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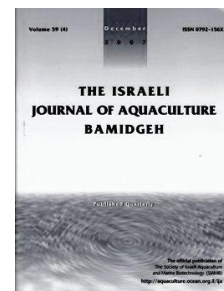
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## Survival of Juvenile Silver Pomfret, *Pampus argenteus*, Kept in Transport Conmditions in Different Densities and Temperatures

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### Abstract

Optimum conditions for the transportation of juvenile silver pomfret, *Pampus argenteus* (Euphrasen 1788) were investigated under simulated conditions. Juveniles ( $5.22 \pm 1.09$  g) were kept in 10-l plastic bags containing oxygen and 3 l water at 15, 20, and 25°C in (a) low loading densities (5, 10, 20 g/l) for 8 h and (b) high loading densities (20, 30, 40 g/l) for 4 h. Following simulations, water was sampled to measure dissolved oxygen, pH, and total ammonia. Both survival rates and dissolved oxygen levels decreased when the temperature and loading density increased; pH decreased significantly under all transportation conditions. High loading density (30-40 g/l) and temperature (25°C) resulted in high total ammonia and mortality (54-64%). The ideal temperature for transporting juvenile silver pomfret in plastic bags was 15°C. At this temperature, the highest survival rate was recorded, even at loading densities of 40 g/l. Transport at high temperature (25°C) and loading density (20 g/l) should not exceed 8 h, due to raised mortality (>30%) and ammonia levels.

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## Introduction

Silver pomfret (*Pampus argenteus* Euphrasen) are of high commercial value for consumption worldwide. As a result, this species has an important economic role in the fisheries of China, India, Kuwait, Iraq, and Iran (Al-Husaini, 2003). However, overfishing has caused silver pomfret stocks to decline (Wen et al., 2006). Due to the depletion of wild stock, market demand, and high prices, technological research for the development of silver pomfret aquaculture was initiated in China in 2004 by the East China Sea Fisheries Research Institute.

Silver pomfret have a high growth rate, good adaptability to culture conditions, and no serious disease problems (James and Almatar, 2008). Further, silver pomfret is a promising species for aquaculture production because there is adequate existing knowledge about its biological and technological requirements (Almatar and James, 2007; James and Almatar, 2007). Due to the success of captive breeding and larval rearing (James and Almatar, 2007), the need for transporting silver pomfret to national and international markets has increased. Within China, fingerlings (i.e., juveniles) are usually transported in plastic bags via truck or air freight. However, transport in a closed system leads to the accumulation of potentially harmful toxic nitrogenous waste (Handy and Poxton, 1993). This arises as a result of normal protein metabolic processes that cause fish to excrete total ammonia, which takes the form of highly toxic un-ionized ( $\text{NH}_3$ ) and ionized ( $\text{NH}_4^+$ ) ammonia (Fivelstad et al., 1993; Tomasso, 1994).

In general, these problems can be reduced by lowering the temperature. Temperature can affect the rate of chemical reactions in the body (Hazel, 1993), and influence oxygen consumption by the fish and the oxygen-carrying capacity of the water (Ross and Ross, 1984). Lowering the temperature would allow for greater loading densities within a given volume of water during transport. However, the transportation loading density of fish may be influenced by fish species, fish size, and water quality (Berka, 1986; Kaiser and Vine, 1998). Hence, while a low ambient temperature may reduce the metabolic rate of fish, the extent of cooling that can be applied may be limited and vary with species (Ross and Ross, 1984; Lim et al., 2003).

This study focuses on delineating the optimal conditions for transport of silver pomfret by evaluating water quality parameters and performance of silver pomfret in different loading densities and water temperatures. The results were compared with other commercially important aquacultured fish stock. The development of effective and economically viable transportation systems for aquacultured species is essential to reduce the pressure on wild stocks that are becoming seriously depleted worldwide.

## Materials and Methods

*Experimental fish.* Eggs of silver pomfret were collected from the wild in May 2009, hatched in captivity after 36 h, and reared for two months until the onset of the study when the fish averaged  $5.22 \pm 1.09$  g and had a mean total fork length of  $5.46 \pm 1.15$  cm. The fish were fed four times daily at approximately 07:00, 11:00, 15:00, and 18:00 to apparent satiation.

*Transport simulation experiments.* In the first experiment, the effects of low fish density (5, 10, 20 g/l) and water temperature (15, 20, 25°C) on water quality parameters and transport performance for a duration of 8 h were investigated (i.e., 9 combinations). Treatments were replicated three times, hence nine 10-l plastic transportation bags were prepared for each loading density, and three were kept at each temperature (27 bags in total). In the second experiment, transportation performance and water quality parameters at high fish density (20, 30, 40 g/l) and the same temperatures (15, 20, 25°C) were investigated for 4 h. Treatments were replicated three times, hence nine 10-l plastic transport bags were prepared for each loading density, and three were placed in each temperature (27 bags in total).

