Received: 8 March 2020 | Revised: 20 July 2020 | Accepted: 8 September 2020

DOI: 10.1111/jwas.12741

APPLIED STUDIES

Journal of the World Aquaculture Society Open Access | WORLD AQUACULTURE Society | WILEY

Effects of salinity on growth characteristics and osmoregulation of juvenile cobia, *Rachycentron canadum* (Linnaeus 1766), reared in potassium-amended inland saline groundwater

Jose Antony[1] | **Appidi Krishna Reddy**[1] | **Arun Sudhagar**[1] | **Hari Krishna Vungurala**[1] | **Luke A Roy**[2]

[1]Rohtak Centre, ICAR-Central Institute of Fisheries Education, Rohtak, Haryana, India

[2]School of Fisheries, Aquaculture & Aquatic Sciences, Auburn University, Auburn, Alabama

Correspondence
Jose Antony, ICAR—NGRC of CIBA, NAU Campus, Eru Char Rastha, Navsari, Gujarat 396 450, India.
Email: batista.antony4@gmail.com, jose.antony@icar.gov.in

Funding information
Department of Science and Technology (DST), Govt. of India; Indian Council of Agricultural Research (ICAR)

Abstract

The suitability of inland saline groundwater as a medium to culture juvenile cobia, *Rachycentron canadum*, was assessed. In the first experiment, juvenile cobia stocked in raw (unamended) saline groundwater at salinities of 5, 10, and 15 g/L exhibited complete mortality after 108, 176, and 195 hr, respectively. The second experiment evaluated the rearing of juvenile cobia (mean weight ~9.23 ± 0.12 g) in potassium (K^+)-amended saline groundwater (100% K^+ fortified) and reconstituted seawater at salinities of 5, 10, and 15 g/L to assess growth and osmoregulation in distinct culture media. Following 60 days of culture, all fish survived the experimental period. Final mean bodyweight of cobia reared in K^+-amended saline groundwater (103.2–115.8 g) and seawater (111.2–113.8 g) of different salinities did not vary significantly ($p > .05$). No differences ($p > .05$) were observed in specific growth rate, weight gain (%), and feed conversion ratio between treatment groups. Serum osmolality increased with salinity and was significantly higher ($p < .05$) for fish in K^+-amended saline groundwater (353–361 mOsmol/Kg) than in reconstituted seawater (319–332 mOsmol/Kg), although differences were not observed between salinities by water type. Cobia stocked in saline groundwater of different salinities were osmoregulating normally, and the higher values observed may be because of variations in ionic composition and other interfering ions in saline groundwater. Trial results suggest that juvenile cobia can achieve optimal growth in K^+-amended saline groundwater of low and intermediate salinities.

KEYWORDS

cobia, groundwater, inland, osmoregulation, potassium, salinity

1 | INTRODUCTION

Arid and semiarid regions of northwestern India possess abundant resources of underlying inland saline groundwater. In India, an estimated area of 0.2 million km^2 is affected by inland saline groundwater at a salinity over 2 g/L (CGWB, 2018). Such regions are unsuitable for traditional agriculture and are considered nonproductive land (Barman, Jana, Garg, Bhatnagar, & Arasu, 2005; Fielder, Bardsley, & Allan, 2001; Forsberg, Dorsett, & Neill, 1996). Alternatively, inland saline groundwater has been used to culture marine and brackish water fish on both experimental and commercial scales (Fielder et al., 2001; Forsberg et al., 1996; Ingram, McKinnon, & Gooley, 2002; Partridge & Lymbery, 2008). Saline groundwater of inland origin is often deficient in specific ions, such as potassium (K^+), compared to seawater of equivalent salinity (Fielder et al., 2001; Forsberg & Neill, 1997; Ingram et al., 2002; Partridge & Lymbery, 2008; Roy et al., 2010; Saoud, Davis, & Rouse, 2003). Potassium is an essential intracellular cation required for several physiological processes in all animals, including fish (McDonough, Thompson, & Youn, 2002). For instance, maintenance of acid–base balance and osmoregulation are significant functions of K^+ in fish (Evans, Piermarini, & Choe, 2005; Wilson & El Naggar, 1992). Reduced survival and, often, total mortality are observed when marine fish are reared in K^+-deficient raw (unamended) inland saline groundwater (Fielder et al., 2001; Ingram et al., 2002; Partridge & Creeper, 2004). The supplementation of aqueous K^+ levels in inland saline water has been demonstrated to improve the growth and survival of several species of euryhaline marine fish (Doroudi, Fielder, Allan, & Webster, 2006; Fielder et al., 2001; Mourad, Kreydiyyeh, Ghanawi, & Saoud, 2012; Partridge & Lymbery, 2008; Partridge & Lymbery, 2009). Marine fish are a priced commodity and are in great demand in inland regions, thus making its farming a lucrative option for prospective farmers. However, because of the characteristic subtropical climate in semiarid regions of northwestern India, the growing season is short (180–240 days), and the species of choice for farming needs to be a fast-growing euryhaline marine finfish capable of attaining marketable size within a short growing season.

Cobia, *Rachycentron canadum* (Linnaeus, 1766), is a large migratory pelagic fish widely distributed in tropical, subtropical, and warm temperate seas around the world, except the Mediterranean Sea and the central and eastern Pacific Ocean (Hassler & Rainville, 1975; Shaffer & Nakamura, 1989). Cobia are an important species for cage and other intensive aquaculture production systems because of their large size (Denson, Stuart, Smith, Weirlch, & Segars, 2003), excellent meat quality (Chen, 2001), high market value (Liao et al., 2004), fast growth rate (Chou, Su, & Chen, 2001; Franks, Warren, & Buchanan, 1999), adaptability to production systems (Santos et al., 2012), ease of captive reproduction (Liao, Su, & Chang, 2001), and high demand for fillets and sashimi (Chou et al., 2001; Gopakumar et al., 2010). Salinity is an important environmental variable affecting the growth, survival, and feed intake of fishes (Arunachalam & Reddy, 1979; Boeuf & Payan, 2001; Lambert, Dutil, & Munro, 1994). The growth of euryhaline fish can be improved by rearing them at salinities that expend minimal energy for osmoregulation

(Sampaio & Bianchini, 2002). The requirement of aqueous K^+ for marine fish and shrimp also increases with salinity, thus making culture at higher salinities less economically sustainable in K^+-deficient inland saline groundwater because of higher costs incurred on K^+ supplementation (Partridge & Lymbery, 2008; Roy et al., 2010). The suitability of cobia as a candidate for aquaculture in semiarid saline groundwater regions in India would depend on its production performance at low and intermediate salinities of 5–15 g/L.

Although a few studies have been conducted to evaluate the performance of cobia in seawater of different salinities, results were highly variable. Denson et al. (2003) reported reduced growth in juvenile cobia reared in seawater of salinities of 5 and 15 g/L. Juvenile cobia subjected to salinity acclimation of 2 g/L day^{-1} demonstrated significant mortality at salinities below 8 g/L (Atwood, Young, Tomasso, & Smith, 2004). Chen et al. (2009) reported the maximum and minimum growth rate for cobia at 30 and 5 g/L respectively. Alternatively, Resley, Webb Jr, and Holt (2006) reported similar growth and survival rates of juvenile cobia reared in seawater of salinities of 5, 15, and 30 g/L, while Santos et al. (2012) observed optimal growth of cobia at 5 g/L. The euryhaline nature of cobia is often debated, and further expansion of production systems would require a better understanding of its performance at low salinities. Inland saline groundwater has been successfully used in the past to rear marine fish (Fielder et al., 2001; Forsberg et al., 1996; Partridge & Lymbery, 2008; Partridge & Lymbery, 2009). However, the suitability of inland saline groundwater as a medium to grow juvenile cobia has never been assessed. The objective of the present study was to evaluate the effects of different salinities (5, 10, and 15 g/L) on the production characteristics and osmoregulation of juvenile cobia reared in the saline groundwater of Haryana, India, in comparison to seawater of equivalent salinity.

