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Risk analysis of non-native three-spot cichlid, *Amphilophus trimaculatus*, in the River Cauvery (India)

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Abstract

The three-spot cichlid *Amphilophus trimaculatus* (Günther) is an ornamental fish known to be invasive but has not been assessed for India. The present study confirmed this non-native species' identity using conventional and molecular methods, as well as its occurrence in the River Cauvery, the first for this species in a lotic environment outside its native range. Gut content analysis indicated a diet of predominantly insects. The river's water quality and habitat were conducive for the species' growth and reproduction. Using the Aquatic Species Invasiveness Screening Kit, *A. trimaculatus* was ranked as posing a "high" risk of being invasive in the River Cauvery, with climate match analysis showing 58% similarity with the species' native range. Vector and pathway analysis found that *A. trimaculatus* was introduced to India via the aquarium trade, although consignment origin remains unknown, and the most likely means of entry into the river and its tributaries was the species' escaping from holding facilities. Subsequent dispersal and impact of the species on native fauna are discussed. The study's outcomes demonstrate the importance of using risk analysis protocols to inform management of non-native species in India, with suggestions provided for immediate and long-term measures for the species' management.

KEYWORDS
aquarium trade, biological invasions, climate match, invasiveness, vector/pathway analysis

1 | INTRODUCTION

The risks associated with unauthorised and accidental introductions of non-native species pose a potential threat to the conservation of native species and ecosystems (França et al., 2017; Pimentel, 2011; Wilcove et al., 1998), and the aquarium fish trade is one of the major vectors for non-native fish species (NNS) introductions outside their native ranges (Chan et al., 2020). Cichlid fishes (family Cichlidae, order Cichliformes) are hardy and attractive, which makes them commercially important in aquaculture and the aquarium trade (Chan et al., 2020; Pullin, 1991). These attractive traits, coupled with their euryphagous diet, facilitate their successful establishment in novel environments (Liew et al., 2012). One such species of Cichlidae is the three-spot cichlid *Amphilophus trimaculatus* (Günther), also known as trimac cichlid. It is native to Central America, where its distribution extends from Laguna Coyuca in Mexico through El Salvador to Rio Lempa in Guatemala (Nico & Loftus, 2019). *Amphilophus trimaculatus* has a preference for muddy and sandy bottoms amongst roots of macrophytes in lakes and river catchments that discharge into the Pacific Ocean (Corfield et al., 2008). A fish of ornamental value, *A. trimaculatus* is believed to be one of the cichlids used to develop the hybrid cichlid "flowerhorn" in the mid-1990s by the aquarium industry in Malaysia (Nico et al., 2007). Flowerhorn is an ornamental fish traded globally and popular amongst aquarium hobbyists, especially followers of the Chinese geomancy "Feng-Shui" (Knight & Devi, 2009).

Amongst the cichlids, *A. trimaculatus* is one of the most aggressive (Baensch & Riehl, 1993), and elsewhere it has been ranked as posing "very high" and "moderate" risks of being invasive (Arthington et al., 1999; Bomford & Glover, 2004). The threats posed by *A. trimaculatus*, similar to those posed by some other invasive NNS, include changes in the behaviour, abundance and genetic composition of native species or their extinction (Blackburn et al., 2014). A negative impact of *A. trimaculatus* has been reported from Sempor Reservoir in Indonesia, where the species has become dominant, having replaced economically valuable native fish species (Hedianto et al., 2014). *Amphilophus trimaculatus* has been introduced, including release into open waters, to several countries in Asia (Amin et al., 2019; Chakraborty et al., 2019; Hedianto et al., 2014; Knight & Devi, 2009; Lecera et al., 2015; Ng & Tan, 2010), Australia (Arthington et al., 1999) and the USA (Nico & Loftus, 2019) (Figure 1). Establishment of self-sustaining populations of *A. trimaculatus* has been reported for Australia, Singapore and Indonesia (Arthington et al., 1999; Database of Introduced Aquatic Species, 2019; Hedianto et al., 2014). Although *A. trimaculatus* is very common in the aquarium trade in the countries of South Asia, its occurrence in natural waters is relatively poorly documented (Nico et al., 2007).

