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Growth performance of fry and fingerling Asian Seabass (*Lates calcarifer*) from Cambodian brood stock reared at different salinities

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Abstract

Coastal aquaculture of Asian seabass *L. calcarifer* in Cambodia is in an early phase of development and salinity of coastal waters varies both seasonally and geographically based on terrestrial influx and rainfall. We evaluated performance of fry and juvenile Asian Seabass of Cambodian origin in terms of growth, feed conversion (FCR), condition factor (CF) and mortality, at differing salinities found in Cambodian coastal aquaculture. Fry were reared for 14 days in duplicate treatment tanks at salinities of 0, 5, 10 and 20psu with a start and final weight of 0.32 and 1.9g, respectively across all salinity treatments. Fingerling fish were reared for 56 days in triplicate treatment tanks at salinities of 10, 20 and 30psu, with a mean start and final weight of 7 and 60g, respectively; there was no significant effect of salinity on final weight. In the fry experiment, mortality was below 1% and not related to salinity. In the fingerling experiment, mortality was zero. No consistent or significant effect of salinity treatment were noted on growth, measured either as increase in total weight or as specific growth rate (SGR). Neither was any consistent effect of salinity level determined for FCR, or CF. We conclude that farming of early life stages of Cambodian Asian seabass can be performed in a wide range of salinities from as small as 0.3g. We also note that there is very high variance in growth rates amongst the fingerling cohort suggesting potential for improvement through selective breeding in this nascent industry in Cambodia.

Keywords: *barramundi, fish, Giant Sea perch, mortality, recirculating system*

Introduction

Marine aquaculture in Cambodia is still in an early phase but is expected to increase in importance as returns from capture fisheries are declining (Joffre et al 2016; Sen et al 2018). In order to succeed, this emerging industry is in need of relevant country-specific knowledge. Asian seabass, also called giant sea perch or barramundi, is an economically important fish for food and sport in the tropical and subtropical areas of the Western Pacific Ocean and the Indian Ocean, and one of the focus species for the developing Cambodian marine aquaculture industry. Asian seabass is fast growing, with potential to gain in excess of 1kg per year and attain market size within 8 months. It has firm, white flesh and commands a high market price, making it desirable for farming. Asian seabass is also highly resilient, tolerating handling, high stocking densities, and accepts a wide range of artificial feeds. Consequently, Asian sea bass have been widely domesticated for aquaculture throughout tropical and subtropical Asia and Oceania (Boonyaratpalin 1997).

Asian seabass is a euryhaline fish tolerating a wide range of salinity. In the wild they are catadromous and generally ascend coastal rivers and creeks into freshwater during young adulthood, but they can be found in brackish estuarine, lagoon, rivers and in the coastal area throughout their adult life (Grey 1987). Newly-hatched fry at 15–20 days old or 0.4–0.7cm are distributed along the coastline of brackish water estuaries while at the size of about 1cm can be found in freshwater bodies e.g. rice fields and lakes (Bhatia and Kungvankij 1971). Maturing fish migrate downstream to higher salinity waters where they spawn with the fertilized eggs requiring seawater in order to develop (Davis 1986). Consequently, eggs but also fry and juvenile stages have a narrower range of tolerance of salinity and temperature than adults (Russell and Garret 1983, 1985).

In Thailand, Asian seabass is farmed in fresh water or brackish water ponds either as monoculture or in polyculture with forage fish like tilapia *(Oreochromis mossambicus* or *O. niloticus)*, which may serve as food for seabass. Asian seabass in Cambodia are predominantly farmed in fixed or floating net cages in coastal waters, most commonly in salinities ranging from 10-30psu (Practical Salinity Units) depending on location along the coast, with salinity in coastal waters also fluctuating during rainy and dry season (Cheong 1989; Sen et al 2018). However, in Tahiti, seabass is successfully grown in seawater at 35psu salinity (Cheong 1989; Fuchs 1989) indicative of the flexibility of this species to different salinity farming conditions.

Comparative performance of seabass at different salinities is less well documenteds. Dunstan (1959) reported that wild Asian seabass in fresh water are more sluggish, contain more visceral fat and are generally heavier than those of the same length found at higher salinity, indicating that a lower salinity could be beneficial for farming. In aquaculture it has been proposed that farming fish at isotonic salinity (i.e. close to 10psu) reduces the energetic cost of ion and water exchange and might therefore be favorable, resulting in increased growth and/or improved feed conversion. As Asian sea bass responds to changes in salinity by synthesis of new salt-transporting proteins (Kidder et al 2006), one would expect this energetic cost to also hold true for this species.