2 | MATERIALS AND METHODS

2.1 | Experimental fish

This study was conducted at the Central Institute of Fisheries Education (CIFE), Rohtak Centre, Haryana, India, from May 2015 to October 2015. Cobia fry (35 days old, mean bodyweight of 1.78 ± 0.41 g, total length of 65.8 ± 8.3 mm) produced from the captive reproduction of wild brooders were obtained from the Marine Finfish Hatchery Project of Rajiv Gandhi Centre for Aquaculture (RGCA) in Kerala, India. The fry were transported via air freight following a 48-hr fast in a flow-through system (salinity ~29 g/L). Cobia fry were subjected to screening for Viral Nervous Necrosis Virus (VNNV) before transportation via Polymerase Chain Reaction (PCR) techniques. Upon arrival at the Centre, cobia fry were stocked in 1,200-L fiber-reinforced plastic (FRP) tanks filled with K^+-amended saline groundwater of salinity of 29 g/L at 100 fish per tank following an acclimation period of 2 hr (N = 800). Saline groundwater (34 g/L salinity) obtained from a deep tube well in the Centre was diluted using freshwater to obtain a salinity of 29 g/L and was supplemented using fertilizer-grade Muriate of potash to raise K^+ levels. The K^+-amended saline groundwater (29 g/L salinity) was gradually added to tanks in a flow-through mode to acclimate fishes from seawater to saline groundwater. The experimental fish started feeding immediately after stocking and were fed ad libitum four times a day using a commercial semisinking crumbled feed (size – 0.8 mm, Prince™ wean #2, Lucky Star Feeds, Taiwan) containing more than 55% crude protein (CP). Experimental fish were allowed to acclimate to the new system for 3 days, during which temperature, pH, salinity, and dissolved oxygen (DO) were maintained at 28.5°C, 8.23, 29 g/L, and > 5.8 mg/L, respectively, before salinity reduction.

2.2 | Treatment water types and ionic profile

The study's rearing media included two water types: K^+-amended inland saline groundwater (ISGW) and reconstituted seawater (RSW) of different salinities: 5, 10, and 15 g/L. Raw inland saline groundwater (IW) of salinity of

TABLE 1 Ionic profile of the amended inland saline groundwater and reconstituted seawater treatments, raw saline groundwater, and freshwater used in the trial expressed as mean ± SD (n = 20)

Water type	Treatments	Salinity (g/L)	Na^+ (mg/L)	K^+ (mg/L)	Mg^{2+} (mg/L)	Ca^{2+} (mgL)	$Na^+:K^+$ ratio	$Mg^{2+}:Ca^{2+}$ ratio	K^+ (%)
Inland saline groundwater	IW5	5.0 ± 0.2	1,390 ± 24	5.8 ± 0.7	287.5 ± 2.5	157.9 ± 3.4	241.3:1	1.82:1	10.5
	IW10	10.1 ± 0.1	2,795 ± 13	7.9 ± 0.5	564.4 ± 3.1	318.3 ± 2.9	354.4:1	1.77:1	7.2
	IW15	15.0 ± 0.2	4,130 ± 53	10.2 ± 0.4	843.1 ± 1.4	435.1 ± 4.7	412.1:1	1.93:1	6.3
	ISGW5	5.1 ± 0.2	1,443 ± 35	54.5 ± 1.2	318.1 ± 4.8	158.1 ± 1.9	27.1:1	2.01:1	100.0
	ISGW10	10.2 ± 0.1	2,890 ± 24	110.5 ± 2.4	636.4 ± 3.7	317.2 ± 3.3	26.7:1	2.00:1	100.4
	ISGW15	15.2 ± 0.1	4,247 ± 33	163.9 ± 1.7	885.6 ± 7.9	439.3 ± 5.1	26.4:1	2.01:1	100.1
Reconstituted Seawater	RSW5	5.1 ± 0.1	1,538 ± 21	55.0 ± 1.4	188.8 ± 4.5	54.2 ± 2.4	27.9:1	3.49:1	100.4
	RSW10	10.2 ± 0.2	3,191 ± 12	110.2 ± 1,1	376.5 ± 3.6	109.6 ± 2.1	28.7:1	3.45:1	99.9
	RSW15	15.1 ± 0.1	4,485 ± 46	162.8 ± 0.5	569.4 ± 2.1	161.7 ± 3.6	27.6:1	3.50:1	99.6
Freshwater	FW	0.5 ± 0.2	340 ± 6	5.4 ± 0.3	33.4 ± 1.2	38.5 ± 4.5	60.1:1	0.92:1	101.7

Abbreviations: Ca^{2+}, calcium ion; FW, freshwater; ISGW-K^+, amended inland saline groundwater; IW, raw inland saline groundwater; K^+, potassium ion; K^+ (%), percentage of potassium compared to seawater of the same salinity; Mg^{2+}, magnesium ion; $Mg^{2+}:Ca^{2+}$ ratio, magnesium ion to calcium ion ratio; Na^+, Sodium ion; $Na^+:K^+$ ratio, sodium ion to potassium ion ratio; RSW, reconstituted seawater.

15 g/L (IW15) was obtained from a tube well at CIFE. The water was pumped into a settling basin where it remained for 2 days and was then transferred to the indoor wet lab facility following filtration and UV sterilization. Freshwater (FW) of salinity ~0.7 g/L, obtained from a shallow aquifer, was used to dilute raw saline groundwater (15 g/L) to 10 g/L (IW10) and 5 g/L (IW5). The raw saline groundwater of different salinities was treated with liquid bleach (NaOCl) at an active chlorine concentration of 10 mg/L in 9,000-L epoxy-coated cement tanks and was subsequently stored. RSW of different salinities (5, 10, and 15 g/L designated as RSW5, RSW10, and RSW15, respectively) was produced using artificial sea salt (Instant Ocean® sea salt, Blacksburg, VA, USA). The artificial seawater (Table 1) was further reconstituted using laboratory-grade potassium chloride (KCl) and magnesium chloride flakes ($MgCl_2 \cdot 6H_2O$) so that the sodium-to-potassium ionic ratio (Na^+:K^+) and magnesium-to-calcium ionic ratio (Mg^{2+}:Ca^{2+}) of the culture medium remained similar to seawater, as described by Goldberg (1963). The raw saline groundwater of different salinities was fortified using technical-grade KCl to increase aqueous potassium (K^+) levels to 100% equivalent of seawater of the same salinity. Furthermore, $MgCl_2 \cdot 6H_2O$ flakes were also added to the culture medium to increase the Mg^{2+}:Ca^{2+} ratio to 2:1. The K^+-amended saline groundwater of salinities 5 (ISGW5), 10 (ISGW10), and 15 g/L (ISGW15) and the RSW were also bleached and stored in 9,000-L reservoir tanks. The ionic profiles of IW, RSW, and ISGW of different salinities are shown in Table 1. Salinity (g/L) of the culture medium was measured using a hand-held (Master S/Millα, Atago Co. Ltd., Tokyo, Japan) and a digital refractometer (PR-100SA, Atago Co. Ltd., Tokyo, Japan). The Na^+ and K^+ levels in the water were measured using flame photometry (Microprocessor flame photometer, Model 1382, Esico, Parwanoo, India). Total hardness and Ca^{2+} concentrations in the water were analyzed using ethylenediaminetetraacetic acid (EDTA) titrimetry (APHA, 1992), and the Mg^{2+} levels were determined through the calculation method (APHA, 1992). Furthermore, the concentrations of Na^+, K^+, Ca^{2+}, and Mg^{2+} were periodically measured (n = 5) using an atomic absorption spectrophotometer (AAnalyst 800, Perkin Elmer, Waltham, MA, USA) at CIFE, Mumbai.

