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Orange Chromide, *Pseudetroplus maculatus* (Bloch., 1795): A Potential Euryhaline Fish Model to Evaluate Climate Change Adaptations in Fishes

Suresh Babu Padinhate Purayil[1*], *Shilta Madathampady Thomas*[2], *Anuraj Anirudhan*[1], *Jeena Nikarthil Sidhick*[3], *Asokan Pillaru Kandiyil*[2], *Sanal Ebeneezar*[3], *Boby Ignatius*[3] *and Gopalakrishnan Achamveetil*[3]

[1] Mariculture Division, Karwar Regional Station of Indian Council of Agricultural Research (ICAR) -Central Marine Fisheries Research Institute, Karwar, India , [2] Mariculture Division, Calicut Regional Station of Indian Council of Agricultural Research (ICAR) - Central Marine Fisheries Research Institute, Calicut, India, [3] Marine Biotechnology Fish Nutrition and Health Division, Indian Council of Agricultural Research (ICAR), Central Marine Fisheries Research Institute, Kochi, India

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***Correspondence:**
Suresh Babu Padinhate Purayil
sbabukkd@rediffmail.com

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Orange chromide, *Pseudetroplus maculatus* is a euryhaline species with both ornamental and food value. The species has several attributes similar to other fish model organisms such as smaller size, repeated breeding, ease of maintenance, and higher fecundity. A salinity tolerance study was performed in different salinities (0, 15, and 35 ppt) in triplicate introducing 10 fishes each (5.4 ± 0.08 g) in 12 plastic tanks of 60 L water-holding capacity. Fish were fed with commercial feed (1.2 mm and 40% protein) at 5% of body weight twice daily for 45 days. No significant variation ($p < 0.05$) in growth and survival was observed during the study indicating the wide salinity tolerance for the species. Experimental breeding of the species in freshwater and seawater (35 ppt) revealed the ability of the species to breed in varying salinities. Lenience in captive broodstock development, pair formation, and year-round natural breeding makes the seed production of the species easier. Characteristics such as multiple spawnings, a prolonged incubation period (48 to 72 hours) useful for elaborative embryonic studies, shorter larval development cycle (25 to 30 days), and better acceptance of live feed (*Artemia* nauplii and flakes) and commercial feed by the larvae make the species a potential euryhaline ornamental fish model to assess the physiological changes at different salinities. Minimal input requirements and lower capital and operational investments for the seed production of the species make it an ideal model organism for studying the impact of climatic and environmental changes on fish farming in different habitats.

Keywords: marine fish, breeding, orange chromide, salinity, euryhaline fish model

INTRODUCTION

Modern biological research depends heavily on model organisms to reproduce and ensure the impact of research findings on a biological system. These 'models' help to evaluate complex biological processes related to human health or provide a powerful window into fundamental

biological principles shared across the tree of life (Matthews and Vosshall, 2020). Model organisms have become reference points for an array of researchable issues for biological interventions (Ankeny and Leonelli, 2020). Put simply, model organisms are non-human species that are studied extensively in order to understand a range of biological phenomena. The US National Institutes of Health (NIH, 1999) listed the most important model organisms for biomedical research as mouse (*Mus musculus*), rat (*Rattus norvegicus*), zebrafish (*Danio rerio*), fruit fly (*Drosophila melanogaster*), nematode (*Caenorhabditis elegans*), baker's yeast (*Saccharomyces cerevisiae*), and Thale cress (*Arabidopsis thaliana*).

Climate change has a direct impact on various habitats related to fishes. Major physical impacts of climate change on aquaculture are fluctuations in precipitation, salinity, temperature, dissolved oxygen level, pH, and nutrient concentration (Whitney et al., 2016). Variations in these parameters directly or indirectly affect the growth and physiology of fishes in all salinity regimes such as freshwater, brackishwater, and marine habitats. Sundell et al. (2021) reported metabolic changes in euryhaline fish due to reduced water salinity. Whitney et al. (2016) reviewed the impact of various climate change factors on the physiology of inland fishes from North America. But in order to attain a clear picture of the impact of climate alteration implications on all these salinity regimes a model organism that thrives well in all these habitats is the need of the hour.

Orange chromide, *P. maculatus* is a euryhaline fish with a wide distribution that can survive in fluctuating salinity. This species is endemic to brackish streams, lagoons, estuaries, and the lower reaches of rivers in peninsular India and Sri Lanka. The colour and body patterns of the species make it an important fish for the ornamental trade (Bindu and Padmakumar, 2012; Raghavan et al., 2013; Shilta et al., 2016). Some earlier studies (Pampapathi Rao, 1958; Virabhadrachari, 1961) reported the ability of this species to thrive in extreme salinities ranging from freshwater to salinities up to 100 ppt due to the structural changes in the gills, kidney, and intestine. It is reported that the species can have pair formation, mating, and breeding in different salinities ranging from freshwater (Bindu and Padmakumar, 2012) to marine conditions (Shilta et al., 2016)

The present report is an attempt to evaluate the merits of the ornamental fish *P. maculatus* as a potential candidate to become a model organism to evaluate the impact of climate change on fishes living in different salinity regimes.

MATERIALS AND METHODS

Evaluation of the Characteristics of the Model Organism

Before initiating the breeding and salinity tolerance studies, the characteristics of the selected species as a model organism were evaluated by comparing the criteria to other two well-established fish models such as zebrafish (*Daneo rerio*) and Medaka (*Oryzias latipes*), using published data. Furthermore, the major objectives of the current study were highlighted in the list of important steps involved in the development of a new model organism as described by Matthews and Vosshall (2020).

Fish

Akalapuzha is a backwater connected to the Korapuzha estuary which flows through Calicut, Kerala, India that drains into the Arabian sea. Two hundred and fifty live fish of *P. maculatus* were caught from Akalapuzha (11° 49.498286 N and 75°75.670861 E) using a trap operated at 4 m depth and were brought to Calicut Regional Station of ICAR- Central Marine Fisheries Research Institute (CMFRI) for the purpose of the research.

Species Identification and Confirmation

For species identification based on conventional methods, counts and measurements were carried out according to Jayaram (2002); Jayaram (2010), and Mathews (2016). Morphometric measurements were accurately recorded using a digital vernier caliper (accuracy of 0.01 mm). Total length (TL- distance from the snout to the tip of the caudal fin), fork length (FL -distance from the snout to the end of the middle caudal fin rays), and standard length (SL distance from the snout to the posterior end of the last vertebra) were measured to the nearest mm using a measuring board and the total weight of the fish (W, wet weight) were recorded to the nearest 0.1 g using a digital analytical balance. In addition, various morphometric characters such as the proportion of Head length (HD), Body depth (BD), Snout length (SNL), Pre-dorsal length (PDL), Pre- pectoral length (PPL), Pre-ventral length (PVL), Pre-anal length (PAL) and Caudal depth (CD) to the standard length were calculated. Counts and measurements were compared to the data reported by Mathews (2016) for *P. maculatus*.