Considering that Asian sea bass is farmed in, and transferred between, water of varying salinities (Sen et al 2018), coupled with the possibility of improved energy physiology by matching life stage and salinity level, the lack of systematic data in the literature regarding salinity tolerance in this species is unexpected. In order to provide information of use to the nascent Cambodian aquaculture industry, we evaluated the effect of different salinity levels on growth, feed conversion, condition factor and survival rate for Asian sea bass at fry and fingerling stages in water of differing salinities. We employed salinities reflecting those found in Cambodian coastal farming areas (Sen et al 2018) where salinity fluctuates between 30-32psu in the dry season and 20-25psu in the rainy season. Our studies had two main aims. Firstly, to evaluate if salinity is an important variable in selection of farm locations for fry and fingerlings and to determine whether rearing of fry and fingerlings at close to physiological salinity would improve performance compared to those reared at higher salinity and therefore subject to higher energetic cost via osmotic load. We accomplish this using strains and salinities relevant for Cambodian marine aquaculture.

Material and methods

Aquarium systems and general husbandry

Experiments were conducted at the Marine Aquaculture Research and Development Centre (MARDeC), Preah Sihanoukville Province, Cambodia. Experimental systems comprised 200 L circular (650 mm d x 750 mm h) tanks connected to individual canister-type biofilters (Eheim) with continuous aeration supplied via a central compressor and air lines. Asian sea bass *L. calcarifer* fry and fingerlings were produced at MARDeC using local broodstock.

Fish were fed by hand twice a day at 08:00 AM and 05:00 PM. Uneaten feed was collected after each feeding by siphon and water replaced as necessary with water of identical salinity from a pre-prepared stock. Temperature, dissolved oxygen (YSI 556 MSP, YSI Environmental, USA), pH (ECO Test pH2, Cole-Parmar, USA) and NH_3 and NO_2 (Sera test kit, Germany) were measured in each tank every second day at 8:00 AM and at 5:00 PM. Salinity was measured and adjusted where necessary daily using a digital refractor meter (ATAGO, Japan).

Growth experiments

Fry experiment

For the fry experiment, four different salinity treatments were prepared by mixing seawater and freshwater. Salinity was determined by means of a refractometer. Treatments comprised 0 (fresh water), 5, 10 and 20psu (Practical Salinity Unit) in duplicate 200L recirculating tanks with a stocking density of 100 fish per tank at an average size of 0.3g (with an estimated fork length (FL) of 21-25 mm) and an age of 42 days. The fry were fed at 10% biomass per day with commercial dry pellet, NRD 5/8 (500-800µm) (INVE Thailand Company Ltd.) with a proximate composition of moisture 8%, protein >55%, fiber < 1.9% and ash <14.5%, as given by the manufacturer. Fry were reared for 14 days.

Fingerling experiment

In the fingerling experiment, a complete randomized design was used with 3 treatments and 3 replicates in nine independent recirculating tanks. The salinities of the treatments were 10, 20 and 30psu, respectively. Ten fingerlings were distributed to each tank with a stocking density equivalent to 50 fingerlings per m^3 and were acclimatised for 1 week. The fish were fed to satiation with a commercial dry pellet Ocialis C1 (Ocialis-BERNAQUA ASIA, Ho Chi Minh, Vietnam). The proximate composition (provided by the manufacturer) was moisture 14, crude protein 58% (min), digestible protein 55% (min), metabolisable energy 5000 kcal/kg (min), crude fiber 1%, calcium 1.5-2.5% lysine 3.2%, cys+met 2% (min). Fingerlings were reared for 56 days.

Data collection, calculations and statistics

In the fry experiment, weight was measured by weighing the whole tank population (all 100 fish from each tank) and individual weight was estimated by dividing the total weight with number of individuals. Length was measured by random selection of a subsample (n = 10) from each tank to minimise handling stress and physical damage to fine structures in small animals

In the fingerling experiment, individual body weight and total length of all fish in each tank were measured on days 14, 28, 42 and day 56 and the average daily weight gain was calculated as SGR. Total feed eaten and uneaten were recorded and the feed conversion ratio (FCR) was calculated.