Amphilophus trimaculatus was first reported from India in 2009 (Knight & Devi, 2009). Although evidence is insufficient to delineate the exact pathway used by *A. trimaculatus* to enter India, one

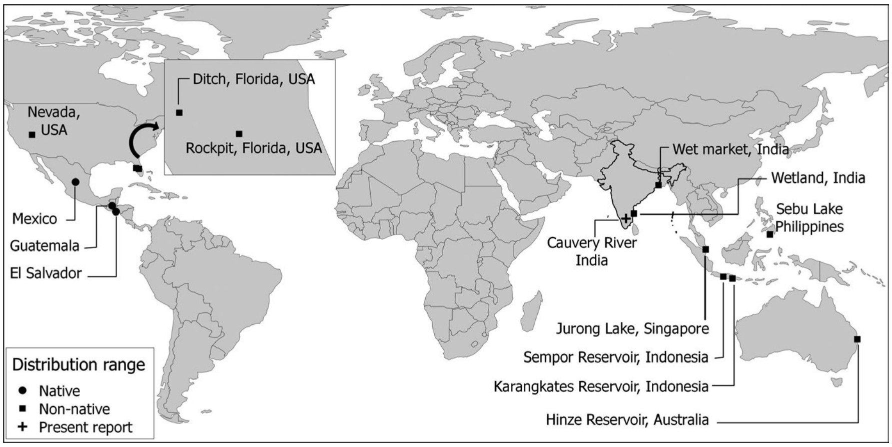

FIGURE 1 Map showing the native and non-native distribution of *A. trimaculatus*

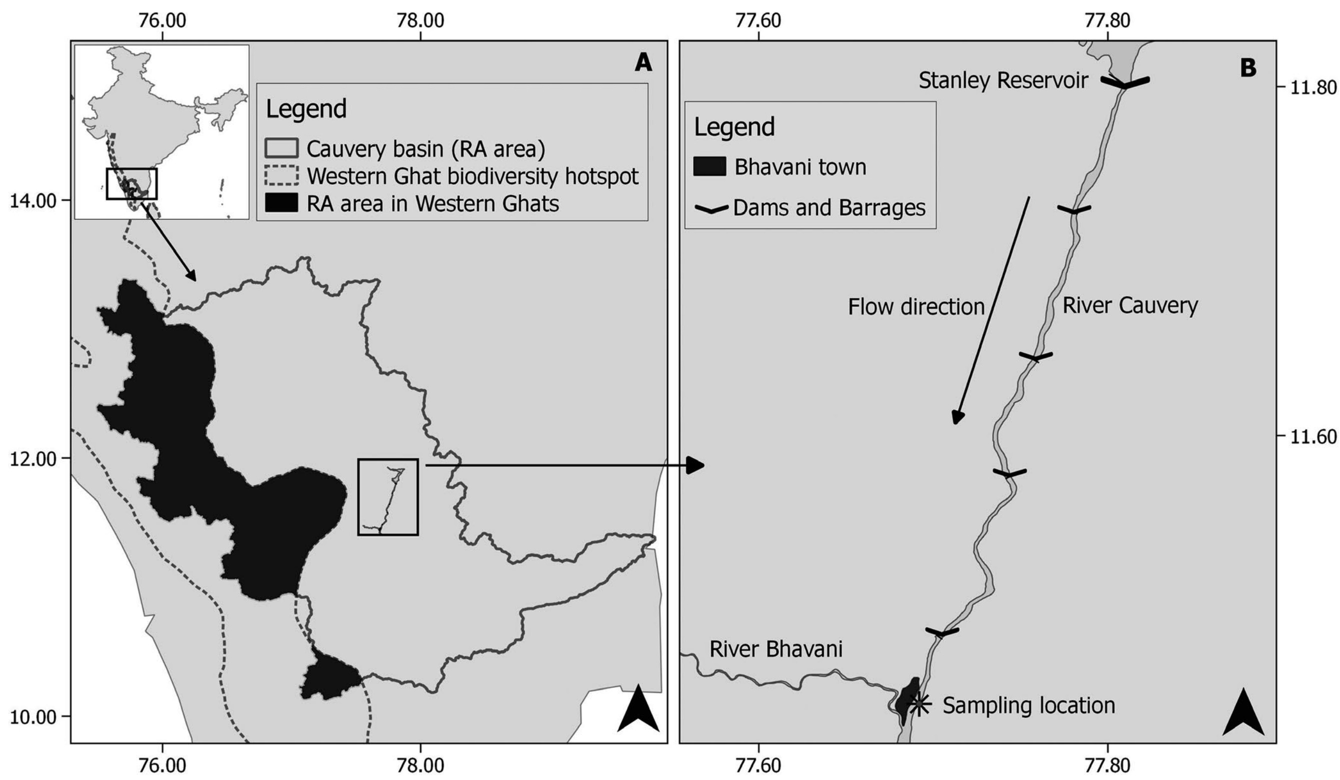

FIGURE 2 a, Boundary of the Western Ghats Biodiversity Hotspot in the River Cauvery basin and the area of the Western Ghats lying within the basin; b, sampling location and location of dams and barrages on the River Cauvery upstream of Bhavani town