2.3 | Acclimation

Salinity reduction was carried out in 1,200-L FRP tanks stocked with 50 cobia fry per tank. The salinity of the medium was reduced using FW (salinity ~0.7 g/L). Briefly, FW stored in 100-L containers above the acclimation tanks was allowed to flow into the tanks through a hose (5 mm inner diameter), and simultaneously, water was discharged from the acclimation tanks through a hose set opposite to the inlet hose. The addition of FW to tanks was carried out 2 hr after the first water exchange and feeding on all days during the experimental period. The reduction of salinity from 29 to 15 g/L was carried out at a rate of 3 g/L day^{-1}. After the completion of salinity reduction on each day, the fresh culture medium of the new salinity was prepared for the next water exchange of the day and the first exchange for the next day. The subsequent drop in salinity was achieved at 2 g/L day^{-1} from 15 to 7 g/L, followed by 1 g/L day^{-1} to attain 5 g/L. The fishes were observed to feed normally following salinity reduction, and no mortality was observed during the acclimation process. The fish were held at the treatment salinities for a minimum of 2 days before the commencement of experimental trials.

2.4 | Experiment 1: Effects of raw saline groundwater

This experiment was carried out to assess the effects of raw (unamended) saline groundwater (IW) on the behavioral pattern and survival of juvenile cobia in comparison to fish reared in K^+-amended saline groundwater (ISGW). Juvenile cobia (42 days old, mean bodyweight of 8.3 ± 0.93 g) were randomly distributed to 18 static 300-L FRP tanks (10 fish per tank): 9 filled with raw saline groundwater (IW) and the remaining 9 filled with K^+-amended saline groundwater (ISGW-100% K^+ fortified) of salinities of 5, 10, and 15 g/L in triplicate. Before the initiation of the study, juveniles were maintained in K^+-amended saline groundwater of different salinities from which the fish were harvested and restocked in the new media. The experimental tanks were continuously observed for fish mortality, and dead fish were immediately removed when noticed. Timings of the occurrence of fish mortality and cumulative mortality for each 24-hr period were recorded. The fish behavior was studied through visual assessment of the swimming pattern and the external morphology, but it was not quantified. Experimental fish were fed a commercial crumbled diet twice a day for the first 4 days, after which feeding was stopped in the raw saline groundwater tanks as the fish failed to accept the feed. However, feeding continued in the K^+-amended saline groundwater tanks throughout the experimental duration. The tanks were provided with continuous aeration, and the DO, pH, and water temperature were recorded twice daily. Cleaning and water exchange in all the tanks were performed daily at a rate of 70%. The experiment was terminated after 10 days because of complete mortality in all three raw saline groundwater treatments (IW).

2.5 | Experiment 2: Effects of K^+-amended saline groundwater

This experiment was conducted to determine the production characteristics and osmoregulation of juvenile cobia in K^+-amended saline groundwater (ISGW-100% K^+ fortified) and RSW of different salinities. The study was comprised of six treatment salinities, namely, ISGW5, ISGW10, and ISGW15 (K^+-amended inland saline groundwater of salinities 5, 10, and 15 g/L respectively) and RSW5, RSW10, and RSW15 (reconstituted artificial seawater of salinities 5, 10, and 15 g/L, respectively). Juvenile cobia (45 days old, mean bodyweight ~9.23 ± 0.120 g) were individually weighed and graded to a uniform size before random distribution to 24 static 550-L circular FRP (diameter 1.2 m) tanks (10 fish per tank) filled with water of different treatment salinities. The study followed a completely randomized design where each treatment salinity was replicated in four tanks. The complete experimental setup was connected to two magnetic air pumps (HiBlow, HP200, Techno Takatsuki, Osaka, Japan), which worked by alternately supplying air to individual tanks through two air diffusers, thus maintaining acceptable DO levels. Each experimental unit was equipped with a protein skimmer (Coral Life Super Skimmer 65G, Central Aquatics, Franklin, WI, USA) and an external canister filter (HW 507A, SunSun, Zhejiang, China) to maintain optimal water quality. Water temperature-regulating equipment was not used as the study was conducted during the summer where ambient temperature remained high. A natural photoperiod (14–15 hr light) was maintained in the wet lab during the study. All the tanks were covered with stretched polyethylene netting to prevent the loss of fish from jumping. Experimental fish were fed three times a day ad libitum using a commercial semisinking crumbled feed (size 1.2 mm, Prince™ wean #3, CP > 55%) for the first 15 days, followed by floating pellets (1.8 mm, Nutrila™, Growel feeds, Krishna, India, CP ~45%) for 30 days and larger pellets (3.0 mm, Nutrila™, CP ~40%) for the final 15 days. The feed was weighed before feeding, and uneaten feed, if any, was removed immediately. The experimental tanks were cleaned 3 hr following the first feeding, and 100% water exchange was carried out daily using fresh media stored in 9,000-L cement tanks. The DO, pH, salinity, and water temperature of the treatment groups were measured twice daily using a multiparameter probe (YSI 556, YSI Incorporated, Yellow Springs, OH, USA). Total ammonia nitrogen (TAN) and nitrite–nitrogen (NO_2-N) were determined once daily (1,800 hr) using the phenate method (APHA, 1992) and colorimetry (APHA, 1992), respectively. Water quality parameters during the trial did not vary significantly, and mean values along with ranges of temperature, pH, DO, TAN, and NO_2-N were 29.3 ± 0.11°C (28.0–30.7°C), 8.22 ± 0.08 (8.03–8.35), 6.39 ± 0.14 mg/L (5.92–7.60 mg/L), 0.06 ± 0.001 mg/L (0–0.1 mg/L), and 0.11 ± 0.02 mg/L (0–0.19 mg/L), respectively. The salinity of different treatment groups was monitored daily, and measured values did not vary by more than 0.3 g/L of the original target treatment salinity.

2.6 | Sampling and analysis

In the first study, swimming behavior, response to external stimuli, and morphological changes of juvenile cobia reared in raw saline groundwater were recorded. Dead and moribund fish were removed immediately following observation, and the timing (date/time) of fish mortality was noted for each treatment group. In the second study, all experimental fish were individually weighed and counted at the end of the 30th and 60th days after being anesthetized using AQUI-S (Aqui S New Zealand Ltd., Lower Hutt, New Zealand) at a dose of 20 mg/L. Survival rate (%) of the treatment groups was calculated as the number of fish at the end of the 60th day divided by the number of fish stocked on the 0th day multiplied by 100. Weight gain (WG) (%) and specific growth rate (SGR) (%/day) were determined to assess growth and were measured as final weight − initial weight/initial weight × 100 and [ln(final weight) − ln(initial weight)]/experimental duration × 100, respectively. Feed conversion ratio (FCR) was measured as the dry weight of feed consumed divided by the wet weight gain.

On the 30th and 60th days, 12 fish from each treatment group (3 fish per replicate) were collected and anesthetized (AQUI-S, 40 mg/L) for blood collection. Blood (1–2 mL) was obtained from the caudal peduncle using a 2-mL syringe (22G × 1″ needle, Dispo Van, HMD Ltd., Haryana, India) and transferred to untreated 2-mL tubes that were placed in a refrigerator at 5°C for 30 min. Blood samples were centrifuged at 3,000 rpm (4°C, 10 min) using a refrigerated centrifuge (RA-2314, REMI, Mumbai, India) to obtain serum. Serum samples were stored at −80°C for further analysis. Serum and medium osmolality were measured using a cryoscopic osmometer (Osmomat® 030, Gonotec GmbH, Berlin, Germany). Serum Na^+ and K^+ levels were determined using flame photometry (Esico). Serum total protein levels of the different treatments groups were estimated through the colorimetric method described by Bradford (1976) using a total protein kit (TP0100, Sigma Aldrich, Saint Louis, MO, USA). Deskinned muscle separated from the dorsal region of fish from each treatment group (n = 8) was used to obtain muscle moisture (%) by drying muscle samples at 105°C for 3 hr until a constant weight was obtained according to established techniques (AOAC, 1999).