Daily Weight Gain (DWG), Specific Growth Rate (SGR), Survival Rate (SR), Feed Conversion Ratio (FCR), and Condition Factor (CF) were calculated as follow:

$$\text{DWG (g)} = \frac{W1 - W0}{d}$$

Where: W_0: Mean weight of fish in first sampling; W_1: Mean weight of fish in the following sampling; d: Number of days between sampling.

$$\text{SGR (\%)} = \frac{(\text{Ln}(W1) - \text{Ln}(W0)) \times 100}{d}$$

$$\text{SR (\%)} = \frac{(N0 - N1)}{N0} \times 100$$

Where: N_o: The initial number of the fish; N_1: The remaining number of the fish

$$\text{FCR} = \frac{\text{Total feed intake by fish (g, wet weight)}}{\text{Weight gain by fish, g}}$$

$$\text{CF} = 100 \times \frac{(\text{Weight, g})}{(\text{Length, cm})^3}$$

Data were analysed using General Linear Model analysis of variance (ANOVA) in Minitab version 17 and p <0.05 was considered significant.

Results and discussion

Both fry (Fr) and fingerlings (F) at all salinities displayed an even growth trajectory throughout respective experiment (Figure 1A) and a concomitant decrease in SGR with increasing body size (Figure 1B). No significant (*p*>0.05) difference was found between any of the treatment groups at any of the sampling times (not shown).

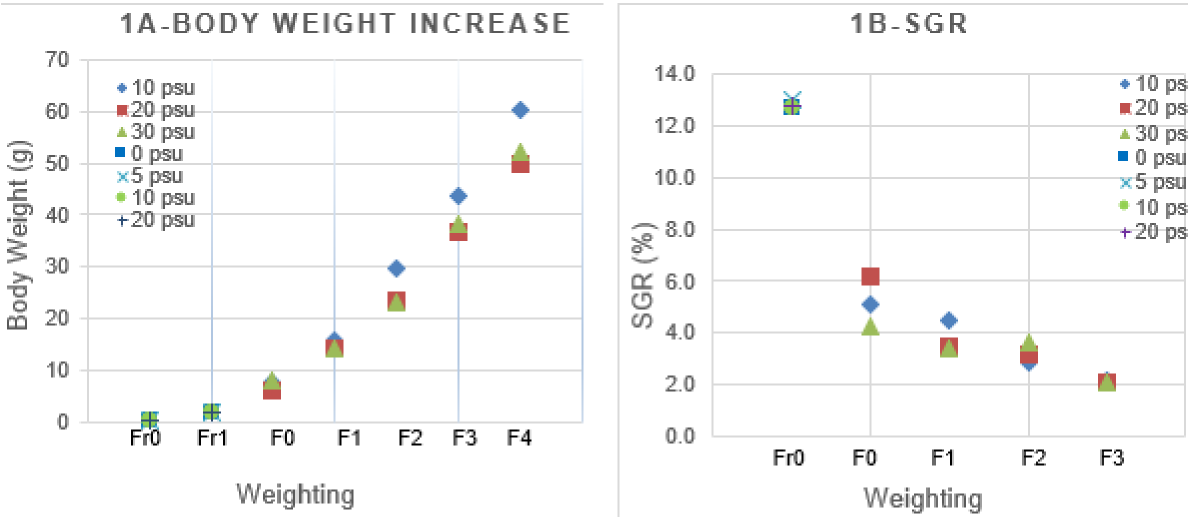

Figure 1. A-B. Effect of varying salinity levels on body weight (1A) and specific growth rate (SGR) (1B) per treatment in fry (Fr)
and fingerling (F) Asian sea bass. The data are derived from two separate experiments, both using 14 days weighing
intervals where fry experiment is denoted Fr0 and Fr1, and the fingerling experiment F0- F4. No significant differences (p>0.05) were detected between any of the treatment at a given sampling time.

Only in the fry stage was any mortality observed, but not consistently with respect to salinity (Table 1). The fry experiment did not include three replicates of each treatment, eliminating statistical power (Thorarensen et al 2015). This is a valid criticism and the underlying rational was that the fry experiment was conducted as a pilot study to explore the broadest range of salinities within the limited number of experimental units available locally in Cambodia. However, each experimental unit in the fry experiment included 100 individuals and the fish increased in weight six-fold in the two-week period, yet no consistent differences between duplicates or salinity levels were detected. Moreover, the data are directly complementary to, and consistent with, the fingerling study. We contend that the data provide useful information to the industry in Cambodia thereby warranting inclusion of fry results together with the fingerling data.

During the period between weighing F3 and F4 of the fingerling experiment, one tank of 20psu and one tank of 30psu, deviated markedly from their growth trajectory, displaying stunted growth, but maintained apparent feed intake. This resulted in unexpected high FCR of 5.2 and 4.1 in the respective tanks and affected the average FCR for this period in the fingerling experiment at 20psu and 30psu. However, regardless of whether these two deviating tanks were included or excluded from the analyses, no significant differences (p>0.05) were detected between 20 and 30psu and the 10psu treatment for FCR in the final feeding period (Table 2). Omitting these two tanks from the calculation yielded reduced FCR of 1.38 and 1.34 in 20 and 30psu treatments respectively during this period. Final average body weight was also affected by these two deviating tanks, yielding a final average weight in 20 and 30psu treatments of 53.5g and 61.55g, respectively, when these outliers were removed, although this did not affect the statistical outcomes of these treatments (no significant effect) (Table 2).