*Simulated transport conditions and post-transport assessments.* Three liters of salt water were added to each 10-l plastic bag. Before adding the fish, dissolved oxygen was 8.17 mg/l, salinity  $\sim 25$ -26 g/l, temperature 27.8°C, pH 8.17, and total ammonia was undetected. Feed was withheld from the fish for 24 h prior to the experiment. The fish

were weighed and packed in the respective bags. The bags were inflated with oxygen and tied with rubber strings. Each experimental bag was enclosed in a 20-l black plastic bag to replicate dark transport conditions. For the 15°C groups, temperature was maintained by placing the plastic bags in a 500-l tank that had a cooling system connected to a thermostat. For the 20°C and 25°C groups, temperature was maintained using the air conditioning system of the laboratory. The temperature of each group was checked at 30-min intervals throughout the experiment.

At the completion of the experiment, mortality was verified, and survivors were transferred to aerated 1000-l tanks for an additional 24 h observation period to record any delayed mortality. Water samples were taken before and after simulated transportation for each group. Dissolved oxygen and pH were determined using a YSI 6600 multiparameter instrument (Yellow Spring Instrument, Yellow Spring, OH, USA). Total ammonia nitrogen was determined using the direct Nesslerization method (Greenberg et al., 1976). Concentrations of un-ionized ammonia were calculated based on equations of the China Sea Water Quality Standard (GB3097-1997):  $c(\text{NH}_3) = 14 \times 10^{-5} \text{ TAN} \times 100 / (10pK_a^{S \times T - \text{pH}} + 1)$  and  $pK_a^{S \times T} = 9.245 + 0.002949 \times S + 0.0324 \times (298 - T)$ , where  $c(\text{NH}_3)$  is the concentration of un-ionized ammonia in mg/l; TAN is total ammonia nitrogen in  $\mu\text{mol/l}$ ;  $pK_a$  is the acidity constant; S is salinity in g/l; T is water temperature (Kelvin) calculated as  $273 + t$ , in °C; and pH is the pondus hydrogenii.

*Statistical analysis.* The physicochemical water parameters and mortality were analyzed using analysis of variance and the Tukey test (Tukey, 1977) with the level of significance set at  $p < 0.05$ .

## Results

*Experiment 1: Eight-hour simulation of transport at low density.* At the lowest loading density (5 g/l), all juvenile silver pomfret survived the 8-h simulated transport conditions at all three temperatures (Fig. 1). At the 20 g/l loading density, a significant decrease in survival was recorded at the highest temperature. Following 8 h simulated transport, dissolved oxygen ranged 11.89-21.04 mg/l (Table 1). Dissolved oxygen decreased as the loading density and temperature increased. There were no significant differences in water pH although it tended to decrease with increasing loading density. Total ammonia significantly increased with increased loading density at all temperatures. Un-ionized ammonia significantly increased with loading density at 25°C.

*Experiment 2: Four-hour simulation of transport at high density.* Survival after 4 h in transport conditions exceeded 95% at the loading density of 20 g/l but was low (36-75%) at densities of 30 and 40 g/l. At all temperatures, dissolved oxygen decreased significantly as loading density increased. Water pH significantly dropped as the temperature rose at the density of 40 g/l. Total ammonia significantly increased with loading density and tended to increase with temperature. Un-ionized ammonia significantly increased with density and temperature.