For molecular confirmation, five samples of each of the species in the study *P. maculatus*, and Pearlspot, *Etroplus suratensis* of its closely related genus *Etroplus* were collected and specific morphological characteristics were examined. Fin clip of each specimen was preserved in 90% ethanol for DNA extraction. Total genomic DNA was extracted from fin using DNeasy Blood and Tissue Kit (Valencia, CA) following the manufacturer's protocol. A 650 bp region from the COI gene was amplified using the primer pair Fish F1 [5'-TCAACCAACCACAAAGACATTGGCAC-3'] and Fish R1 [5'-TAGACTTCTGGGTGGCCAAAGAATCA-3'] (Ward et al., 2005). PCR amplifications were performed in a volume of 25 µl containing 12.5 µl of TaKaRa EmeraldAmp GT PCR Master Mix, 0.5 µl of each primer (10mM), and 1 µl of 50 ng/µl DNA template. The PCR conditions consisted of initial denaturation at 95°C for 5 min; followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 45 s; a final extension step at 72°C for 10 min, and then held at 4°C. The PCR products were visualised in 1.5% agarose gel, purified, bi-directionally sequenced, and aligned in MEGA X (Kumar et al., 2018). Sequence similarity search was performed using the BLAST algorithm in GenBank and COI sequences of *P. maculatus* and members of its related genus *Etroplus* (Pearlspot, *E. suratensis*, and Canara pearlspot, *E. canarensis*) were mined

from GenBank. The data was aligned with *Polymixia japonica* (AB034826) as an outgroup and phylogenetic tree was produced using MrBayes (GTR+G+I substitution model, 500,0000 chain length with 25% burn-in) (Huelsenbeck and Ronquist, 2001). Genetic divergence (uncorrected p-distance under uniform rates) was calculated using MEGA X (Kumar et al., 2018) with COI data of 606bp trimmed length. All sequences in this study were submitted to the NCBI database GenBank (https://www.ncbi.nlm.nih.gov/) under accession nos. OM992292-OM992296 and OM988400- OM988401.

Biology of the Species

The sex ratio of the moribund specimens collected from the wild was determined by dissecting the gonad.

The total length of the fish from the tip of the snout to the tip of the caudal fin was measured using a 1 m wooden scale with an accuracy of 1 mm. The total weight of the fish was measured using an electronic weighing balance (Sartorius, Germany) with an accuracy of 0.001 g. The length-weight relationship was calculated using the formula Log (WEIGHT) = a + [b x log (LENGTH)] where 'a' is the intercept and 'b' is the slope of the linear regression on the log-transformed weight (g) and length data (cm). The condition factor (k) of the fish in their habitat was determined using the equation as per Gomiero and Braga (2005). $k = (W \times 100)/L^3$ where k =condition factor; W=the weight of the fish in gram (g); L= the total length of the fish in centimetres (cm). The relative length of the gut of *P. maculatus* was measured to the nearest 0.1 cm as described by Euzen (1987) through the following equation: RLG= Length of gut/total body length

For ova diameter studies ovarian biopsy samples were analysed using a stereo zoom microscope (Zeiss, China) with digital imaging facility (software: Zen 3.0, Germany) and a compound microscope (Zeiss, Germany) with digital imaging facility (ProgRes, Germany) for identifying the maturation stages based on the diameter and developmental stage of the oocytes present in the ovary.

Salinity Tolerance Study

The present study was conducted at the marine hatchery complex of Calicut Research Centre of ICAR-CMFRI to determine the salinity tolerance of *P. maculatus* to know the scope of this species as a marine ornamental fish. For the determination of salinity tolerance, the fish were reared in different salinities such as 0, 15, and 35 ppt respectively. The salinities were checked using a hand refractometer. Twelve plastic tanks of 60 L capacity were filled with water of varying salinities (50 L). All tanks were provided with uniform aeration. Ten numbers of *P. maculatus* were directly stocked in freshwater (0 ppt), brackish water (15 ppt), and seawater (35 ppt) and reared for 45 days. The study was carried out at room temperature (28.5C) under natural photoperiods. Fish were fed with commercial pellet feed (Nutrila 1.2 mm, Growel Feeds Pvt Ltd. India; 40% protein) at 5% of initial biomass. The fish were fed twice a day during daytime (8 am and 4 pm). At the end of the experiment, the number of fish in each tank was counted and the survival rate (%) was calculated by the following formula: Survival (%) = (Total number of fish present x 100)/Total number of fish stocked.

After 45 days, fish from each unit were weighed and sampled for tissue analysis after 24 h of fasting. Fish were anaesthetized with clove oil (50 μL L^{-1}) and blood was collected from the caudal vein. Serum was collected by centrifuging the blood for 10 minutes at 0.5 g and stored at -20°C for subsequent analysis. For the collection of liver tissue, fish were euthanized and dissected. The liver tissue was weighed and collected in labelled tubes, added and homogenized with 0.25 00M sucrose buffer, and stored at -20°C until analysis. Triglycerides, cholesterol, total protein, albumin, globulin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels in the liver and serum samples were measured using diagnostic kits (Tulip Diagnostics Ltd., Goa, India) according to the manufacturer's instructions as per Ebeneezar et al. (2020).

Breeding in Different Salinities

About 150 count of adult fish (total length ranging from 5.2 to 8.7 cm, total weight ranging from 3.4 to 10.6 g) were stocked in two FRP tanks (1-ton capacity filled with 800 L chlorine-treated water) containing 0 and 35 ppt saline water with sufficient aeration. The fishes were fed with pellet feed (Nutrila 1.2 mm, Growel Feeds Pvt Ltd. India; 40% protein) at 2.5% of biomass. The fish were fed twice a day during daytime (8 am and 4 pm). Daily water exchange (20% of total volume) and tank bottom cleaning was performed. Two weeks after stocking the fish started pair formation. The fish that formed breeding pairs were directly stocked into plastic tanks (60 L breeding tanks) filled with 0 and 35 ppt water (50 L) with replications. No recirculating system was provided for the breeding tanks. The fish were fed with commercial pellet feed (Nutrila 1.2 mm, Growel Feeds Pvt Ltd.) containing 40% protein at 5% of biomass till spawning. The pellet feeds were given twice daily during daytime (8 am and 4 pm) at 2.5% of biomass for each of the two feeding times.

The breeding tanks were not provided with separate enclosures for brooders since the fish shows a high degree of parental care. Since *P. maculatus* is a substrate spawner, pieces of tile were introduced into the spawning tanks to facilitate spawning. Daily water exchange (20% of total volume) and tank bottom cleaning was performed. All tanks were provided with uniform aeration. The parents were retained in the breeding tank after spawning until the fourth day post-spawning (dps). Water quality parameters were monitored weekly during the breeding and larval rearing time.