Table 1. Performance variables in Asian seabass fry reared for 14 days at different salinity levels given as arithmetic mean ± SD (standard deviation).

Variable		Treatments				*p* -value	SD
		0 psu	5 psu	10 psu	20 psu		
Fish (Number)	Initial	100	100	100	100	-	-
	Final	97	100	99	100	0.27	1.46
BW (g)	Initial	0.32	0.32	0.32	0.33	>0.1	0.01
	Final	1.88	1.99	1.86	1.98	>0.1	0.07
DWG (g/d)*		0.11	0.12	0.11	0.12	0.25	0.004
FCR		0.63	0.60	0.64	0.63	>0.1	0.01

*BW (Body Weight), DWG (Daily Weight Gain), FCR (Feed Conversion Ratio). *In this experiment, the relative growth is expressed as daily weight gain rather than SGR, based on including only one weighing period.*

Although there were no differences between any treatment groups at any time during the experiments (*p=0.25)*, the fish reared at 10psu had continually higher body weight at all weighing intervals perhaps indicating that, over time, close to physiological salinity may offer a more favourable environment for growth of this species, at least during the early life stages. This is supportive of the general contention that farming in isotonic or close to isotonic salinities yields a physiological energy advantage compared to salinities demanding management of a larger osmotic load (Cardona 2000). It is also consistent with the reports of Dunstan (1959) and Robins (2006), both recording higher growth in freshwater and lower estuary salinities in wild populations of Asian sea bass. Our data are also supportive of the previous observations that seabass adapt well to seawater and freshwater once they reach a size of 13.9 - 17.7mm (Boonyaratpalin 1997) as fish in the current fry experiment had reached a size of 21-25mm at the beginning of the experiment.

Similar to many fish species, FCR in sea bass increased with increasing fish size (Table 1 and 2), possibly due to a relative shift in metabolism from protein accretion to fat deposition, reported in other fish such as e.g. trout (Kiessling et al 1991). In the final growth period, the deviating FCR of 20 and 30psu tank point to a practical result concerning the difficulty in discerning the true satiation in fish at handfeeding and underlines the need including feed waste traps in experiments using handfeeding in order to be able to separate between apparently hungry fish not feeding and fish feeding, but low feed efficiency. The data also suggest that Asian sea bass, at least the strains available to Cambodian fish farmers, display a large variation in both growth and FCR, which indicate a substantial potential for improvement by systematic genetic selection, perhaps through a future breeding program.

Table 2. Performance variables in fingerling Asian sea bass reared 56 days at different salinity levels given as arithmetic mean ± SD (standard deviation).

Variable		Treatments			*p*-value
		10 psu	20 psu	30 psu	
Fish (number)	Initial	10	10	10	-
	Final	10	10	10	-
BW (g)	Initial	7.7±1.82	6.0±0.22	7.9±1.37	0.25
	Final	60.4±14.0	49.8±13.3	52.3±16.4	0.67
SGR (whole period)		3.67±1.33	3.73±1.74	3.34±0.91	0.75
FCR	Initial	0.53±0.03	0.46±0.05	0.55±0.05	0.10
	Final	1.70±0.61	2.74±2.38	2.27±1.61	0.77
CF	Initial	1.52±0.13	1.55±0.03	1.40±0.01	0.11
	Finals	1.41±0.14	1.53±0.09	1.42±0.17	0.52

BW (Body Weight), SGR (Specific Growth Rate), FCR (Feed Conversion Ratio), CF (Condition Factor)

Finally, Dunstan (1959) reported that wild Asian sea bass in freshwater habitats are heavier than those fish of the same length in salt water. We found no effect of salinity on condition factor in our experiment (Table 2). This may be a result of differing life stages in the two studies but may also reflect the types and availability of natural prey in the differing habitats in wild fish rather than any direct physiological driver of the observed differences.

Conclusions

- The salinities available to Cambodian coastal fish farms are suitable for early life stages of local strains of barramundi.
- A possible favorable effect on growth at close to physiological salinity, warrants further investigation.
- The high variability in the growth performance of juveniles in the Cambodian strain of barramundi indicates potential for selective breeding to greatly improve performance and consistency.

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