possibility is that the species may have been introduced from the Southeast Asian countries through the ornamental fish trade, entering the risk assessment (RA) area by accidental escape from the ornamental fish trade facilities in the nearby townships. To manage bioinvasions, rapid, early-stage risk identification and assessment of the newly introduced species is necessary to aid environmental managers in their decisions to prevent the species introduction and/or aiding its control and containment (Kolar & Lodge, 2001). For containment, it is crucial to identify and understand the vectors and pathways of the dispersal of a species to prevent spread and reduce or mitigate impacts (Crooks & Soulé, 1999). The aim of the present study was to report on the occurrence of *A. trimaculatus*, as provisionally identified, and to assess the environmental risks posed by the species with regard to the River Cauvery (India) catchment, the risk assessment (RA) area. The specific objectives of the present study were to: 1) confirm the species identity as *A. trimaculatus* in morphological and genetic analyses; 2) carry out risk screenings of *A. trimaculatus* using an internationally applied decision-support tool to assess the species' likelihood of being invasive in the RA area; and 3) undertake vector and pathway analysis to identify means by which *A. trimaculatus* entered India, the likely geographical origin of the import consignments, and the species' subsequent dispersal in the RA area. The outcome of the present study is intended to inform the decision-making process, with particular reference to environmental managers for the control and/or eradication of the species. The potential impacts of the species in the RA area and possible management measures are also discussed.

TABLE 1 Minima (Min) and maxima (Max) of morphometric and meristic characteristics of *Amphilophus trimaculatus* specimens (n = 29) from the River Cauvery, India

Character	Min	Max (mm)	Mean	± *SD*
Standard length (SL)	88.00	157.00		
In percent of SL				
Body depth	41.97	51.84	46.47	2.92
Head length	31.78	33.73	33.73	1.62
Anal fin base length	22.62	32.30	27.47	2.92
Pectoral fin base length	06.81	08.40	07.28	0.51
Pelvic fin base length	04.36	06.35	05.44	0.58
Dorsal fin base length	50.13	56.96	53.33	1.92
Pre-dorsal length	31.59	46.88	36.83	4.31
Pre-pectoral length	32.99	39.68	36.12	2.02
Pre-pelvic length	37.11	43.16	39.92	2.06
Pre-anal length	56.40	65.51	61.23	3.11
Caudal depth	14.93	17.47	15.82	0.75
In percent of Head Length				
Snout length	30.94	40.34	36.59	2.51
Eye width	18.01	25.38	20.99	2.58
Pre orbital length	35.59	25.38	41.13	2.77
Post-orbital length	56.58	68.53	60.18	3.29
Inter-orbital width	30.38	38.62	35.69	2.84
Mouth width	27.56	37.06	34.07	2.73
Meristics				
Dorsal	XVII/ 10–11			
Anal	VII/ 7–8			
Pelvic	I/ 5			

2 | MATERIALS AND METHODS

The River Cauvery flows in an easterly direction across the Indian peninsula. The river's basin, the RA area (Figure 2a), extends over an area of 0.089 million km^2 and is one of the five river basins that drain the Western Ghats (Abell et al., 2008). The cichlid fish specimens (n = 29) from the River Cauvery were collected between May 2019 and January 2020 from commercial fishers at Bhavani in Tamil Nadu (Figure 2b). Following collection, the fin clips and muscle samples were preserved in 90% ethanol for molecular studies and the specimens were dissected to determine sex, based on macroscopic gonad inspection, as per Costa (2009). Specimens and their gut contents were preserved in 10% formaldehyde for later taxonomic identification and diet composition studies, respectively (Manko, 2016). The meristic and morphometric characteristics (Regan, 1905) were recorded for taxonomic identification of the fish.

Total DNA was extracted from preserved fin and tissue samples using standard proteinase K digestion, phenol/chloroform isolation and ethanol precipitation (Sambrook et al., 1989). Amplification of mitochondrial gene, cytochrome c oxidase I (*COI*) fragment, was carried out in a 25 µL reaction volume containing 50 ng template DNA, 10 pmol of each specific primer, 200 µM of each dNTPs, 0.75 units of *Taq* DNA polymerase and 1x *Taq* buffer containing 1.5 mM $MgCl_2$. The *COI* gene (FishF2 and FishR1) were amplified using primers (Ward, Zemlac, Innes, Last, & Hebert, 2005). Thermal cycling conditions consisted of an initial denaturation step at 95°C for four minutes, followed by 35 cycles of a denaturation step at 94°C for one minute; an annealing step at 44°C for one minute and an extension step at 72°C for one minute, with a final extension step at 72°C for ten minutes. The product was checked on 1.5% agarose gel and both sense and antisense strands were sequenced commercially by AgriGenome (India) using the corresponding PCR primers. The nucleotide sequences were subjected to homology analysis using BLASTn programs against the nucleotide database at NCBI (www.ncbi.nlm. nih.gov/BLAST).

