambient lake water with the addition of tropical almond, fresh *Terminalia catappa* leaves to further enhance the production of natural food. Rearing tanks is to be filled with ambient lake water. Tropical almond leaves is added two days before stocking of larvae and for two consecutive weeks thereafter. The leaves (180 g /tank) are to be rinsed and dried prior to the application in the rearing tank. Water is not changed throughout the 40 day rearing period.

The following examples are given by way of illustration and therefore should not be construed to limit the scope of the present invention.

EXAMPLE 1

Growth and survival of silver therapon at different stocking densities

Parameter	Stocking density		
	0.9 larvae L ^{-1*}	0.6 larvae L ^{-1**}	0.4 larvae L ^{-1**}
Total Length (mm)	24.37 ± 5.95	12.03 ± 2.02	20.04 ± 3.75
SGR (% d ⁻¹)	5.39 ± 0.90	5.15 ± 0.61	6.97 ± 0.68
AGR (mm d ⁻¹)	0.59 ± 0.18	0.33 ± 0.67	0.61 ± 0.13
Survival (%)	11.58 ± 6.56	14.05 ± 8.40	35.41 ± 15.32

*Larvae were reared in outdoor tanks for 40 days with natural food grown 2 weeks prior to start of larval rearing;

**Larvae were reared in tanks fertilized with inorganic and organic fertilizers for 30 days

SGR; specific growth rate. AGR; absolute growth rate.

Result showed a higher survival rate, AGR and SGR of therapon larvae at lower stocking density (0.4 larvae L⁻¹).

EXAMPLE 2

Effect of various types of fertilizers on survival and growth and survival of silver therapon larvae stocked at an initial density of 0.5 larvae L⁻¹ in outdoor concrete tanks (OF: Organic Fertilizer;

IF: Inorganic Fertilizer; NF: No Fertilizer)

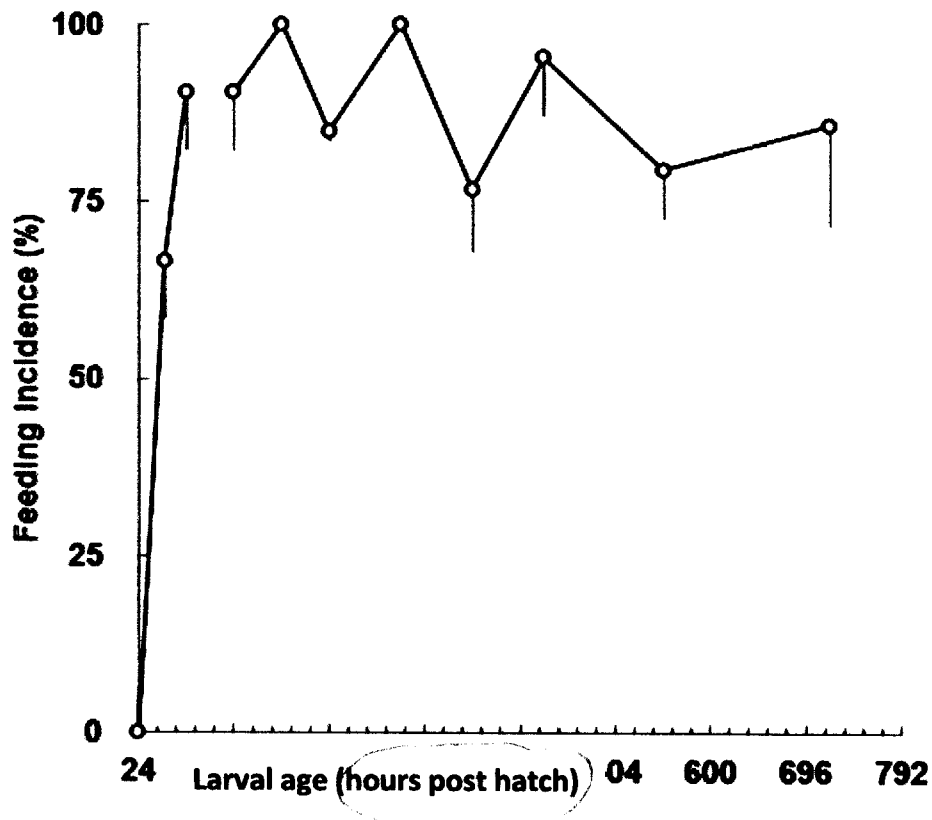
Parameter	Treatments		
	OF	IF	NF
Total Length (mm)	16.77 ± 7.04	11.56 ± 2.18	12.89 ± 1.84
SGR (% d ⁻¹)	7.20 ± 1.36	5.98 ± 0.60	6.34 ± 0.52
AGR (mm d ⁻¹)	0.49 ± 0.23	0.32 ± 0.07	0.39 ± 0.07
Survival (%)	5.97 ± 6.80	6.07 ± 7.09	2.10 ± 2.87

Total length and SGR were significantly improved when an organic and inorganic fertilizer was added in the rearing water.

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EXAMPLE 3

Feeding incidence of larval silver therapon provided initially with rotifers and *Moina* sp.



- 5 Therapon larvae start to feed exogenously on rotifers at 54 hph (hours post hatch). At 24 h guts of the larvae were empty. About 57-71% of larvae had food items in the gut at 54 hph and this fraction increased to 100% by 168 hph.