The larvae rearing was done in a clear water system, since the yolk sac absorption and parental care were continued until the third dps, no external feeding was provided. From 4^{th} dps onwards the larvae were provided with artemia nauplii at 5 numbers/ml rearing volume. From 10^{th} dps artemia nauplii were gradually replaced with artemia flakes at 0.5 g/day/tank.

Statistical Analysis

Data presented in the text, figures, and tables are expressed as mean ± standard error. Statistical significance for various

parameters was fixed at $p<0.05$. The mean values were compared using Student's t-test for significant difference ($p<0.05$) among the stunted and normal fish using SPSS 16.0 VERSION.

RESULTS

Evaluation of the Characteristics of the Species to be a Model Organism

Among the important steps involved in the development of a new model organism described by Matthews and Vosshall (2020) (**Table 1**.) initial two steps such as choosing the organism and rearing the organism in laboratory conditions were attempted in the study. In addition, some of the important applications of the model organism (step 10) such as salinity tolerance and breeding adaptations in different salinities also were attempted. For the selection of the organism, the major characteristics of the fish were compared with established model fishes and the results are given in **Table 2**. Attributes pertaining to size, feeding habits, breeding behaviour, embryo development, and larval rearing almost matched well-established model fishes such as Zebrafish and Medaka.

TABLE 1 | Important steps involved in the development of a new model organism as described by Matthews and Vosshall (2020) and the targets for the present work.

Step	Description of the step	Targets
1	Choose an interesting and/or important organism.	(✓)
2	Learn how to rear and work with the organism in the lab.	(✓)
3	Assemble its genome + profile its gene expression	
4	Develop a method to introduce genetic material into the organism	
5	Make transgenic animals	
6	Knock-down and knock-out genes	
7	Develop precise mutagenesis for tagged mutants, gene replacements	
8	Develop binary effector systems	
9	Generate effectors of interest (label, image and manipulate cells)	
10	Grow a field with interesting questions using your new model organism.	(✓)

Species Identification

Morphometric measurements and meristic counts of 50 P. *maculatus* samples collected from Akalapuzha, Calicut, India are given in **Table 3**. The prominent characters observed in the specimens were as follows: Body with 1- 4 black blotches on the dorso- posterior half of the body; anal and pelvic fins with dark black border; orange colour small dots more prominent in the mid-portion of the body and in the dorsal fins. Meristic characters: Dorsal fin spines: XVIII-XIX (mostly XVIII); Dorsal fin soft rays: 8- 10 (mostly 8-9); Anal fin spines: XII-XIV (mostly XII- XIII); Anal fin soft rays: 8-9 (mostly 8); Pectoral fin- I, 12- 13; pelvic fin- I, 5. Metric characters: Standard

TABLE 2 | Comparison of characteristic of established model fishes to *E. maculatus*.

Attributes	Species		
	Zebra fish	**Medaka**	**Orange chromide**
Approximate size of fish	2.2 to 4 cm	2.5 - 3 cm	6.5 to 9 cm
Distribution	Tropical (South Asia)	Asia (Japan, Korea, China)	Asia (Indian subcontinent)
Habitat specificity	Freshwater	Freshwater Eurythermal	euryhaline
Sexual dimorphism	Less prominent	Easy	Easy breeding pair formation
Feeding habit adult	omnivore	omnivore	omnivore
Feeding habit larvae	Artificial diet and artemia	Artificial diet and artemia	Artificial diet and artemia
Mature of breeding	Oviparous, external fertilisation and external embryogenesis	Oviparous, external fertilisation and external embryogenesis	Oviparous, external fertilisation and external embryogenesis
Spawning behaviour	Asynchronous	Asynchronous	Asynchronous
Spawning frequency	10 days	Daily	7 to 14 days interval
Photoperiod	16 L 8 D	14 h L 10 h D	12 L 12 D
Breeding period	Throughout the year	Throughout the year	Throughout the year
Egg quality	Pelagic, non-adhesive	Adhesive eggs	Demersal adhesive
Egg transparency	Optically clear	transparent	opaque
blastula	5 h	8 h 15 min	6h 30 min
gastrulation	10 h	21 h	24 h
Hatching time	48 - 72 h	3.5 to 7.5 days	48 to 72 h
Generation time	10 weeks	2 - 3 months	
Parental care	nil	nil	present
Fecundity	200 eggs/week	38 to 141 eggs/female	350 to 400 eggs/female
Rearing efforts	easy	easy	easy
Nature of fish	Hardy	hardy	hardy
Maintenance	Low space and cost	Low space and cost	Low space and cost
References	Kimmel et al., 1995; Nasiadka and Clark, 2012; Khan and Alhewairini, 2018; Abdollahpour et al., 2020	Shima and Mitani, 2004; Iwamatsu, 2004.	Bindu and Padmakumar, 2012; Shilta et al., 2016,

TABLE 3 | Comparison of the counts and morphometric data of *P. maculatus* collected from Korapuzha river, Calicut, Kerala with *P. maculatus* collected from Manimala river, Travancore, Kerala (**Mathews, 2016**).

PARAMETERS	Present study	(Mathews, 2016)
	AVERAGE (Range) (n = 50)	
Dorsal fin spine& ray	XVIII-XIX,8-10	D- XVII- XX, 8- 10;
Anal fin spine &ray	XII-XIV,8-9	XII-XV, 8- 9.
Pectoral fin ray	I, 12 to 13	I, 15 to 16
Pelvic fin & rays	1,5	1,5
Scales raw on cheeks	5 to 6	
Scale raws on operculum	5 to 6	
Gill rackers	13-16	
Standard length (mm)	55.51	61
Proportions (% SL):		
Body depth highest	55.6 (50.0-59.6)	
Body depth at dorsal-fin origin	49.0 (48.5-50.3)	49.2
Body depth at 1st anal spine	54.3 (50.0-59.6)	
Head length	34.1 (31.4-35.8)	39.3
Snout length	11.7 (9.4-14.7)	
Orbit diameter	9.7 (8.0-12)	9.8
Bony interorbital width	13.2 (11.1-15.3)	
Upper jaw length	8.1 (7.0-9.6)	
Caudal peduncle length	8.1 (7.0-9.8)	10.7
Caudal peduncle width	15.0 (13.4-16.9)	14.8
Pre - dorsal length	35.6 (32.0-38.8)	
Pre - anal length	52.0 (50.0-57.3)	
Pre - pelvic length	35.6(31.4-39.5)	
Pre - pectoral length	35.4 (31.2-39.5)	
Caudal fin hight	32.5 (30-37.7)	
Caudal fin length	27.5 (24.1-30.6)	
Dorsal fin base length	63.0 (60.0-69.3)	
Anal fin base length	47.3 (45.4-49.1)	
Pectoral fin length	27.8 (24.5-30.0)	
1st dorsal spine length	6.0 (5.1-7.6)	
1st dorsal fin ray length	12.0 (9.4-18.0)	
1st anal fin spine length	7.01 (5.8-8.3)	
1st anal fin ray length	11.9 (10-14)	
1st pelvic fin spine length	11.4 (8.8-13.7)	
1st pelvic fin ray length	17.2 (15.6-19.2)	

Length (SL) (mm)-55.51; % SL: Head Length-34.1; orbit diameter-9.7; Caudal peduncle length-8.1; Caudal peduncle width-15.0.