2.7 | Statistical analysis

All values were expressed as mean ± standard error (SE) unless otherwise mentioned. Normality and homogeneity of variances were analyzed using the Shapiro–Wilk test and Levene's test, respectively, where necessary. Differences between treatment groups (salinities) were tested using one-way analysis of variance (ANOVA) at $p < .05$, and means were compared using Tukey's multiple comparison tests ($p < .05$). All statistical analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) unless otherwise stated. The slope of the relationship between medium and serum osmolality were determined using linear regression analysis (Ferraris, Parado-Estepa, Ladja, & de Jesus, 1986). The significance of the linear relationship between the two variables and the correlation coefficients was tested using a LinReg T-test on Microsoft Office Excel (MS Office Professional Plus 2016, Microsoft, Redmond, RCW).

3 | RESULTS

In the first study, juvenile cobia reared in raw saline groundwater (15 g/L salinity) exhibited abnormal swimming behavior (swimming with head pointed toward the water surface), loss of equilibrium, excess mucus secretion, changes in coloration (dark patches with intermittent shining spots), and flaring of the operculum following 18 hr of exposure. Similar behaviors were exhibited by fish in 10 and 5 g/L after 26 and 54 hr, respectively. Feed intake was significantly poor, and fish completely stopped feeding after 12, 16, and 40 hr at salinities of 15, 10, and 5 g/L, respectively. Initial mortalities were observed within 48 hr of exposure for the 15 g/L raw saline groundwater

TABLE 2 Cumulative mortality (replicates combined, *n* = 3) of juvenile cobia at 24 hr intervals during exposure to raw saline groundwater (IW) and K^+-amended saline groundwater (ISGW) of salinities 5, 10, and 15 g/L

				Exposure time intervals (24 hr)								
				24 hr	48 hr	72 hr	96 hr	120 hr	144 hr	168	192	216
Water type	Treatment	Salinity (g/L)	Number of fish	Cumulative mortality (%)								
Raw inland saline groundwater	IW5	5	30	0	0	0	0	6.66	26.66	53.33	85.66	100
	IW10	10	30	0	0	0	13.33	36.66	66.66	90	100	-
	IW15	15	30	0	10	36.66	83.33	100	-	-	-	-
K^+-amended inland saline groundwater	ISGW5	5	30	0	0	0	0	0	0	0	0	0
	ISGW10	10	30	0	0	0	0	0	0	0	0	0
	ISGW15	15	30	0	0	0	0	0	0	0	0	0

Abbreviations: ISGW-K^+, amended inland saline groundwater; IW-raw inland saline groundwater.

TABLE 3 Production characteristics of juvenile cobia reared in K^+-amended saline groundwater and reconstituted seawater of salinities of 5, 10, and 15 g/L. Values expressed as mean ± SE

Treatment	Initial weight (g)	Bodyweight 30th day (g)	Bodyweight 60th day (g)	WG (%)	SGR (%/day)	FCR
ISGW5	9.31 ± 0.26[a]	51.60 ± 1.46[a]	103.23 ± 3.58[a]	1,031.6 ± 47.4[a]	7.53 ± 0.06[a]	1.14 ± 0.018[a]
ISGW10	9.15 ± 0.25[a]	54.00 ± 1.52[a]	113.44 ± 2.77[a]	1,156.0 ± 43.0[a]	7.72 ± 0.04[a]	1.13 ± 0.018[a]
ISGW15	9.20 ± 0.26[a]	52.40 ± 1.49[a]	115.82 ± 3.54[a]	1,178.2 ± 54.9[a]	7.75 ± 0.06[a]	1.14 ± 0.005[a]
RSW5	9.29 ± 0.27[a]	55.75 ± 1.64[a]	111.22 ± 3.40[a]	1,109.9 ± 37.7[a]	7.69 ± 0.05[a]	1.10 ± 0.015[a]
RSW10	9.30 ± 0.43[a]	55.88 ± 2.57[a]	113.83 ± 5.35[a]	1,156.5 ± 70.4[a]	7.71 ± 0.08[a]	1.10 ± 0.010[a]
RSW15	9.15 ± 0.35[a]	51.55 ± 1.96[a]	113.23 ± 3.24[a]	1,163.3 ± 59.6[a]	7.71 ± 0.05[a]	1.14 ± 0.015[a]
F statistic	0.0625	1.1726	1.7987	1.2582	1.9212	1.4775
p value	.9973	.3256	.1171	.2857	.0947	.2874

Note: Different superscript letters with the same column indicate a significant difference ($p < .05$). Survival rate of fishes in all the treatment groups across all relocation ($n = 4$) were 100% as all fish survived the experiment. Abbreviations: ISGW5, ISGW10, and ISGW15 are amended saline groundwater (100% potassium fortified) of salinities of 5, 10, and 15 g/L, respectively, and RSW5, RSW10, and RSW15 are reconstituted seawater of salinities of 5, 10, and 15 g/L respectively.

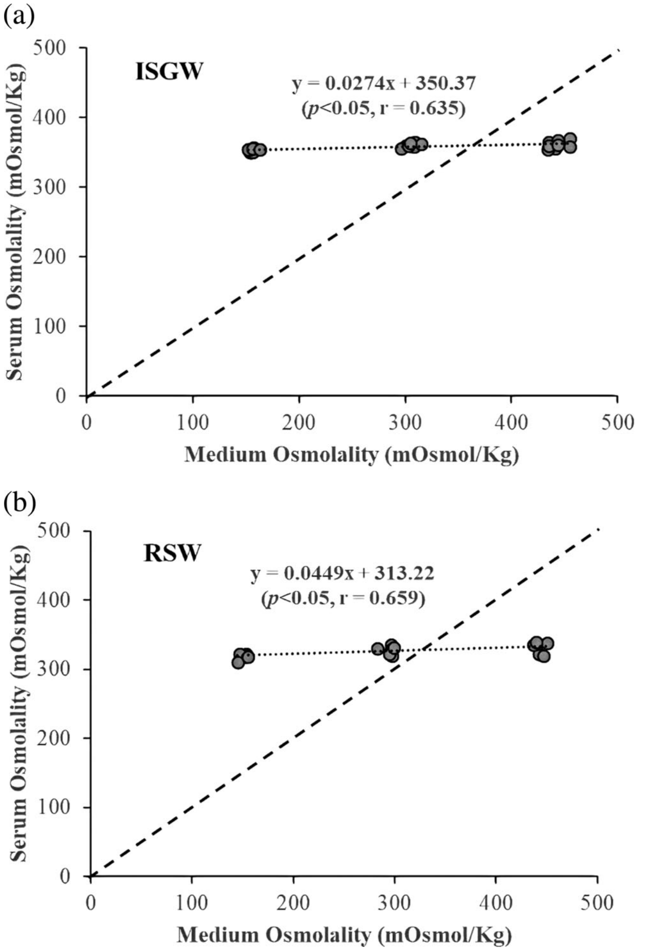

FIGURE 1 Linear relationship between medium osmolality (*x*) and serum osmolality (*y*) for juvenile cobia reared at different salinities in two water types: (a) K^+-amended inland saline groundwater and (b) reconstituted seawater. The linear regression equation and correlation coefficient for the two water types are shown in the figure

TABLE 4 Physiological characteristics of juvenile cobia reared in K^+-amended saline groundwater and reconstituted seawater of different salinities following 60 days of culture. Values expressed as mean ± SE