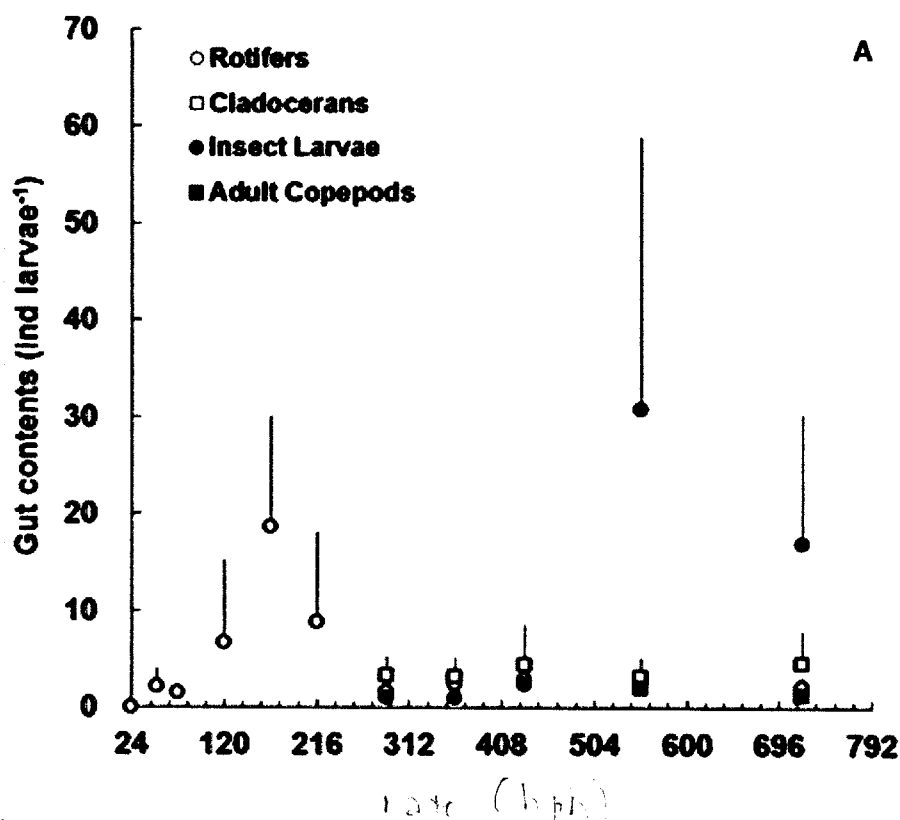
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EXAMPLE 4

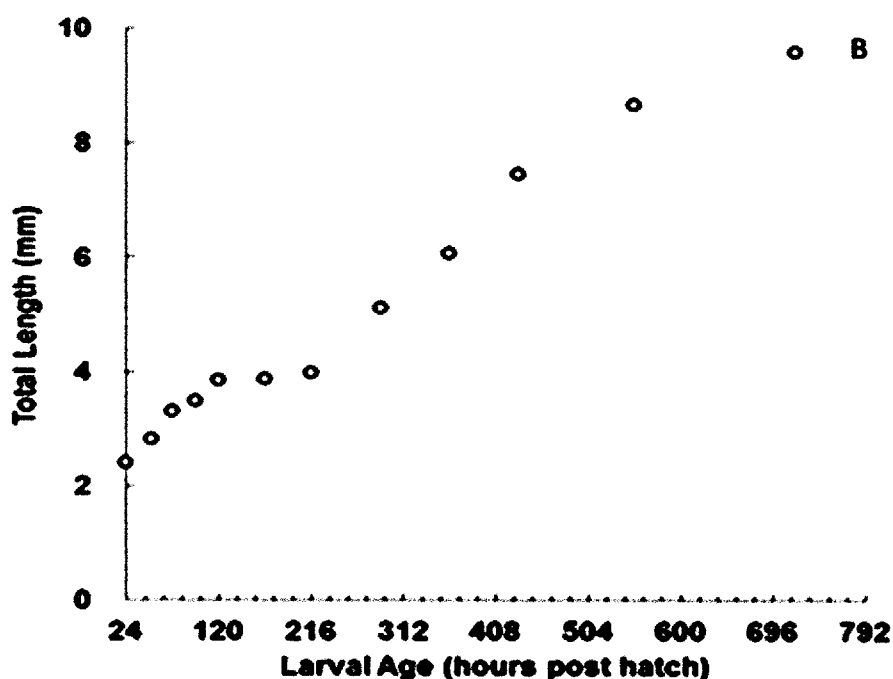
Gut contents (A) and early growth (B) of silver therapon, *Leiopotherapon plumbeus* larvae provided initially with rotifers and *Moina* sp. in outdoor tanks

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The amount of rotifers ingested by the Silver therapon larvae increased almost ten fold from the onset of feeding up to 168 hph. As the larvae grows, rotifer abundance decreased sharply at 288 hph, where larger preys such as cladocerans, which were offered at 240 hph, became more frequent in the gut. The amount of insect larvae in the gut increased more rapidly at 552 hph up to 720 hph.

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Mean total length (TL) of Silver therapon larvae during the rearing period ranged from 2.42 mm to 9.57 mm and a daily growth rate of 0.25 mm d^{-1} .

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EXAMPLE 5

Growth and survival of Silver therapon larvae reared in ambient lakewater with and without tropical almond *Terminalia cattapa* leaves

Parameter	Treatments	
	LW	LW + T
Total Length (mm)	16.25 ± 3.04	15.63 ± 1.51
SGR ($\% \text{ d}^{-1}$)	5.58 ± 0.41	5.23 ± 0.25
AGR (mm d^{-1})	0.56 ± 0.10	0.34 ± 0.04
Body weight (mg)	55.50 ± 41.72	39.33 ± 13.87
Survival (%)	26.73 ± 1.60	48.44 ± 7.85
Final density (larvae L^{-1})	0.10 ± 0.01	0.18 ± 0.03

Results showed that larvae reared in ambient lake water (LW) for 40 days had an improved growth rate. Higher survival rate was observed in larvae reared in lake water and *T. cattapa* leaves (LW+T).

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