Key identification features of *P. maculatus* were derived in comparison with *Etroplus suratensis* and given in **Figure 1**. **Figure 1A** shows *E. suratensis* with a blunt snout with a steeply sloping profile; a prominent black patch on the pectoral fin and (**Figure 1B**) *P. maculatus* with *a* snout profile markedly acute. **Figure 1C** shows *E. suratensis* with seven prominent dark lateral bars and (**Figure 1D**) indicate an absence of bars but the presence of one or more black blotches on the side of the body in *P. maculatus*. **Figure 1E** shows lateral oral dentition unicuspid, spatulate in *E. suratensis* while **Figure 1F** shows a tricuspid tooth pattern in *P. maculatus*

The sequence analysis confirmed the identity of *P. maculatus* which is used as a model organism in this study. The Bayesian phylogram (**Figure 2**) indicated two clusters in which the monotypic genus *Pseudetroplus* formed a sister clade to the genus *Etroplus* and showed 17.8% and 19.3% p-distance with *E. suratensis* and *E. canarensis* respectively. The interspecific distance in genus *Etroplus* was 15.7%. The comparative genetic analysis of these three South Asian cichlids revealed that they are evidently discriminated by their genetic distances and also by the criterion of cluster formation of COI barcodes in the phylogenetic tree.

Biology of the Species

The sex ratio observed for the collected stock was 1: 1.76 (male: female). Length-weight relationship of orange chromide, *P. maculatus* was investigated from 100 specimens collected from Akalapuzha estuary, Calicut, Kerala, India. The fish collected ranged from 4.7 - 9. 5 cm (mean 7.15 ± 0.95) in total length and 1.9 – 12.6 g (6.99 ± 2.56) in weight. Length-weight relationships with the descriptive regression parameters of the equations are presented in **Table 4.** Length-weight relationship for males and females and the total sample population were determined as $W=0.1605L^{3.1172}$, $W=0.3075L^{2.3418,}$ and $W=0.2396L^{2.6347}$ respectively. The overall b value ranged from 2.3 to 3.1 with a mean value of 2.63 ± 0.02. This indicated a negative allometric growth pattern ($b<3$) in *P. maculatus* and the r^2 values ranged from 0.73 to 0.91. The condition factor for males was 1.98 and for females it was 1.74, overall condition factor was 1.91.

Biopsy samples of the ovary of *P. maculatus* sample from an ovary (**Figure 3**) revealed the presence of developing oocytes

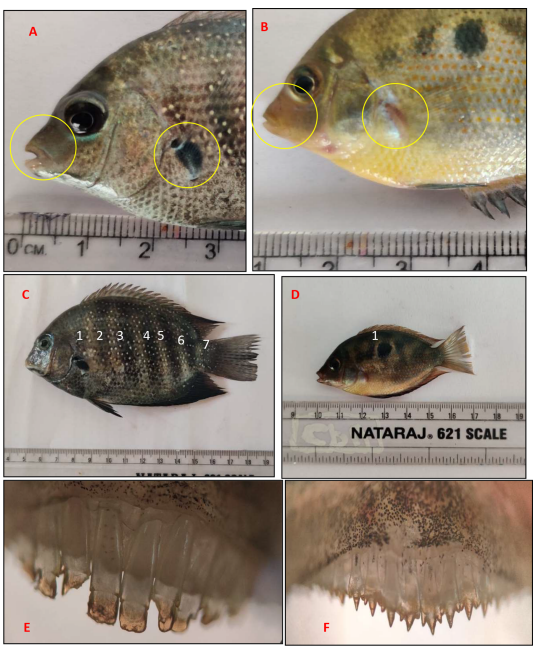

FIGURE 1 | Key identification points for *P. maculatus* in comparison with *E suratensis* **(A)** *E suratensis* with blunt snout with a steeply sloping profile; a prominent black patch on the pectoral fin **(B)** *P. maculatus* with snout profile markedly acute; pectoral fin hyaline. **(C)** *E suratensis* with seven prominent dark lateral bars **(D)** an absence of bars but the presence of one or more black blotches on the side of the body in *P. maculatus.* **(E)** Lateral oral dentition unicuspid, spatulate in *E suratensis* **(F)** Tricuspid in *P. maculatus.*

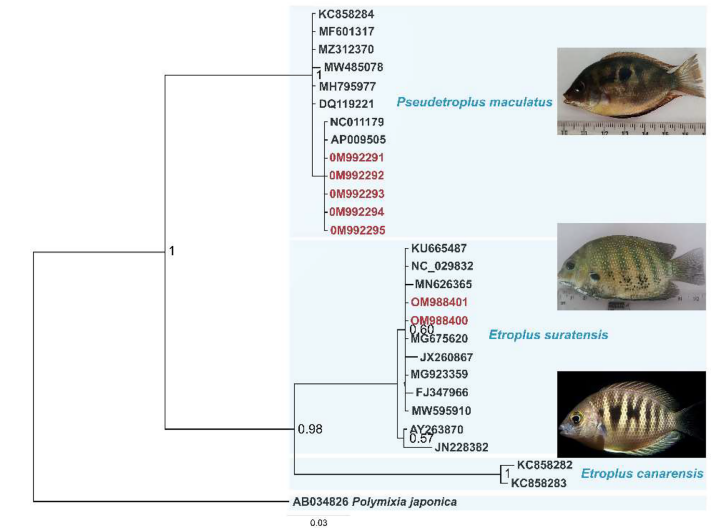

FIGURE 2 | Bayesian phylogenetic tree based on COI sequences retrieved from GenBank (accession numbers indicated) and the specimens from this study (red text). Branch labels are posterior probability and the scale bar is the number of substitutions per site. *Polymixia japonica* is used as the outgroup.

(small spherical), immature oocytes (small oval), and ripe oocytes (large oval) in a single ovary indicating asynchronous development of oocytes in the ovary. Details of the maturation stages of oocytes observed in female *P. maculatus* are given in **Table 5**. As the oocyte matures the shape changes from spherical to oval with yolk deposition and the oocyte grows from an average diameter of 181.5 ± 10.64 µm to a size of 845.92 ± 57.39 x 1159.96 ± 59.6 µm.

Salinity Tolerance Study

The fish could be successfully reared in different salinities 0, 15, and 35 ppt and the fishes have attained a final weight of 9.4 ± 0.12 g, 8.6 ± 0.18 g, 8.2 ± 0.08 g ($p>0.05$) respectively from the initial average weight of 5.4 ± 0.08 g. The average survival of the fish (98%, 96%, and 96% respectively for 0%, 15%, and 35% ppt also was not different among different salinities. The fish when exposed for long periods to higher salinities at 28.5C, exhibited no change in survival and no sign of loss of appetite was observed, which is an indication that there is no imbalance in the physiological mechanisms of the animals.