Water variables, including temperature, pH, salinity, dissolved oxygen (DO), DO saturation and conductivity, were measured in-situ using an Aquaread portable millimetre (model no: multi-probe 2000). Turbidity was measured with an Oakton t-100 turbidity metre (minimum–maximum = 0.2–800 NTU), water depth with a Hondex BS-7 Echo-sounder. Water velocity was measured using flow metre (Global instruments make, model No: FP 111). Total alkalinity was determined chemically following standard methods (Baird & Bridgewater, 2017). Transparency was measured by employing a Secchi disc (Strickland & Parsons, 1972) and soil texture using a Bouycos hydrometer. Observations on habitat features were also made.

TABLE 2 Non-native distributional records of *A. trimaculatus*, including the present study

No.	Country	Name	Environment	Reference
1	Australia	Hinze	Reservoir	Arthington et al., 1999
2	India	Rettai Eri	Wetland	Knight & Devi, 2009
3	Singapore	Jurong	Lake	Ng & Tan, 2010
4	Indonesia	Sempor	Reservoir	Hedianto et al., 2014
5	Philippines	Sebu	Lake	Lecera et al., 2015
6	USA	Rockpit	Rockpit	Nico & Loftus, 2019
7	USA	NA	NA	Nico & Loftus, 2019
8	USA	Ditch	Ditch	Nico & Loftus, 2019
9	Indonesia	Karangkates	Reservoir	Amin et al., 2019
10	India	Fish market	NA	Chakraborty et al., 2019
11	India	Cauvery	River	present study

TABLE 3 Percentage area under each climate class in the native range and Cauvery basin

	Köppen–Geiger Climate classes	Percentage area under each climate class		
			Cauvery basin	
		Native	Present	Predicted
1	Tropical, rainforest	14.73	0	0
2	Tropical, monsoon	29.05	5.16	5.80
3	Tropical, savannah	41.44	44.78	75.61
4	Arid, steppe, hot	1.47	47.84	17.33
5	Arid, steppe, cold	0.36	0	0
6	Temperate, dry summer, warm summer	0.05	0	0
7	Temperate, dry winter, hot summer	0.24	0	0
8	Temperate, dry winter, warm summer	10.82	1.54	1.19
9	Temperate, no dry season, hot summer	0.30	0	0
10	Temperate, no dry season, warm summer	1.54	0.68	0.07

Invasiveness risk was evaluated using the Aquatic Species Invasiveness Screening Kit (AS-ISK: Copp et al., 2016). The AS-ISK (available at www.cefas.co.uk/nns/tools) consists of 55 questions available to assessors in 32 languages including Urdu (Copp et al., 2021): 49 questions comprise the Basic Risk Assessment (BRA) and six questions the Climate Change Assessment (CCA). The output is in the form of BRA and BRA + CCA (composite) scores. The cut-off score between medium and high-risk levels depends on setting a "threshold" value. However, due to the absence of threshold values for freshwater fishes, both in India generally and specifically in the RA area, the resulting scores were evaluated against the global threshold value (10.25) for freshwater fishes (Copp et al., 2020, unpublished).

The Köppen–Geiger climate classification maps of present climate (1986–2016) and future climate (2076–2100) at 1-km resolution (Beck et al., 2018) were used to compare climate in the species native range and the RA area. The Central American landmass between latitudes 13 °N and 18 °N (i.e. from the River Lempa, Guatemala, in the south to Coyuca Lagoon, Mexico, in the north) was considered as the species' native range. The boundary of the Cauvery basin downloaded from the database of the world's river basins (http://riverbasins.wateractionhub.org/) was considered as the introduced range for climate analysis. The areas under the influence of each climate class in the native and introduced range were extracted in the GIS platform, and percentage area under each climate class was calculated. The similarity of climate between native and introduced range was evaluated by cluster analysis. Preparation and analysis of maps were done on the GIS platform using QGIS (v2.18)—an open-source GIS Software (www.qgis.org).