Treatment	Serum osmolality (mOsmol/Kg)	Serum Na^+ levels (mmol/L)	Serum K^+ levels (mmol/L)	Serum total protein (g/dL)	Muscle moisture (%)
ISGW5	353.75 ± 3.64^a	166.05 ± 2.05^{a,b}	5.11 ± 0.23 ^{b}	3.44 ± 0.12^a	75.76 ± 0.47^a
ISGW10	361.62 ± 3.47^a	170.46 ± 2.08^a	6.07 ± 0.33^{a,b}	3.50 ± 0.07^a	76.09 ± 0.73^a
ISGW15	361.55 ± 4.43^a	172.42 ± 3.68^a	6.71 ± 0.16 ^{a}	3.57 ± 0.11^a	76.80 ± 1.02^a
RSW5	319.16 ± 3.43^b	151.81 ± 0.87^c	5.21 ± 0.21^b	3.40 ± 0.09^a	75.76 ± 1.06^a
RSW10	328.83 ± 3.26^b	156.41 ± 1.19^{b,c}	6.06 ± 0.22^{a,b}	3.44 ± 0.15^a	76.50 ± 0.57^a
RSW15	332.33 ± 5.21^b	156.60 ± 1.09^{b,c}	6.69 ± 0.25^a	3.48 ± 0.11^a	76.12 ± 1.00^a
F statistic	20.22	11.53	8.04	0.2583	0.2398
p value	<.0001	<.0001	<.0001	.9328	.9408

Note: Different superscript letters with the same column indicate a significant difference ($p < .05$). Abbreviations: ISGW5, ISGW10, and ISGW15 are amended saline groundwater (100% potassium fortified) of salinities of 5, 10, and 15 g/L, respectively, and RSW5, RSW10, and RSW15 are reconstituted seawater of salinities of 5, 10, and 15 g/L, respectively.

treatment, and 100% mortality occurred 108 hr following stocking in this treatment. In raw saline groundwater of salinities of 10 and 5 g/L, the first fish mortality was observed 86 and 106 hr after stocking, respectively. All the fish died following 176 and 195 hr of exposure in raw saline groundwater of salinities of 10 and 5 g/L, respectively. Cumulative mortality rates (replicates combined) of juvenile cobia at 24-hr intervals following exposure to raw saline groundwater of different salinities are shown in Table 2. During the same period, no fish died in K^+-amended saline groundwater, and all fish were feeding normally.

The production performance of juvenile cobia reared in K^+-amended saline groundwater and seawater of different salinities in the second experiment is shown in Table 3. The mean final weight of juvenile cobia at the end of 30 and 60 days did not vary significantly ($p > .05$) between treatments. Final mean bodyweight of fish reared in 5 g/L seawater (RSW5 ~111.2 g) and saline groundwater (ISGW5 ~103.2 g) was similar to that observed at higher salinities for both water types (113.2–115.8 g). During the experimental period (60 days), no fish mortality was observed in any of the treatment groups, and all fish survived. The coloration, general activity, and feed intake of fish during the experiments were found to be similar for all treatment groups. WG (1,031–1,178%) and SGR (7.53–7.75%/day) of cobia juveniles also did not vary significantly ($p > .05$) among treatment salinities. The FCRs following 60 days of culture were similar for all treatments.

Serum osmolality of juvenile cobia reared in K^+-amended saline groundwater (353.7–361.6 mOsmol/Kg) was significantly higher ($p < .05$) than for fish raised in seawater (319.1–332.3 mOsmol/Kg). However, no differences ($p > .05$) were observed in serum osmolality of fish reared at different salinities (5, 10, and 15 g/L) within the two water types (RSW and K^+-amended saline groundwater). Serum osmolality increased with salinity in both water types. The relationship between serum and medium osmolality for juvenile cobia reared in K^+-amended saline groundwater of different salinities followed a slope of 0.0274 (Figure 1(a)). In the case of cobia reared in RSW of different salinities, the relationship between medium and serum osmolality resulted in a slope of 0.044 (Figure 1(b)). Serum osmolality was hyper-regulated at salinities of 5 g/L and 10 g/L and hyporegulated in media of salinity of 15 g/L for fish reared in both water types.

Serum Na^+ levels were significantly higher ($p < .05$) in fish reared in K^+-amended saline groundwater (166.05–172.42 mmol/L) than those reared in seawater (151.8–156.6 mmol/L) except for treatment ISGW5, which did not show differences with treatments RSW10 or RSW15. Serum Na^+ concentrations, however, did not vary between different salinities (5, 10, and 15 g/L) in either water type. Serum K^+ levels increased with salinity, and fish at 15 g/L (6.69–6.71 mmol/L) salinity exhibited significantly higher ($p < .05$) K^+ levels than fish stocked at 5 g/L (5.11–5.21 mmol/L) for both seawater and K^+-amended saline groundwater. However, differences were not observed in the serum K^+ levels between salinities of 5 and 10 g/L or 10 and 15 g/L in either water type. Serum Na^+ levels were hyper-regulated at salinities of 5 and 10 g/L and hyporegulated at a salinity of 15 g/L for fish reared in both RSW and K^+-amended saline groundwater. However, serum K^+ levels were hyper-regulated in all the salinities (5, 10, and 15 g/L) in either water type. Serum osmolality and the Na^+ and K^+ levels of fish reared in different treatment groups are shown in Table 4. Serum total protein levels (3.44–3.57 g/dL) were similar ($p > .05$) for fish reared in seawater and K^+-amended saline groundwater at all salinities (Table 4). Muscle moisture levels did not vary significantly ($p > .05$) among treatment groups following 60 days of culture (Table 4).

4 | DISCUSSION

Saline groundwater at Rohtak, Haryana is deficient in K^+ (6.3–10.5% equivalent to seawater of identical salinity). The results of the first experiment suggest that K^+-deficient raw saline groundwater is not a suitable medium to grow marine teleosts such as cobia as total mortality was observed in 4.5–8 days at different salinities. Fielder et al. (2001) reported complete mortality of Australian snapper, *Pagrus auratus*, reared in K^+-deficient raw saline groundwater of salinity of 19.6 g/L (K^+ – 9.2 mg/L; Na^+:K^+ ratio of 458:1) within 4 days and observed similar behavioral changes in fish to those seen in our experiment. Similarly, juvenile barramundi, *Lates calcarifer*, reared in K^+-deficient saline groundwater of salinity of 45 g/L encountered complete mortality after a few days (Partridge & Creeper, 2004; Partridge & Lymbery, 2008). Total mortality was also reported for juvenile rabbitfish, *Siganus rivulatus*, and mulloway, *Argyrosomus japonicus*, reared in saline groundwater of equivalent K^+ levels below 15 and 38%, respectively (Doroudi et al., 2006; Mourad et al., 2012). The complete mortality of juvenile cobia in raw saline groundwater is likely explained by the acute deficiency of K^+ in the medium. Being a major intracellular cation, K^+ plays a significant role in osmoregulation in fish through the modulation of Na^+ and K^+ adenosine triphosphatase activity and the maintenance of constant osmolality through homeostasis with other extracellular ions (Marshall & Grosell, 2006; McCormick, 2001; Teeter, 1997). The requirement of K^+ in fish is primarily fulfilled through the diffusion of K^+ ions via the gills and gut (Wilson & El Naggar, 1992), and marine teleosts have evolved in an environment (seawater) containing high levels of K^+ from which the requirements for osmotic and ionic regulation were realized. Under circumstances of environmental K^+ deficiency (low aqueous K^+), osmoregulation would consume a more substantial portion of the animal's energy budget, resulting in reduced growth and survival (Bryan, Ham, & Neill, 1988; Partridge & Creeper, 2004; Partridge & Lymbery, 2008). Partridge and Creeper (2004) observed degeneration and necrosis of skeletal muscle (hypokalemic myopathy) for juvenile barramundi reared in K^+-deficient saline groundwater, resulting in high mortality. Sustained K^+ deficiency in the rearing medium may have caused the lowering of blood K^+ levels in juvenile cobia, resulting in an osmotic disruption that ultimately led to muscular myopathy and subsequent mortality, as described by Partridge and Creeper (2004).