Biochemical indices in the liver and serum of orange chromide reared in different salinities are given in **Table 6**. In liver tissue the maximum level of triglycerides was found in the 15 ppt salinity group, while the lowest was found in the 35 ppt group, while the highest level of triglycerides in serum was found in the 0 ppt group, followed by the 35 and 15 ppt groups. In the present study, the highest level of cholesterol was found in liver tissue in the 15 ppt group, while the lowest level was found in the serum in the 15 ppt group. The protein concentration of the liver was highest in the 15 ppt group, followed by the 0 and 35 ppt groups in our study. The highest amount of protein in serum was found in the 0 and 35 ppt groups, with no significant differences between them, followed by the 15 ppt group. In the current study, the 35 ppt group had the highest ALT activity in liver tissue, while the 0 and 15 ppt groups had no significant changes. In serum, the 15 ppt group had the highest ALT activity, with no significant differences between the 0 and 35 ppt groups. In liver tissue, AST activity was highest in the 15 and 35 ppt groups, whereas in serum, it was highest in the 20 ppt group, followed by 0 ppt and 35 ppt groups.

Variation in GSI and HSI in *P. maculatus* reared in different salinities at the end of the experiment is given in **Figure 4**. GSI and HSI were showing similar trends in different salinities with highest values in fish reared in 15 ppt and lower values for the other salinities.

TABLE 4 | Descriptive statistics and estimated parameters of Length-weight relationships for *P. maculatus* collected from the Korapuzha estuary.

Category	Length (cm)		Weight (g)					
	Range	Mean +SD	Range	Mean ± SD	N	a	b	r^2
Male	4.7-8.5	6.95 ± 0.93	1.9-11.79	6.68 ± 2.81	50	0.1605	3.1172	0.90893
Female	5.5-9.5	7.26 ± 0.95	2.97-12.6	7.15 ± 2.42	50	0.3075	2.3418	0.73562
Over all	4.7-9.5	7.15 ± 0.95	1.9-12.6	6.99 ± 2.56	100	0.2396	2.63472	0.80438

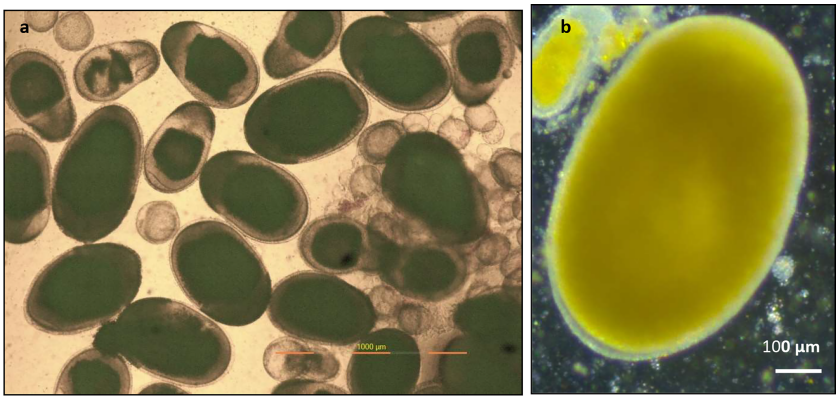

FIGURE 3 | Biopsy samples of ovary of *P. maculatus* **(A)** sample from a female ovary indicating developing oocytes (small spherical), immature oocytes (small oval), and ripe oocytes (large oval) observed under a compound microscope. **(B)** image of a ripe oocyte with clear zona radiata and cytoplasm filled with yolk granules observed under a steriozoom microscope.

Breeding in Different Salinities

Spawning was observed in both the salinities maintained. Details of the spawning obtained are given in **Table 7**. The process of spawning is completed within 15- 30 minutes in all the salinities. The higher spawning fecundity about 350 to 400 eggs per female was observed in 35 ppt (**Figure 5**) when compared to freshwater (150-250 eggs/female). After hatching most of the hatchlings remained in the substrate itself. The hatchlings were found to sink to the bottom after a few hours and they were mouth-picked by the female and transferred to one of the darker corners of the plastic crates. The hatchlings had heads down and tails up with lashing movements. The newly hatched larvae appeared transparent with a voluminous yolk sac containing large oil globules; presence of large pigmented eyes; prominent pulsing heart located between the head and yolk sac. Yolk absorption was completed in three days, after which the larvae began to accept external food. In a few tanks, it was observed that the two day old larvae were devoured by the parents and they are promptly removed immediately from each spawning tank. The fry become free swimming from the fourth day and are found congregated near the aeration points as swarms in tanks due to the absence of parents in the tanks. In general, fry move in shoals guided by the parents, swimming mostly underneath the parents. From the fourth day immediately after the yolk sac absorption, fry was fed with artemia nauplii, since the yolk sac absorption was completed. From the tenth day, the amount of artemia nauplii is reduced and artemia flakes were added to the tanks. After 25 days of rearing in fresh water and seawater a survival rate of 98 - 99% was observed.

DISCUSSION

Fish are considered as one of the most suitable model organisms for experimental studies. Fish are denoted as an ideal model because of their minimum inputs for maintenance and their ability to produce isogenic and genetically modified lines (Cossin and Crawford, 2005). Because fish live in water, the close physiological relationship with the environment is more intact than in the terrestrial animals making them more appropriate for environmental interaction studies (Cossin and Crawford, 2005). This advantage makes fish an ideal organism to study the impact of climate change on biological processes.

Size is an important factor in the selection of a model organism (Ulloa et al., 2011). Since *P. maculatus* grows only up to 7 to 9 cm (Bindu and Padmakumar, 2012; Shilta et al., 2016), it can be easily maintained in laboratory facilities similar to other model fishes. Smaller fishes such as Zebrafish, *Danio rerio* (Dai et al., 2014), Medaka, *Oryzias latipes* (Wittbrodt et al., 2002), and Goldfish, *Carassius auratus* (Popesku et al., 2008) have been regularly employed as model organisms for several studies. The omnivorous nature of feeding habits of both adults and larvae, and acceptance of artificial feed and live feed by the larvae facilitate rearing in confined conditions. Higher fecundity and hatching time (Shilta et al., 2016) and asynchronous spawning

TABLE 5 | Maturation stages of oocytes observed in female *P. maculatus* based on oocyte dimensions and colour.

	Developing oocytes	Maturing oocytes	Ripe oocytes
Colour	transparent	yellowish	Dark yellow
shape	spherical	oval	oval
Average horizontal width or diameter (µm)	181.5 ± 10.64	385.13 ± 19.85	845.92 ± 57.39
Average vertical width (µm)	–	587.1 ± 22.66	1159.96 ± 59.6
Size range	68 - 296	(197-578) x (335 -753)	(522- 1058) x (815- 1640)

TABLE 6 | Biochemical indices in the liver and serum of Orange chromide reared in different salinities.