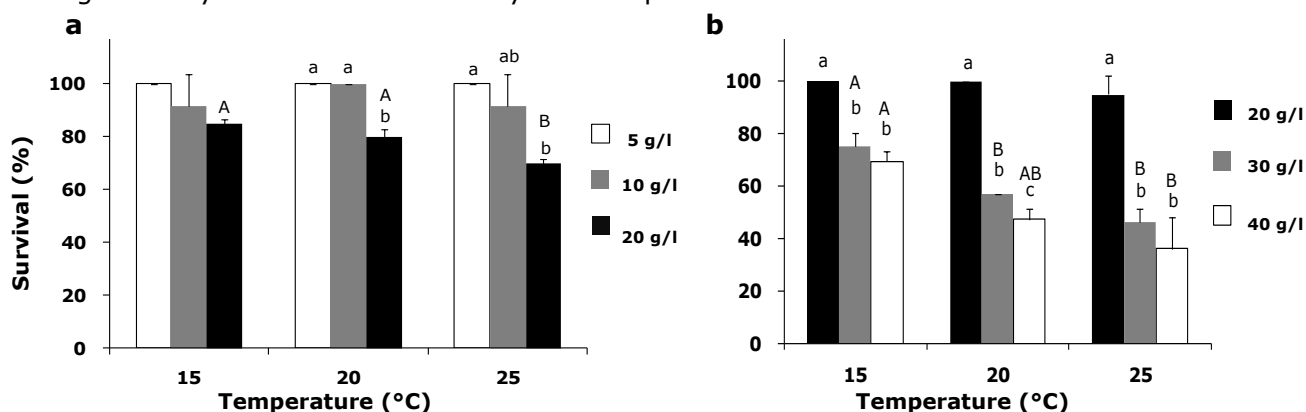


Fig. 1. Mean survival of juvenile silver pomfret after (a) 8 h and (b) 4 h in simulated transport conditions at different loading densities and temperatures. Bars with different lowercase letters denote significant differences in temperature ( $p < 0.05$ ); bars with different capital letters denote significant differences in density ( $p < 0.05$ ).

Table 1. Water parameters after 8 and 4 h in simulated transport conditions for juvenile silver pomfret.

After 8 h	°C	Loading density (g/l)		
		5	10	20
Dissolved oxygen (mg/l)	15	21.04±0.04 <sup>aA</sup>	19.86±1.20 <sup>abA</sup>	17.78±0.52 <sup>b</sup>
	20	17.21±0.49 <sup>B</sup>	16.64±0.08 <sup>B</sup>	15.17±1.02
	25	16.15±0.57 <sup>B</sup>	15.20±0.17 <sup>B</sup>	11.89±2.52
pH	15	7.46±0.13	7.28±0.23	7.04±0.18
	20	7.49±0.13	7.24±0.26	7.03±0.13
	25	7.25±0.15	7.12±0.28	6.91±0.01
Total ammonia nitrogen (mg/l)	15	1.50±0.55 <sup>b</sup>	2.49±0.09 <sup>bb</sup>	5.27±0.28 <sup>abB</sup>
	20	1.89±0.26 <sup>c</sup>	3.27±0.28 <sup>bA</sup>	5.97±0.38 <sup>abB</sup>
	25	2.09±0.40 <sup>c</sup>	3.50±0.02 <sup>bA</sup>	7.05±0.23 <sup>aA</sup>
Un-ionized ammonia (ug/l)	15	10.42±1.95 <sup>B</sup>	11.53±1.85 <sup>B</sup>	13.52±2.04 <sup>C</sup>
	20	19.78±1.17 <sup>A</sup>	20.52±2.71 <sup>A</sup>	20.98±0.02 <sup>B</sup>
	25	17.31±2.49 <sup>ba</sup>	24.26±0.67 <sup>ba</sup>	27.31±0.01 <sup>aA</sup>
After 4 h	°C	20	30	40
Dissolved oxygen (mg/l)	15	18.14±1.07 <sup>aA</sup>	13.48±1.05 <sup>ab</sup>	11.62±1.32 <sup>b</sup>
	20	15.92±0.63 <sup>aAB</sup>	10.25±0.18 <sup>b</sup>	8.77±0.59 <sup>b</sup>
	25	15.05±0.28 <sup>ab</sup>	10.12±0.94 <sup>b</sup>	8.13±0.36 <sup>b</sup>
pH	15	7.12±0.12	7.00±0.00	7.13±0.04 <sup>A</sup>
	20	7.02±0.11	6.95±0.07	7.03±0.04 <sup>AB</sup>
	25	7.03±0.25	6.90±0.14	7.00±0.00 <sup>B</sup>
Total ammonia nitrogen (mg/l)	15	4.78±0.74 <sup>b</sup>	7.13±0.89 <sup>ab</sup>	8.27±0.89 <sup>a</sup>
	20	4.85±0.49 <sup>b</sup>	7.16±1.05 <sup>ab</sup>	8.67±0.47 <sup>a</sup>
	25	5.61±0.72 <sup>b</sup>	7.78±0.45 <sup>ab</sup>	9.18±0.67 <sup>a</sup>
Un-ionized ammonia (ug/l)	15	14.66±0.47 <sup>bb</sup>	16.14±2.02 <sup>bc</sup>	24.88±0.66 <sup>abB</sup>
	20	16.73±2.62 <sup>bb</sup>	20.83±0.30 <sup>bb</sup>	30.13±0.83 <sup>abB</sup>
	25	29.52±1.22 <sup>ba</sup>	29.95±0.81 <sup>ba</sup>	43.69±3.20 <sup>aA</sup>

Values are means±SD.