Survival of juvenile cobia in raw saline groundwater was reduced with the salinity of the medium as fish reared at higher salinities exhibited stress symptoms and mortality within shorter periods of exposure. In the present study, deficiency of K^+ was observed to increase with salinity and is evident from the ascending Na^+:K^+ ratios (5 g/L ~241:1; 10 g/L ~354:1; and 15 g/L ~412:1) (Table 1). The low tolerance of juvenile cobia at 15 g/L may be potentially because of the higher medium Na^+:K^+ ratio, resulting in an inverse relationship between survival and the Na^+:K^+ ratio, as proposed by Forsberg et al. (1996). The mortality of juvenile cobia in raw saline groundwater of different salinities cannot be attributed to high levels of calcium (269–291% equivalence to RSW) and magnesium (148–152% equivalence to RSW), resulting in Mg^{2+}:Ca^{2+} ratios ranging between 1.7:1 and 1.9:1 (Table 1), for example, Forsberg et al. (1996) reported acceptable survival of red drum, *Sciaenops ocellatus*, in raw saline groundwater with Mg^{2+}:Ca^{2+} ratios varying from 0.47:1 to 2.17:1.

In the second experiment, no mortality was observed for juvenile cobia reared in both RSW and K^+-amended saline groundwater (100% K^+ fortified) of salinities of 5, 10, and 15 g/L. Stieglitz, Benetti, and Serafy (2012) reported 60–100% survival of juvenile cobia subjected to abrupt changes in salinity from 35 g/L to 5 and 11 g/L after 24 hr, indicating the salinity tolerance range of the species. High survival rates during long-term growth assays in seawater of low salinities (0–15 g/L) has been previously reported for several species of marine fish, namely, flounder *Paralichthys orbignyanus*, Lebranche mullet *Mugil Liza*, and black bream *Acanthopagrus butcheri* (Lisboa, Barcarolli, Sampaio, & Bianchini, 2015; Partridge & Jenkins, 2002; Sampaio & Bianchini, 2002). Australian snapper exhibited complete survival in K^+-fortified (60–100%) saline groundwater and seawater of the same salinity at the end of 42 days (Fielder et al., 2001). Partridge and Lymbery (2008) observed 100% survival of juvenile barramundi in K^+-fortified saline groundwater of salinities of 5 and 15 g/L. Likewise, rabbitfish and mulloway reared in K^+-fortified saline groundwater displayed high survival similar to that reported in seawater (Doroudi et al., 2006; Mourad et al., 2012). The results of our experiments suggest that juvenile cobia can also thrive in K^+-amended low salinity groundwater.

In this study, cobia reared at 5 g/L exhibited excellent survival, similar to fish reared at 10 and 15 g/L in both water types. Denson et al. (2003) experienced numerically lower survival of cobia at 5 g/L compared to fish reared at 15 and 30 g/L and stated that the fish at 5 g/L displayed eroded fins, ulcerations, and unusual gray coloration. However, the 5 g/L water used by Denson et al. (2003) was saline well water and not seawater diluted to 5 g/L using FW, resulting in an Mg^{2+}:Ca^{2+} ratio of 0.63:1. The Mg^{2+}:Ca^{2+} ratio of seawater and ISGW of 5 g/L used in this study was 3.45:1 and 2.01:1, respectively, and is notably higher than the ratio reported by Denson et al. (2003). In this study, magnesium supplementation was carried out in saline groundwater of all salinities to maintain the Mg^{2+}:Ca^{2+}

ratio at a minimum of 2:1. Juvenile cobia may, therefore, require a minimum Mg^{2+}:Ca^{2+} ratio of 2:1 for normal survival and growth at low and high salinities, and such requirements may be species specific. Forsberg et al. (1996) achieved high survival of red drum in saline groundwater with Mg^{2+}:Ca^{2+} ratios as low as 0.47:1. Cobia reared at 5 g/L in this study did not show signs of body lesions/fin erosion and were as actively feeding as fish reared in other salinities. Resley et al. (2006) reported high survival of juvenile cobia reared in 5 g/L seawater and observed no significant differences between the salinities of 5, 15, and 30 g/L. However, in a second trial, the authors reported a lower survival at 5 g/L. Atwood et al. (2004) also reported a poor survival rate of cobia at salinities below 8 g/L. Although these observations could not be adequately explained, the performance of the fish is a function of its genetics, pathological status, and environmental conditions, including the ionic profile of the rearing medium. Juvenile cobia used in this trial were obtained from wild brooders after VNN screening and reared in disease-free inland saline groundwater (Gong, Jiang, Lightner, Collins, & Brock, 2004), maintaining optimal water quality conditions for the species in both water types. These factors may have resulted in optimal survival at all salinities. Santos et al. (2012) reported complete survival of juvenile cobia reared at 5 g/L during a trial that evaluated the effects of dietary sodium chloride on growth at low salinities. A high survival rate of juvenile cobia at different salinities is not uncommon as several other authors have reported 100% survival of cobia at identical salinities (Burkey, Young, Smith, & Tomasso, 2007:5–14 g/L; Zhou, Wu, Chi, & Yang, 2007:21–23 g/L; Chen et al., 2009:5–35 g/L).

Growth characteristics of juvenile cobia in K^+-amended saline groundwater and RSW of different salinities were very good and did not differ between salinities and water type. Growth and SGR of several marine fish such as Atlantic cod *Gadus morhua*, turbot *Scophthalmus maximus*, black bream, and flounder at low and intermediate salinities (5–15 g/L) have been reported to be similar or even better than oceanic salinities (Gaumet, Boeuf, Severe, Le Roux, & Mayer-Gostan, 1995; Lambert et al., 1994; Partridge & Jenkins, 2002; Sampaio & Bianchini, 2002). Similar observations were made for red drum, Australian snapper, mulloway, and barramundi, where fish reared in K^+-fortified saline groundwater and seawater displayed similar growth characteristics (Doroudi et al., 2006; Fielder et al., 2001; Forsberg et al., 1996; Partridge & Lymbery, 2008). These results suggest that K^+-amended saline groundwater as a medium is suitable for growing cobia in inland regions. The production characteristics of juvenile cobia reared in 5 g/L saline groundwater and seawater were similar to fish stocked at higher salinities. The results of this study are in agreement with previous observations of Resley et al. (2006). Santos et al. (2012) found similar growth rates for cobia fed normal and sodium chloride-supplemented diets when reared at 5 g/L and concluded that mineral requirements of the species were met adequately from the low-salinity culture medium. This further establishes the euryhaline nature of cobia and its adaptability to commercial low-salinity culture. The mean SGR of juvenile cobia reared in seawater (7.7%/day) and K^+-amended saline groundwater (7.6%/day) of different salinities in the present study was 7.68%/day. High SGR is not uncommon in cobia as similar growth rates have been previously reported for the species: 7.1–7.3%/day (Weirich, Smith, Denson, Stokes, & Jenkins, 2004), 5.2%/day (Resley et al., 2006), and 5.99–7.01%/day (Zhou et al., 2007). Resley et al. (2006) reported a significantly lower FCR (0.95–0.88) for juvenile cobia reared at 5, 10, and 30 g/L, although no differences were noted between salinities as observed in this study. The FCR demonstrated by experimental fish in this study (1.1–1.14) was similar to the FCR reported by other authors for cobia fed on diets with similar CP levels: 1.18 (CP 44%, Chou et al., 2001), 1.19 (CP 48%, Chou et al., 2004), and 1.10–1.16 (CP 44%, Zhou et al., 2007).