	Triglycerides (mg/dL)	Cholesterol (mg/dL)	Total Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	ALT (U/L)	AST (U/L)
Biochemical indices in the liver							
0	218.13 ± 0.37^{b}	38.25 ± 1.42^{a}	4.54 ± 0.02^{b}	2.20 ± 0.03^{c}	2.34 ± 0.01^{c}	6.40 ± 0.89^{a}	7.76 ± 1.18^{a}
15	241.19 ± 0.18^{c}	149.56 ± 1.65^{b}	4.82 ± 0.01^{c}	3.99 ± 0.01^{b}	0.84 ± 0.01^{a}	6.60 ± 0.39^{a}	25.80 ± 1.08^{b}
35	165.97 ± 0.38^{a}	37.94 ± 0.31^{a}	3.33 ± 0.01^{a}	1.11 ± 1.47^{a}	2.23 ± 2.08^{b}	21.15 ± 0.85^{b}	25.41 ± 0.19^{b}
Biochemical indices in the serum							
0	69.56 ± 0.21^{c}	116.60 ± 0.81^{b}	4.33 ± 0.04^{b}	2.78 ± 0.02^{c}	1.55 ± 0.06^{a}	11.83 ± 1.18^{a}	283.63 ± 2.90^{b}
15	22.70 ± 0.26^{a}	107.28 ± 0.81^{a}	4.09 ± 0.03^{a}	2.21 ± 0.02^{a}	1.88 ± 0.05^{b}	41.13 ± 2.23^{b}	405.27 ± 0.85^{c}
35	35.59 ± 0.21^{b}	117.07 ± 0.27^{b}	4.28 ± 0.02^{b}	2.67 ± 0.02^{b}	1.62 ± 0.01^{a}	10.67 ± 0.51^{a}	163.74 ± 0.51^{a}

TABLE 7 | Breeding performance of Orange chromide reared in different salinities.

	Freshwater (0 ppt)	Marine (35 ppt)
Number of pairs attempted	4	5
Number of pairs spawned	3	3
Spawning fecundity of the individuals	150-250	350-400
Fertilisation rate (%)	95-100	93-99.5
Hatching rate (%)	97-99.5	96.5-99.5
Incubation period (h)	48(h)	72
Larval survival (%)	99	98

with a shorter spawning frequency of one or two weeks (Bindu and Padmakumar, 2012) makes the fish more ideal to be an experimental animal. Easy pair formation and parental care are unique for this fish. Since the eggs are adhesive in nature handling for the purpose of shifting and incubation is easier. Practical aspects of natural breeding such as lower space requirement, adaptability to environmental conditions, and easy feed management (Matthews and Vosshall, 2020) make *P. maculatus* an ideal candidate to become a model fish. Since all these characteristics are consistent with the established model fishes such as Zebrafish (Kimmel et al., 1995; Nasiadka and Clark, 2012; Khan and Alhewairini, 2018; Abdollahpour et al., 2020) and Medaka (Shima and Mitani, 2004; Iwamatsu, 2004), *P. maculatus* can be considered as a potential candidate to become a model fish.

Species Identification

Orange chromide was placed in the genus *Etroplus* until Pethiyagoda et al. (2014) validated the new genus *Pseudetroplus*. Presently the species is renamed *Pseudetroplus maculatus*. In order to confirm the identity of the specimens collected for the experiments, conventional taxonomic identification using morphometric and meristic characters and identification employing DNA Barcoding was adopted.

Sparks (2008) reported that adults of *E. suratensis* and *E. canarensis* differ from *P. maculatus* by possessing a blunt snout with a steeply sloping profile against the snout profile markedly acute in *P. maculatus*; a prominent black patch on the pectoral fin, near its base in *E. suratensis* against pectoral fin hyaline in *P. maculatus* and seven to nine prominent dark lateral bars against an absence of bars but the presence of one or more black blotches on the side of the body in *P. maculatus*. All these characters mentioned for *P. maculatus* were observed in the specimens collected from Akalapuzha. Bleeker (1862) recognized a higher-level distinction between *P. maculatus* and *E. suratensis*, on the basis that *P. maculatus* possesses tricuspid jaw teeth and scaly sheaths to the dorsal and anal fins. On contrary, Günther (1862),

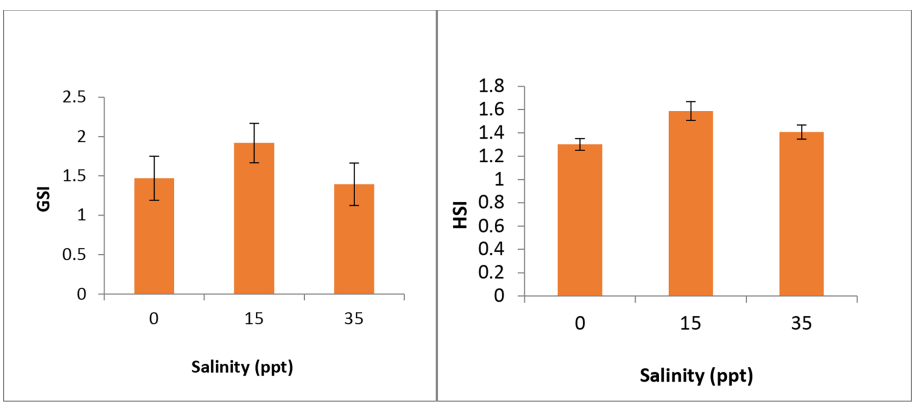

FIGURE 4 | Variation in Gonado-somatic index (GSI) and Hepato-somatic index (HSI) in *P. maculatus* reared in different salinities.

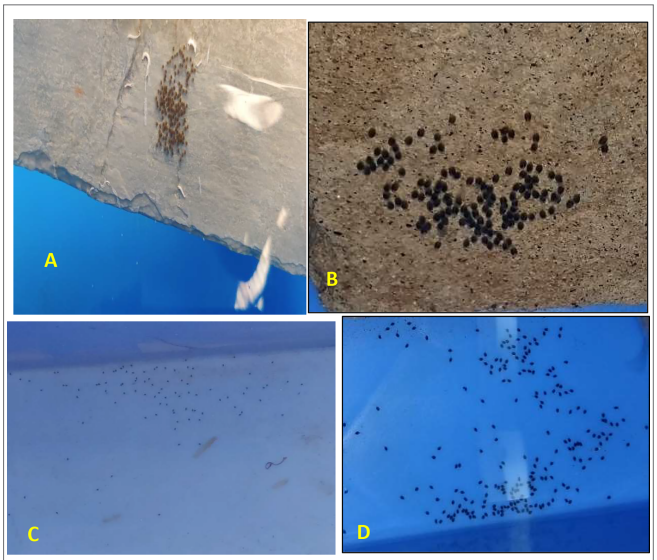

FIGURE 5 | Breeding of *P. maculatus* in different conditions. *P. maculatus* eggs attached to a substrate in **(A)** Freshwater and **(B)** Marine water. One day old hatchlings of *P. maculatus* in **(C)** Freshwater **(D)** Marine water.

was doubtful of the validity of the genus *Pseudetroplus*, stating that the characters of the new genus *Pseudetroplus* mentioned by Bleeker (1862) are equally developed in *E. suratensis* and in *E. maculatus*, and concluded that Bleeker (1862) either described a third species, different from both, or that the author has taken the characters for *Etroplus* from a very old specimen of *E. suratensis*, in which the incisions on the front teeth have become obsolete. Our results on the dentition pattern were consistent with the description of Bleeker (1862) especially the presence of tricuspid teeth in jaws in *P. maculatus*. Similarly, Pethiyagoda et al. (2014) also reported the anterior jaw teeth tricuspid, acuminate in *Pseudetroplus* against the unicuspid, and spatulate teeth in *Etroplus*.