3 | RESULTS

The morphometric and meristic characteristics of the captured specimens (Table 1) were as follows: deep body; compressed head with concave upper profile; mouth slightly upturned with lower jaw slightly projecting and mouth cleft ending before the anterior border of the eyes; upper jaw with two canine teeth in the outer series and lower jaw with the anterior two teeth on each side forming canine; cheek with 5–6 series of scales; upper lateral line with 20–23 scales and lower with 9–11 scales; ventral fin extending little beyond base of the origin of anal fin; caudal fin rounded; caudal peduncle nearly half as long as deep. Also, a dark blackish spot above the origin of lateral line was followed by one in the middle and another on the upper base of caudal peduncle. These characteristics could identify the fish specimens collected from River Cauvery at Bhavani as *Amphilophus trimaculatus*. The conventional taxonomic identity was confirmed using molecular tools. The nucleotide sequence showed 100% identity with *Cichlasoma trimaculatum* (previous name of *A. trimaculatus*) cytochrome oxidase subunit I (COI) gene (GenBank: GU817267.1) reported from the USA and 99.54% similarity with the subfamily Cichlasomatinae (COI) gene (JQ667516) reported from aquarium fish trade in India with an "expect value" (e) of 0. The gene sequences are deposited in GenBank with accession number MN888505. Thus, the specimens were confirmed as the first record of *A. trimaculatus* from a lotic environment in its non-native range (Table 2).

Body total length (TL) and weight of all sampled fish (sexes combined) ranged from 11.0 to 20.1 cm and from 29.4 to 175.0 g, respectively. Males were 16.5–19.5 cm TL and females were 14.0–20.1 cm TL. Gut fullness ranged from empty to quarter-full. Diet consisted of semi-digested items (insect appendages and other parts: 70%), digested matter (25%) and the remainder decaying plant matter and sand particles.

Water temperature and dissolved oxygen at Bhavani ranged from 26 to 33°C and 5.80 to 8.25 mg/L, respectively. Maximum depth recorded was 2.5 m, and the water velocity was between 0.1 and 1.3 m/s. Soil consisted of 89% sand followed by silt (6%) and clay (5%). The River Cauvery at Bhavani was ≈ 400 m wide and the river bed was rocky, forming pools in the dry season, whereas the shores had sandy and muddy bottoms with emergent and submerged macrophytes.

With regard to the climate in the species' native and introduced ranges (present and predicted climate), five of ten climate classes are common between the native range and the current RA area (Table 3). Climate match analysis indicated that the climate in the introduced range is 58% similar to the species' native range. The maximum area in which *A. trimaculatus* experiences a tropical climate is 85% in its native range and about 50% in the RA area under present climate conditions, which may increase up to 81% in the future climate conditions (Table 3, Figure 3).

The AS-ISK basic and climate change scores attributed to *A. trimaculatus* were 43 (BRA) and 55 (BRA + CCA), with confidence values of 0.81 and 0.77, respectively (Table 4). Pathway analysis could not identify the geographical route of *A. trimaculatus* entry into India due to the lack of reliable information. Vector analysis indicated that *A. trimaculatus* entered India through the aquarium trade and subsequently spread into natural waters through escape from holding facilities.

4 | DISCUSSION

The present study confirmed the presence of *A. trimaculatus* in the River Cauvery basin indicating the non-native range extension of the species in India (Chakraborty et al., 2019; Knight & Devi, 2009). The sizes of *A. trimaculatus* recorded from the Cauvery basin corresponded with the species length at first maturity, that is 8–10 cm