Serum osmolality of juvenile cobia followed a positive linear relationship with the salinity of the medium in both water types. Fish stocked in RSE of salinities of 5, 10, and 15 g/L exhibited serum osmolality similar to previously reported data for the species in seawater of identical salinities (Burkey et al., 2007; Denson et al., 2003; Resley et al., 2006). However, juvenile cobia reared in K^+-amended saline groundwater exhibited significantly higher serum osmolality (353–361 mOsmol/Kg) than fish in seawater of identical salinities. Mourad et al. (2012) observed a similar phenomenon for rabbitfish reared in K^+-fortified saline groundwater, where fish displayed significantly higher serum osmolality (426 mmol/Kg) compared to those reared in seawater (383.4 mmol/Kg) of equivalent salinity. The variability in the ionic composition between RSW and K^+-amended saline groundwater may have caused the differences in serum osmolality between the two water types, as assumed by Mourad et al. (2012). Higher serum osmolality of experimental fish in saline groundwater did not appear to affect the growth performance. Moreover, as the slopes of the relationship between serum and ambient water osmolality in either water type were comparable, and salinity only affected serum osmolality slightly, juvenile cobia exhibits physiological euryhalinity for osmoregulating normally in different salinities and water types. Comparable serum osmolality was reported for barramundi and mulloway reared in K^+-supplemented saline groundwater of 5 g/L and 15 g/L (Partridge & Lymbery, 2008; Partridge & Lymbery, 2009). Supplementation of K^+ and Mg^{2+} at the requisite levels in saline groundwater may have caused similar metabolic energy expenditure for osmoregulation in RSW and saline groundwater, thus resulting in consistent growth performance of juvenile cobia in both water types.

Serum Na^+ (166–172 mmol/L) and K^+ (5.11–6.71 mmol/L) of juvenile cobia reared in K^+-amended saline groundwater were similar to those reported for barramundi (Na^+: 161–162 mmol/L, K^+: 4.9–7.5 mmol/L), mulloway (Na^+: 165–167 mmol/L, K^+: 5–7 mmol/L), and rabbitfish (Na^+: 176 mmol/L, K^+: 6.5 mmol/L) reared in 100% K^+-fortified saline groundwater of similar salinities (Mourad et al., 2012; Partridge & Lymbery, 2008; Partridge & Lymbery, 2009). Serum K^+ decreased with the medium K^+ levels, although differences were not observed between the water types at identical salinities. Similar serum K^+ levels for fish raised in either water type may be because of the maintenance of equivalent aqueous K^+ levels in RSW and saline groundwater at a particular salinity. It is difficult to explain the significantly higher serum Na^+ levels of experimental fish reared in K^+-amended saline groundwater compared to RSW despite near-identical aqueous Na^+ levels in both water types. However, as serum Na^+ levels did not vary between salinities by water type, such an adaption by fish in saline groundwater may be associated with the interaction of other ions, which was not analyzed as part of this study. Serum total protein, an indicator of fish health that can be influenced by environmental salinity (Verdegem, Hilbrands, & Boon, 1997), did not vary among treatment groups. This would suggest that overall normal fish metabolism occurred in experimental fish during this study at low salinities using K^+-amended saline groundwater.

Overall, the collective results of this study suggest that cobia exhibit typical euryhaline behavior under the reported culture conditions and can be successfully reared in low-salinity (5–15 g/L) K^+-amended saline groundwater. The growth performance of juvenile cobia makes them an attractive candidate for commercial low and intermediate salinity culture operations in inland and coastal regions. Cobia is ideally suited for shorter growing seasons, such as those available in northern India. Production of cobia using saline groundwater in areas where this unique water resource is available would provide a commercial aquaculture development opportunity for inland mariculture in this region.

ACKNOWLEDGMENT

We thank the technical staff of Rohtak Centre of CIFE, Rohtak, Haryana, India for their support during the research work. We also thank Mr. Johnson D'Cruz, Project manager, RGCA, Pozhiyoor, Kerala, for supplying cobia fry for the study. This study was funded by the Indian Council of Agricultural Research (ICAR), New Delhi, and partly by an INSPIRE scholarship of the Department of Science and Technology (DST), Govt. of India.

CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

ORCID

Jose Antony https://orcid.org/0000-0002-1805-4459
Luke A Roy https://orcid.org/0000-0003-4498-6871

REFERENCES

American public health association. (1992). *Standard methods for the examination of water and wastewater* (18th ed.). Washington, DC: APHA.

Arunachalam, S., & Reddy, S. R. (1979). Food intake, growth, food conversion, and body composition of catfish exposed to different salinities. *Aquaculture*, *16*(2), 163–171.

Association of official analytical chemists. (1999). *Official methods of analysis* (16th ed.). AOAC: Washington, DC.

Atwood, H. L., Young, S. P., Tomasso, J. R., & Smith, T. I. J. (2004). Resistance of cobia, *Rachycentron canadum*, juveniles to low salinity, low temperature, and high environmental nitrite concentrations. *Journal of Applied Aquaculture*, *15*(3–4), 191–195.

Barman, U. K., Jana, S. N., Garg, S. K., Bhatnagar, A., & Arasu, A. R. T. (2005). Effect of inland water salinity on growth, feed conversion efficiency and intestinal enzyme activity in growing grey mullet, *Mugil cephalus* (Linn.): Field and laboratory studies. *Aquaculture International*, *13*(3), 241–256.

Boeuf, G., & Payan, P. (2001). How should salinity influence fish growth? *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *130*(4), 411–423.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1–2), 248–254.

Bryan, J. D., Ham, K. D., & Neill, W. H. (1988). Biophysical model of osmoregulation and its metabolic cost in red drum. *Contributions in Marine Science*, *30*, 169–182.

Burkey, K., Young, S. P., Smith, T. I. J., & Tomasso, J. R. (2007). Low-salinity resistance of juvenile cobias. *North American Journal of Aquaculture*, *69*(3), 271–274.

CGWB (2018). Ground water quality features of the country [pdf file]. Retrieved from http://cgwb.gov.in/WQ/GROUND%20WATER%20QUALITY%20SCENARIO%20IN%20INDIA.pdf

Chen, B. S. (2001). Studies on the net-cage culture and disease control technology of cobia *Rachycentron canadum*. In *Sixth Asian fisheries forum book of abstracts*. Manila: Asian Fisheries Society.

Chen, G., Wang, Z., Wu, Z., Gu, B., Wang, Z., Wang, Z., & Wu, Z. (2009). Effects of salinity on growth and energy budget of juvenile cobia, *Rachycentron canadum*. *Journal of the World Aquaculture Society*, *40*(3), 374–382.

Chou, R. L., Her, B. Y., Su, M. S., Hwang, G., Wu, Y. H., & Chen, H. Y. (2004). Substituting fish meal with soybean meal in diets of juvenile cobia *Rachycentron canadum*. *Aquaculture*, *229*(1–4), 325–333.

Chou, R. L., Su, M. S., & Chen, H. Y. (2001). Optimal dietary protein and lipid levels for juvenile cobia (*Rachycentron canadum*). *Aquaculture*, *193*(1–2), 81–89.

Denson, M. R., Stuart, K. R., Smith, T. I., Weirlch, C. R., & Segars, A. (2003). Effects of salinity on growth, survival, and selected hematological parameters of juvenile cobia *Rachycentron canadum*. *Journal of the World Aquaculture Society*, *34*(4), 496–504.

Doroudi, M. S., Fielder, D. S., Allan, G. L., & Webster, G. K. (2006). Combined effects of salinity and potassium concentration on juvenile mulloway (*Argyrosomus japonicus*, Temminck and Schlegel) in inland saline groundwater. *Aquaculture Research*, *37*(10), 1034–1039.

Evans, D. H., Piermarini, P. M., & Choe, K. P. (2005). The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiological Reviews*, *85*(1), 97–177.

Ferraris, R. P., Parado-Estepa, F. D., Ladja, J. M., & de Jesus, E. G. (1986). Effect of salinity on the osmotic, chloride, total protein and calcium concentrations in the hemolymph of the prawn *Peneaus monodon* (Fabricius). *Comparative Biochemistry and Physiology Part A: Physiology*, *83*(4), 701–708.

Fielder, D. S., Bardsley, W. J., & Allan, G. L. (2001). Survival and growth of Australian snapper, *Pagrus auratus*, in saline groundwater from inland New South Wales, Australia. *Aquaculture*, *201*(1–2), 73–90.