The morphometric measurements and meristic counts of the specimens collected in the present study were similar to those specimens reported from Manimala river, Travancore, Kerala, India with few variations (Mathews, 2016). The variations observed in comparison with specimens from the Manimala river are the counts of dorsal-fin spines (18-19 vz 17-20); anal-fin spines (12– 14 vz 12-15); pectoral-fin rays (12- 13 vz 15-16) (Mathews, 2016). Day (1877) reported that specimens of *P. maculatus* from Madras, Tamil Nadu, India possessed 17–18 dorsal-fin and 11–12 anal-fin spines, whereas those in southern Karnataka, India possessed 19–20 and 14–15 spines, respectively. In the present study *P. maculatus* possessed 18-19 dorsal-fin spines and 12-14 anal fin spines. The difference observed in morphometric and meristic characters owes to the origin of species from two different geographical regions or genetic differences or environmental impact, or a combination of all the above.

Confirmation of the species identification was carried out using molecular tools. The sequence analysis confirmed the identity of *P. maculatus*. The Bayesian phylogram indicated two clusters in which the monotypic genus *Pseudetroplus* formed a sister clade to genus *Etroplus* and showed 17.8% and 19.3% p-distance with *E. suratensis* and *E. canarensis* respectively. The interspecific distance in genus *Etroplus* was 15.7%. The comparative genetic analysis of these three South Asian cichlids revealed that they are evidently discriminated by their genetic distances and also by the criterion of cluster formation of COI barcodes in the phylogenetic tree. Finally, the identity of the specimens collected was confirmed as *P. maculatus*.

Biology of the Species

Sexual dimorphism is not conspicuous in the species during the breeding season but the natural pair formation will help to identify breeding pairs for experimental breeding programmes. Bindu and Padmakumar (2012) reported intensified colouration in males during the pair formation. The fish is monogamous (Bindu and Padmakumar, 2012) and therefore it is easier to maintain the same pairs for multiple breeding cycles. The sex ratio observed in the collected fish is female dominant (male: female 1: 1.76). Roshni and Renjithkumar (2021) reported a female dominant population from the Cochin estuary and opined that the sex ratio varies with stock.

The *b* values of the length-weight relationships of *P. maculatus* exhibited notable variations from the isometric value (Froese, 2006) and all values of parameter *b* for the *P. maculatus* collected from Akalapuzha varied within the range of 2.3–3.1. The values of parameter *b* in the LWR equation were estimated as 2.63 for *P. maculatus* in the present study. Similarly, Roshni et al. (2016) reported *b* value ranging from 2.662 to 2.794 for *P. maculatus* from the Vembanad lake, Kerala, India and Remya et al. (2021) reported *b* value ranging from 2.779 to 3.012 for *P. maculatus* from the Kayamkulam lake in Kerala, India. Karna et al. (2012) also reported similar results for fish collected from Chilka lake, India.

The average relative length of gut in *P. maculatus* is measured to be 2.08 ± 0.27 cm. Taki (1978) suggested that fish having a relative length of digestive tract shorter than 1.5 times the standard length were judged to be carnivorous and those with a relative length of digestive tract longer than three times the standard length were regarded as herbivorous. This ratio is intermediate between 2 and 3 times the standard length. Therefore, the feeding habit of *P. maculatus* could be considered as an omnivore. Condition factor (above 1) for male, female, and overall groups indicates that the fish have grown in good condition. Condition factor 'K' is a good indicator of the degree of the well-being of the fish in their habitat based on the isometric growth pattern (Gomiero and Braga, 2005; Datta et al., 2013).

Biopsy samples of ovary of *P. maculatus* (a) sample from a female ovary revealed the presence of developing oocytes (small spherical), immature oocytes (small oval), and ripe oocytes (large oval) in a single ovary indicating asynchronous development of oocytes in the ovary. Thus, *P. maculatus* is a multiple spawner with a shorter spawning interval. Bindu and Padmakumar (2012) reported a spawning interval ranging from 8 to 16 days in different pairs of the species in freshwater.

Roshni and Renjithkumar (2021) reported the presence of ripe gonads throughout the year indicating the possible spawning throughout the year. The histological section of ovary of *P. maculatus* depicted by Sajla et al. (2019) also represented oocytes of various developing stages on a single section of the ovary. Lamon and Ward (1983) also reported repeated spawning in *P. maculatus*. As the oocyte matures, the shape changes from spherical to oval with yolk deposition and the oocyte grows from an average diameter of 181.5 ± 10.64 µm to a size of 845.92 ± 57.39 x 1159.96 ± 59.6 µm. Selman et al. (1993) reported a diameter of about 0.75 mm for the matured oocytes of Zebrafish. Iwamatsu (2004) reported an oocyte diameter of above 800 µm for medaka. The bigger size of the matured oocytes poises greater advantages for the model organism since micromanipulation of the oocytes can be managed with greater precision.

Absolute fecundity obtained in the present study was 350 to 500 eggs/female. Roshni and Renjithkumar (2021) reported an absolute fecundity of 508 -830 for *P. maculatus*. The absolute fecundity was ideal for the continuous breeding of the species.

Salinity Tolerance Studies

In order to confirm the salinity tolerance of the species, the fish were directly exposed to various salinities in freshwater (0 ppt), brackishwater (15 ppt), and marine conditions (35 ppt). The growth and survival of the fish in different salinities did not differ much indicating the ability of the fish to perform well in all these salinities. Pampapathi Rao (1958) and Virabhadrachari (1961) reported the ability of this species to thrive in extreme salinities ranging from freshwater to salinity up to 100 ppt. According to Parvatheswararao (1967) this euryhaline nature may perhaps be due to the high blood chloride content and the high blood-tissue chloride gradient in blood-tissue.

Haemato-biochemical parameters are indications of fish health in general (Soltanian and Fereidouni, 2019). In this study, in liver tissue the maximum level of triglycerides was found in the 15 ppt salinity group, while the lowest was found in the 35 ppt group, while the highest level of triglycerides was found in the 0 ppt group, followed by the 35 and 15 ppt groups in serum. Triglycerides increased in shi drum between 4 and 10 ppt (3.1 to 7.1 mM, respectively) according to Mylonas et al. (2009), but dropped at 40 ppt (4.7 mM). Arjona et al. (2009), on the other hand, observed that plasma triglycerides increased in the sole, *Solea senegalensis*. Between 15 and 39 ppt, ranging from 2.8 to 10.7 mM.