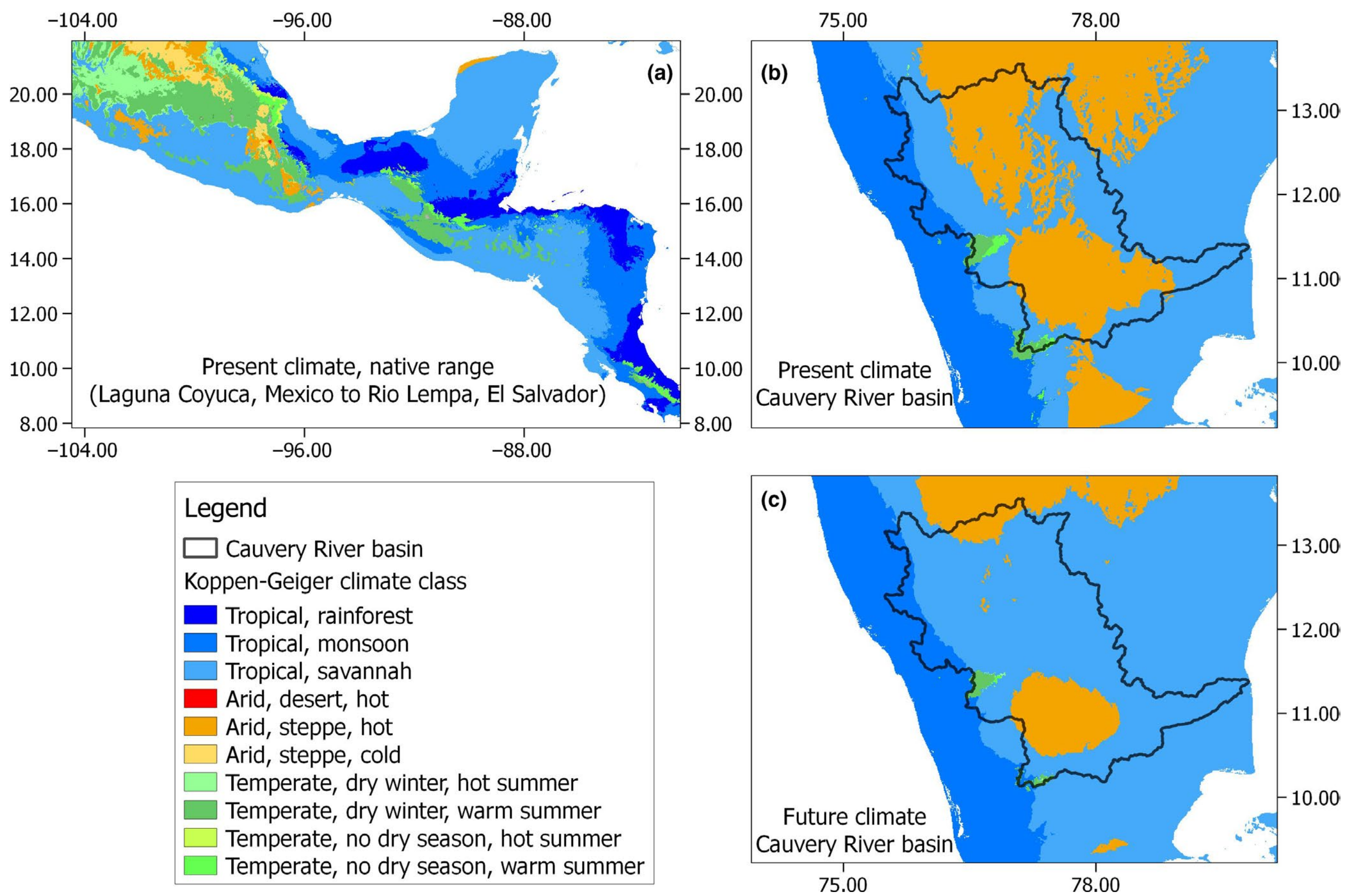

FIGURE 3 a, Present climate (1986–2016) in the native range of *A. trimaculatus*; b) present climate in the Cauvery basin; c, future predicted climate (2076–2100) in the Cauvery basin

TABLE 4 Outcome Basic Risk Assessment (BRA), Climate Change Assessment (CCA) and combined (BRA + CCA)_scores for *Amphilophus trimaculatus* in the River Cauvery basin using Aquatic Species Invasiveness Screening Kit (AS-ISK)

Questions/parameters	Score
BRA	43.0
BRA Outcome	High
BRA + CCA	55.0
BRA + CCA Outcome	High
Score Partition	
A. Biogeography/Historical	20.0
1. Domestication/Cultivation	*4.0*
2. Climate, distribution and introduction risk	*2.0*
3. Invasive elsewhere	*14.0*
B. Biology/Ecology	23.0
4. Undesirable (or persistence) traits	*8.0*
5. Resource exploitation	*7.0*
6. Reproduction	*2.0*
7. Dispersal mechanisms	*2.0*
8. Tolerance attributes	*4.0*
C. Climate change	12.0
9. Climate change	*12.0*
Answered questions	
Total	*55.0*
A. Biogeography/Historical	13.0
1. Domestication/Cultivation	*3.0*
2. Climate, distribution and introduction risk	*5.0*
3. Invasive elsewhere	*5.0*
B. Biology/Ecology	36.0
4. Undesirable (or persistence) traits	*12.0*
5. Resource exploitation	*2.0*
6. Reproduction	*7.0*
7. Dispersal mechanisms	*9.0*
8. Tolerance attributes	*6.0*
C. Climate change	6.0
9. Climate change	*6.0*
Sectors affected	
Commercial	17.0
Environmental	17.0
Species or population nuisance traits	26.0
Thresholds	
BRA	34.0
BRA + CCA	40.0
Confidence	
BRA + CCA	0.77
BRA	0.81
CCA	0.50