Means in columns identified by different capital letters and means in rows identified by different small letters significantly differ ( $p < 0.05$ ).

with species and size, and is affected by water quality (Berka, 1986; Kaiser et al., 1998). When silver catfish (*Rhamdia quelen*) fingerlings (5-10 cm) were stocked at low densities (50, 67, 87 g/l) and transported at 25°C, 20°C, and 15°C, 100% survived for 24 h (Golombieski et al., 2003). Mortality was 2.3-5% in 100 45-day and 60-day-old grouper larvae (*Epinephelus*) transported for 8 h at 23°C (Estudillo and Duray, 2003). In comparison, survival was 100% in 100 47-day-old *Siganus guttatus* larvae transported for 8 h at 28°C, but significantly dropped (48-18%) as the loading density increased to 200, 300, and 400/l (Ayson et al., 1990). In our study, survival at a loading density of 20 g/l was higher after 4 h than after 8 h, indicating that survival of silver pomfret fingerlings in plastic bags is related to the transport duration.

Water temperature is an important factor during fish transport (Berka, 1986). A decrease in water temperature can reduce the metabolic rate in fishes, thereby reducing oxygen consumption and metabolic waste production. In other words, the lower the temperature, the lower the oxygen consumption (Harmon, 2009). In our study, increased simulated transport temperature and density significantly reduced the final dissolved oxygen level in the water; survival after both 4 h and 8 h in transport conditions was lowest at the highest temperature and density, indicating that depletion of dissolved oxygen may have contributed to fish mortality at the higher loading densities and temperatures. In addition, pH noticeably dropped during transport; increased acidity occurred due to the production of carbon dioxide from fish respiration, leading to the formation of carbonic acid that can dissociate into H<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> (Boyd, 1982).

The increase in total ammonia nitrogen (NH<sub>3</sub> plus NH<sub>4</sub><sup>+</sup>) is another important factor that may have contributed to fish death during transport (Fivelstad et al., 1993). During transport, fish metabolism is higher than normal, which increases the production of metabolic wastes such as ammonia and carbon dioxide, causing water quality to

## Discussion

Ocean shipping is an important mode of transporting fish, however, transport by truck or air is also important and regularly used in many regions. In some regions, 4-h or 8-h transports by truck or air are enough to deliver fingerlings to their destination. This study was conducted to determine the optimum conditions for the transport of juvenile silver pomfret.

To obtain 100% survival during 8-h transport, a loading density of 5 g/l and temperature range of 15-25°C should be used. To obtain 100% survival during 4-h transport, a loading density of 20 g/l and temperature of 15-20°C should be used.

The best loading density for 8-h transport was 10 g/l, with survival ranging 91-100% when transported at 15 or 20°C. Our study therefore supports existing research, whereby the optimum transport density for fish varies

deteriorate (Tomasso, 1994). In the present study, survival after 4 h significantly dropped as the loading density increased. Increased loading density led to significant increases in  $\text{NH}_3$  and  $\text{NH}_4^+$ , indicating that mortality may have been associated with increased  $\text{NH}_3$  and  $\text{NH}_4^+$  and decreased water quality. The ammonia toxicity level for fish varies with water temperature, pH, and dissolved oxygen (Hargreaves and Kucuk, 2001). For example, the median 96-h LC50 of  $\text{NH}_3$  concentration averages 1.7 mg/l (40 mg/l total ammonia nitrogen) for sea bass (*Dicentrarchus labrax*) and 2.5-2.6 mg/l (57-59 mg/l total ammonia nitrogen) for sea bream (*Sparus aurata*) and turbot (*Scophthalmus maximus*), according to Person-Le Ruyet et al. (1995). Constant exposure to  $\text{NH}_3$  exceeding 0.025 mg/l results in increased mortality in red porgy (*Pagrus pagrus*; Pavlidis et al., 2003).

Prior to the current study, data on ammonia toxicity for silver pomfret were lacking; our results indicate that  $\text{NH}_3$  levels exceeding 0.03 mg/l may result in high mortality (>50% for 4 h transport). In the current study, total ammonia and  $\text{NH}_3$  levels after transport increased significantly as the temperature increased, suggesting that decreasing the water temperature may decrease the ammonia excretion rate, resulting in a lower ammonia concentration. Similarly, an increase in water temperature increases the non-ionized ammonia concentration (Emerson et al., 1975).

In conclusion, survival of juvenile silver pomfret transported in plastic bags is related to loading density and the time required for transport. The ideal temperature for transporting juvenile silver pomfret in plastic bags is 15°C, compared to 20°C and 25°C, even at a loading density of 40 g/l. At 15°C, the dissolved oxygen level remained high and the increase in total ammonia level was lowest, compared to the other experimental temperatures. Transport of 20 g/l juvenile silver pomfret is feasible at 25°C, but should not exceed 8 h due to high mortality (>30%) and ammonia level.

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