Cholesterol is required for the growth and development of eukaryotic cells (Kim et al., 2019). Environmental stress such as temperature stress (Malekar et al., 2018) low dissolved oxygen (Maita et al., 1998), and inadequate nutrition, have all been shown to impact plasma cholesterol levels in fish (Krogdahl et al., 1999). In our study, the highest level of cholesterol was found in the liver tissue in the 15 ppt group, while the lowest levels were found in the 0 and 35 ppt groups without significant differences among them. The serum samples recorded an inverse trend with the lowest level in the 15 ppt group and higher levels in both 0 and 35 ppt groups without significant differences among them.

Total plasma protein is a reliable physiological indication of fish health status and environmental stress because it plays a role in osmotic regulation and pathogenic defence (Kim and Kang, 2016). In fish, total protein is significantly linked to a greater innate immune response (Sangiao-Alvarellos et al., 2005). Increased salinity resulted in a considerable drop in protein levels (Martinez-Alvarez et al., 2002). Riche (2007), on the other hand, found that changes in salinity enhanced the total protein of hybrid striped bass (*Morone chrysops* x *M. saxatilis*). The protein concentration of the liver was highest in the 15 ppt group, followed by the 0 and 35 ppt groups in our study. The highest amounts in the serum were found in the 0 and 35 ppt groups, with no significant differences between them, followed by the 15 ppt group.

Plasma ALT and AST are sensitive indicators of liver injury and health status in fish (Kim and Kang, 2014; Kim and Kang, 2015). In the current study, the 35 ppt group had the highest ALT activity in liver tissue, while the 0 and 15 ppt groups had no significant changes. In serum, the 15 ppt group had the highest activity, with no significant differences between the 0 and 35 ppt groups. In liver tissue, AST activity was highest in the 15 and 35 ppt groups, whereas in serum, it was highest in the 15 ppt group, followed by 0 ppt and 35 ppt groups. Under salinity stress, the AST and ALT of goldfish (*Carassius auratus*) was increased significantly, according to Al-Khashali and Al-Shawi (2013). In low-salinity environments, Lee et al. (2016) found a substantial rise in plasma AST of juvenile red-spotted grouper (*Epinephelus akaara*), implying that hepatic or cardiomyocyte function may be compromised. The GSI and HSI values in different salinities after 45 days of rearing were slightly higher in the 15 ppt group indicating higher reproductive activity in this salinity. The higher biochemical indices in the liver in the 15 ppt group also indicate the same.

Breeding in Different Salinities

Spawning was observed in both the salinities maintained. The process of spawning is completed within 15- 30 minutes in both the salinities. The higher spawning fecundity of approximately 350 to 400 eggs per female was observed at 35 ppt when compared to freshwater (150-250 eggs/female). Bindu and Padmakumar (2012) reported that spawning fecundity varied between 140 to 231 per female in freshwater. The mean fecundity in *P. maculatus* was reported to be 1,378 (Jayaprakas et al., 1979). However, Antony and Natarajan (2014) reported a fecundity ranging from 250 to 400 for the species in freshwater indicating less correlation between the fecundity and salinity of the water in which breeding trials were performed. The difference in spawning fecundity might be due to the 'asynchronous' development of oocytes in the ovary of *P. maculatus* and multiple batches of eggs were spawned successively within a spawning season. After spawning, both parents alternately guard over eggs by fanning and mouthing, while the other leaves the territory to forage. Their roles were reversed in every few minutes and this keep the pairs in good health (Perrone and Zaret, 1979). During the experiment, in a

few tanks where the brooders were disturbed, they themselves consumed the developing eggs. Bindu and Padmakumar (2012) also reported similar behaviour. The eggs of *P. maculatus* in fresh water hatched out in 48 hours whereas in saline water eggs hatched within 68 to 72 hours. Similarly, Bindu and Padmakumar (2012) reported that the eggs of *P. maculatus* generally hatched out in 48 hours in freshwater. Parental care ensured high hatching up to 97-99.5% in freshwater and 96.5-99.5% in marine water. Biparental care is reported for this species by several authors (Bindu and Padmakumar, 2012; Shilta et al., 2016). Bindu and Padmakumar (2012) reported a 99% hatching in freshwater with parental care and reported a reduced hatching rate without parental care. Yolk absorption was completed in three days and after which the larvae began to accept external food. The experiment revealed that the species can be bred in both freshwater and marine conditions.

P. maculatus as a Model Fish to Study Climate Change

P. maculatus is considered as an euryhaline species (Virabhadrachari, 1961) which inhabits mostly freshwater and backwaters in the Indian subcontinent. This species has been considered as an excellent ornamental and food fish in this region (Bindu and Padmakumar, 2012; Raghavan et al., 2013; Shilta et al., 2016) since breeding in nature and in captivity is easy. In addition, the present work revealed that the species can tolerate, mature, and reproduce in salinity regimes ranging from freshwater to marine conditions with minimum physiological alterations. These characteristics along with the specific characteristics similar to that of a model organism, such as ease of breeding, easy stock management, and prolonged breeding season make the species an ideal candidate to study the influence of fluctuating climate parameters such as altered temperature, pH, dissolved oxygen level, osmoregulation and nutrient enhancement in different salinity regimes. Changes in metabolic and other physiological functions, reproductive behavior, stress parameters, and disease manifestation in different salinity regimes according to climate changes can be addressed with the new model organism. This will help fish farmers to adopt strategies to mitigate the impact of climate change on fish growth, production, and reproduction.

CONCLUSION

The present work indicates that the euryhaline fish *P. maculatus* is an ideal candidate to become a model organism for studying the changes in habitats, as it thrives and reproduces well in all the salinity regimes. It's worth noting that, while salinity had an effect on the biochemical indices of the liver and serum, the ultimate growth, breeding performance, and survival were unaffected. This demonstrates the species' ability to adapt to a wide range of salinity. Moreover, the fish fulfil most of the criteria to be a model organism similar to the established fish models employed for several biological studies.

DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in the NCBI GenBank repository(https://www.ncbi.nlm.nih.gov/), accession numbers OM992292-OM992296 and OM988400- OM988401.

ETHICS STATEMENT

This animal study was reviewed and approved by ICAR-CMFRI, Kochi, India (Project code MDN/HCY/18).

AUTHOR CONTRIBUTIONS

SP, Concept, Manuscript preparation, data analysis. ST, execution of work, manuscript preparation. AA, Data analysis. JS, execution of work, manuscript preparation. AK, execution of work. SE, execution of work, Data analysis. BI, Manuscript preparation. GA. Over all guidance. All authors contributed to the article and approved the submitted version.

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