(Corfiled et al., 2008), and the largest Cauvery female *A. trimaculatus* (20.1 cm TL) approached the reported female maximum of 25.0 cm TL (Corfiled et al., 2008). The sizes of *A. trimaculatus* reported from the earlier records in India ranged from 3.5 to 16.5 cm (Chakraborty et al., 2019; Knight & Devi, 2009), indicating that the specimens in the present study have maximum TL as recorded from natural waters in India. *Amphilophus trimaculatus* is a euryphagous fish that feeds on small fishes, aquatic macro-invertebrates and both aquatic and terrestrial insects (Corfield et al., 2008). The present study suggests that *A. trimaculatus* is predominantly feeding on the insects in the River Cauvery. The piscivorous fishes in the RA area may be deterred from preying upon *A. trimaculatus* due to the presence of strong spiny fins (especially the dorsal fins). However, the results of the present study are preliminary in nature and further studies are needed to understand the prey and predator relation of *A. trimaculatus* in the RA area.

Normal temperature tolerance in *A. trimaculatus* ranges 21–30°C, although the species can withstand temperatures down to 10.9°C (Shafland & Pestrak, 1982). This indicates that water temperature in the River Cauvery at the Bhavani is well within the species' tolerance limits. On the other hand, salinity tolerance of *A. trimaculatus* remains unclear. Despite statements to its euryhalinity (Nico et al., 2007), *A. trimaculatus* has been reported to be a strictly freshwater species (Baensch & Riehl, 1985). River discharge in the sampled stretch of the RA area is low for most the year because of modification of normal flow discharges by the presence of a dam (Stanley Reservoir) and four hydropower barrages within a stretch of 46 km in the upstream of Bhavani town (Figure 2b). The river has a rocky bottom, with sandy and muddy shorelines, abundant macrophytes and reduced water velocity, which appears to be highly suitable for *A. trimaculatus* (Corfield et al., 2008). The water variables measured seasonally from the River Cauvery were within the species' tolerance limits (Table 5), suggesting that availability of suitable habitat is not likely to be a constraint in the dispersal and establishment of *A. trimaculatus* throughout the Cauvery basin.

The likelihood of an introduced fish species establishing can be predicted by matching climate between a species' native and introduced ranges (Howeth et al., 2016), although only to a certain extent because other biotic factors (e.g. food availability, competitors and predators; Bomford et al., 2009) and abiotic factors (physical habitat) are involved. In the Asia-Pacific region, *A. trimaculatus* has established self-sustaining population in the Hinze Reservoir in Eastern Australia (Arthington et al., 1999) and the Sempor Reservoir in Central Java, Indonesia (Hedianto et al., 2014). In both locations, the climate resembles that of the species' native range, and the same can be said for the River Cauvery basin, where the climate and other biotic and abiotic factors are also favourable for *A. trimaculatus*.

In terms of NNS risks, the Cichlidae family have the second highest number of "high-risk taxa" at global level (Vilizzi et al., 2019), and the output AS-ISK scores for *A. trimaculatus* (Table 4) indicated a species that poses a high risk of being invasive under both current

TABLE 5 Mean and SE values for seasonal water quality variables in the River Cauvery. DO = dissolved oxygen, TDS = total dissolved solids, PRM = Pre-monsoon (April–May), MON = Monsoon (September–October) and POM = Post-monsoon (January–February)

Water variables	PRM SE MON SE POM SE	SE	MON	SE	POM	SE
Water Temp (°C)	32.39	0.44	26.38	1.06	27.13	0.32
pH	8.90	0.09	8.11	0.12	8.96	0.07
Transparency (m)	1.37	0.25	0.78	0.07	1.71	0.08
Conductivity (µs/cm)	594.22	56.40	322.00	24.48	535.00	33.48
DO mg/L	7.21	0.29	5.05	0.11	8.54	0.38
DO saturation %	101.03	3.37	62.69	62.69	108.40	4.09
Alkalinity (mg/L)	142.89	5.35	50.73	18.06	148.00	13.17
Free CO_2 (mg/L)	1.33	0.94	2.40	0.46	0.00	0.00
Turbidity (NTU)	2.91	0.39	7.58	1.39	1.71	0.28
TDS (mg/L)	385.89	36.97	144.00	11.20	348.44	22.82
Salinity (ppt)	0.23	0.03	0.00	0.00	0.22	0.03

and future climate conditions, which are predicted to include warmer water temperatures and altered stream discharge rates. To mitigate the impact of these changes in climate on water services, more water-retention structures are likely to be constructed on India's rivers, and this is expected to facilitate the establishment of invasive species, such as observed in Iberia (Clavero & Hermoso, 2011) by enhancing their competitive and predatory effects (Rahel & Julian, 2008). However, comparing the output scores with AS-ISK threshold scores calibrated for the RA area may make the results more reliable.