Forsberg, J. A., Dorsett, P. W., & Neill, W. H. (1996). Survival and growth of red drum *Sciaenops ocellatus* in saline groundwaters of West Texas, USA. *Journal of the World Aquaculture Society*, *27*(4), 462–474.

Forsberg, J. A., & Neill, W. H. (1997). Saline groundwater as an aquaculture medium: Physiological studies on the red drum, *Sciaenops ocellatus*. *Environmental Biology of Fishes*, *49*(1), 119–128.

Franks, J. S., Warren, J. R., & Buchanan, M. V. (1999). Age and growth of cobia, *Rachycentron canadum*, from the northeastern Gulf of Mexico. *Fishery Bulletin*, *97*(3), 459.

Gaumet, F., Boeuf, G., Severe, A., Le Roux, A., & Mayer-Gostan, N. (1995). Effects of salinity on the ionic balance and growth of juvenile turbot. *Journal of Fish Biology*, *47*(5), 865–876.

Goldberg, E. D. (1963). The oceans as a chemical system. *The Sea*, *12*, 3–25.

Gong, H., Jiang, D. H., Lightner, D. V., Collins, C., & Brock, D. (2004). A dietary modification approach to improve the osmoregulatory capacity of *Litopenaeus vannamei* cultured in the Arizona desert. *Aquaculture Nutrition*, *10*(4), 227–236.

Gopakumar, G., Rao, G. S., Nazar, A. A., Kalidas, C., Tamilmani, G., Sakthivel, M., … Rao, K. S. (2010). Successful seed production of cobia *Rachycentron canadum* and its prospects for farming in India. *Marine Fisheries Information Service*; *Technical and Extension Series*, *206*, 1–6.

Hassler, W. W., & Rainville, R. P. (1975). Techniques for hatching and rearing cobia, *Rachycentron canadum*, through larval and juvenile stages. Univ. N.C. Sea Grant Prog. UNC-SG-75-30.

Ingram, B. A., McKinnon, L. J., & Gooley, G. J. (2002). Growth and survival of selected aquatic animals in two saline groundwater evaporation basins: An Australian case study. *Aquaculture Research*, *33*(6), 425–436.

Lambert, Y., Dutil, J. D., & Munro, J. (1994). Effects of intermediate and low salinity conditions on growth rate and food conversion of Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences*, *51*(7), 1569–1576.
Liao, I. C., Huang, T. S., Tsai, W. S., Hsueh, C. M., Chang, S. L., & Leaño, E. M. (2004). Cobia culture in Taiwan: Current status and problems. *Aquaculture*, *237*(1–4), 155–165.
Liao, I. C., Su, H. M., & Chang, E. Y. (2001). Techniques in finfish larviculture in Taiwan. *Aquaculture*, *200*(1–2), 1–31.
Lisboa, V., Barcarolli, I. F., Sampaio, L. A., & Bianchini, A. (2015). Effect of salinity on survival, growth and biochemical parameters in juvenile Lebranch mullet *Mugil liza* (Perciformes: Mugilidae). *Neotropical Ichthyology*, *13*(2), 447–452.
Marshall, W. S., & Grosell, M. (2006). Ion transport, osmoregulation, and acid-base balance. *The Physiology of Fishes*, *3*, 177–230.
McCormick, S. D. (2001). Endocrine control of osmoregulation in teleost fish. *American Xoologist*, *41*(4), 781–794.
McDonough, A. A., Thompson, C. B., & Youn, J. H. (2002). Skeletal muscle regulates extracellular potassium. *American Journal of Physiology-Renal Physiology*, *282*(6), F967–F974.
Mourad, N., Kreydiyyeh, S., Ghanawi, J., & Saoud, I. P. (2012). Aquaculture of marine fish in inland low salinity well water: Potassium is not the only limiting element. *Fisheries and Aquaculture Journal*, *3*(1), 16–28.
Partridge, G. J., & Creeper, J. (2004). Skeletal myopathy in juvenile barramundi, *Lates calcarifer* (Bloch), cultured in potassium-deficient saline groundwater. *Journal of Fish Diseases*, *27*(9), 523–530.
Partridge, G. J., & Jenkins, G. I. (2002). The effect of salinity on growth and survival of juvenile black bream (*Acanthopagrus butcheri*). *Aquaculture*, *210*(1–4), 219–230.
Partridge, G. J., & Lymbery, A. J. (2008). The effect of salinity on the requirement for potassium by barramundi (*Lates calcarifer*) in saline groundwater. *Aquaculture*, *278*(1–4), 164–170.
Partridge, G. J., & Lymbery, A. J. (2009). Effects of manganese on juvenile mulloway (*Argyrosomus japonicus*) cultured in water with varying salinity—Implications for inland mariculture. *Aquaculture*, *290*(3–4), 311–316.
Resley, M. J., Webb, K. A., Jr., & Holt, G. J. (2006). Growth and survival of juvenile cobia, *Rachycentron canadum*, at different salinities in a recirculating aquaculture system. *Aquaculture*, *253*(1–4), 398–407.
Roy, L. A., Davis, D. A., Saoud, I. P., Boyd, C. A., Pine, H. J., & Boyd, C. E. (2010). Shrimp culture in inland low salinity waters. *Reviews in Aquaculture*, *2*(4), 191–208.
Sampaio, L. A., & Bianchini, A. (2002). Salinity effects on osmoregulation and growth of the euryhaline flounder *Paralichthys orbignyanus*. *Journal of Experimental Marine Biology and Ecology*, *269*(2), 187–196.
Santos, R. A., Bianchini, A., Jorge, M. B., Romano, L. A., Sampaio, L. A., & Tesser, M. B. (2012). Cobia *Rachycentron canadum* L. reared in low-salinity water: Does dietary sodium chloride affect growth and osmoregulation? *Aquaculture Research*, *45*(4), 728–735.
Saoud, I. P., Davis, D. A., & Rouse, D. B. (2003). Suitability studies of inland well waters for *Litopenaeus vannamei* culture. *Aquaculture*, *217*(1–4), 373–383.
Shaffer, R. V., & Nakamura, E. L. (1989). Synopsis of biological data on the cobia *Rachycentron canadum* (Pisces: Rachycentridae). FAO Fisheries Synopsis. 153.
Stieglitz, J. D., Benetti, D. D., & Serafy, J. E. (2012). Optimizing transport of live juvenile cobia (*Rachycentron canadum*): Effects of salinity and shipping biomass. *Aquaculture*, *364*, 293–297.
Teeter, R. (1997). The electrolyte: Acid-base connection. *Feed Mix*, *5*, 32–34.
Verdegem, M. C. J., Hilbrands, A. D., & Boon, J. H. (1997). Influence of salinity and dietary composition on blood parameter values of hybrid red tilapia, *Oreochromis niloticus* (Linnaeus)× *O. mossambicus* (Peters). *Aquaculture Research*, *28*(6), 453–459.
Weirich, C. R., Smith, T. I., Denson, M. R., Stokes, A. D., & Jenkins, W. E. (2004). Pond culture of larval and juvenile cobia, *Rachycentron canadum*, in the southeastern United States: Initial observations. *Journal of Applied Aquaculture*, *16*(1–2), 27–44.
Wilson, R. P., & El Naggar, G. (1992). Potassium requirement of fingerling channel catfish, *Ictalurus puntatus*. *Aquaculture*, *108*(1–2), 169–175.
Zhou, Q. C., Wu, Z. H., Chi, S. Y., & Yang, Q. H. (2007). Dietary lysine requirementof juvenile cobia (*Rachycentron canadum*). *Aquaculture*, *273*(4), 634–640.

How to cite this article: Antony J, Reddy AK, Sudhagar A, Vungurala HK, Roy LA. Effects of salinity on growth characteristics and osmoregulation of juvenile cobia, *Rachycentron canadum* (Linnaeus 1766), reared in potassium-amended inland saline groundwater. *J World Aquacult Soc*. 2021;52:155–170. https://doi.org/10.1111/jwas.12741