With more than 5,000 species traded globally, the aquarium trade represents a pool of potentially invasive species (Chan et al., 2020; McDowall, 2004). The aquarium trade was the vector by which *A. trimaculatus* entered the USA and Australia (Arthington et al., 1999; Nico & Loftus, 2019), although the origin of *A. trimaculatus* consignments remains unknown (Database of Introduced Aquatic Species, 2019; Nico & Loftus, 2019). In India, the seaports of Chennai, Kolkata and Mumbai receive the largest quantity of consignments of live fish primarily from Southeast Asian countries (Directorate General of Commercial Intelligence & Statistics, 2019). Of these, Chennai is the ornamental fish hub closest to the RA area, so consignments of *A. trimaculatus* most likely were transported from Chennai to Bhavani. Other South American ornamental fishes (e.g. guppy *Poecilia reticulate* Peters, Amazon or vermiculated sailfin catfish *Pterygoplichthys* spp., red piranha *Pygocentrus nattereri* Kner, green swordtail *Xiphophorus helleri* Heckel and southern platyfish *Xiphophorus maculatus* (Günther)), *A. trimaculatus* is believed to have entered India through the aquarium trade (Knight, 2010; Sandilyan, 2016). Also, because the aquarium trade is the vector by which India receives ornamental fishes (including species of genera *Cichlasoma* and *Amphilophus*) primarily from Southeast Asian countries, it is likely that consignments of *A. trimaculatus* arrived in India from Southeast Asia for sale as an ornamental fish in Bhavani, where the species most likely gained entry into the river by way of accidental escape.

The present study confirms that *A. trimaculatus* in the River Cauvery is in the second of the four bioinvasion phases (introduction, establishment, dispersal and impact; Andersen et al., 2004). Considering its aggressive behaviour, feeding and reproductive biology, wide temperature tolerance, *A. trimaculatus* stands a good chance of getting established in the RA area, where favourable habitat and climatic conditions exist currently and are expected to remain favourable in the future. Whilst reduction in water discharges may create suitable habitat conditions, that is invasion windows for *A. trimaculatus* (Assis et al., 2017), dams and barrages are likely to pose as a barrier for free movement along the river's longitudinal course even though water drawdowns and spates would be expected to facilitate movements and dispersion within the basin, both between tributaries and between the main river and its flood plains. There are proposals by the Government of India to connect the rivers Mahanadi, Godavari, Krishna, Cauvery and Vaigai in peninsular India under the National River Linking Project (Shah et al., 2008), and this would facilitate the wider spread of *A. trimaculatus* from the River Cauvery to these aforementioned river systems.

In conclusion, the present study confirms the presence and establishment of *A. trimaculatus* in the RA area, and the AS-ISK score is indicative of a species that is likely to exert adverse impacts in the RA area. Studies of *A. trimaculatus* impacts in the invaded ecosystems are scarce (Nico & Loftus, 2019), which emphasises the need for further research on this species in its non-native ranges. The available information suggests that *A. trimaculatus* excavates sediments, which can disturb the habitat of native species, leading to failure in resource use (Radhakrishnan et al., 2020). After spawning, both male and female *A. trimaculatus* guard the nest aggressively (Corfield et al., 2008), which enhances offspring survivorship and thereby facilitates the species' establishment. The aggressive behaviour of *A. trimaculatus* and its dietary preferences, which overlap those of native species (Baensch & Riehl, 1993), indicates the potential for competition between *A. trimaculatus* and native fishes, although this requires combined field and controlled experimental study. The River Cauvery is home to 148 fish species, of which 17 are endemic, this being the largest number for any river in India (Ramakrishna, 2018). The RA area is part of the Western Ghats, which is a biodiversity hotspot characterised by high species richness and endemism of freshwater species (Myers et al., 2000), including 12 critically endangered, 54 endangered and 31 vulnerable fish species, of which 96 are endemic (Molur et al., 2011). Therefore, a precautionary approach should be adopted to avoid any adverse impacts that *A. trimaculatus* could cause.

Management of non-native fish species can include no action, control and/or containment, population extirpation, and/or species eradication (Varley & Schullery, 1995)—the latter often proving a most demanding (and potentially unfeasible) task. Therefore, a rapid-response strategy should include continued monitoring and assessment of the distribution and population structure of *A. trimaculatus* in the RA area. At the same time, steps should be taken to reduce propagule pressure by identifying and closing down the sources of entry. Measures for eradication by physical removal can also be considered. In support of these actions, targeted awareness programmes should be developed and implement to involve fishermen in the region in the culling of *A. trimaculatus* whenever they are captured.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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