

# The enemy of my enemy is still my enemy: the biological invasion and management of *Gambusia* in Peninsular India

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*Aquatic invasive species negatively impact the native aquatic communities in many parts of the world. Every nation faces the issue of introduced species, but the efforts to manage them depend on the varying capacity and willingness to manage invasions globally. Aquatic invasive species have their own set of challenges; it is crucial to understand the invasion dynamics of these species as well as their nature of interaction with humans to devise better mitigation processes. The freshwater aquatic wealth of India is seriously threatened by pollution, flow modification, overexploitation, habitat degradation and invasive alien species. India has several globally known freshwater invasive species. This article explores the invasion dynamics, potential pathways of invasion and impacts of the introduction of two of the world's top 100 invasive species, which remain relatively unknown in India: *Gambusia affinis* and *Gambusia holbrooki*. In the early 19th century, *Gambusia* species were introduced into Lalbagh Lake, Bengaluru, and eventually to other parts of the country. *Gambusia* is now widely distributed in the wild and in many Protected Areas, where it has become a threat to endangered amphibians, fishes and other aquatic invertebrates. This article explores the distribution of these *Gambusia* species in India and the drivers leading to their invasion. It also explains the obscure history of *Gambusia* invasion in India and the lack of awareness of its possible negative impacts. The importance of using molecular tools in taxonomy and their applications in invasion ecology are discussed. This study highlights the research gaps in invasion ecology of this genus in India and suggests scientific methods to manage these invasive species.*

**Keywords:** Aquatic invasive species, biological invasion, *Gambusia affinis*, *Gambusia holbrooki*, molecular tools.

It is widely known that invasive species often cause loss of biodiversity by leading to population declines of native species<sup>1</sup>. An invasive species can be defined as any species that has moved beyond its natural distribution range, and can reproduce and maintain its population over several generations, produce fertile offspring, often in very large numbers at considerable distances from the parent and/or site of introduction, or has the potential to spread over long distances, causing negative effects on the local biota<sup>2</sup>. With the rapid increase in anthropogenic activity in the last few centuries, the earth's biota has been transported at a higher rate than ever before in geological history causing biological invasion<sup>3</sup>. Invasion ecology has helped us understand the dynamics of such populations and their impacts in their non-native range as well as their interaction with the native species in the newly

colonized environment<sup>1</sup>. Understanding the mechanisms underlying such interactions could help in minimizing the negative effects of invasive species.

Biological invasion consists of a series of stages and barriers starting from the introduction, naturalization and establishment of an alien species, to its negative impacts on the environment, society and economy of the invaded region<sup>4</sup>. An alien species can get introduced into a new environment via three different pathways<sup>5</sup>: (a) natural spread, where the species extends its borders due to natural disasters or human-mediated large-scale disturbances which create opportunities for a dominant species to expand its range; (b) accidental introductions through activities such as trade, movement of livestock and people, and supplies during disaster management which can create pathways for alien species to move into new areas and (c) deliberate introductions, wherein a species is deliberately introduced into new localities for agriculture, aquaculture, horticulture, pet trade or for research and biocontrol purposes<sup>6</sup>. Deliberate introductions of such species for the biocontrol of another species have been of

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great concern, as they can have serious impacts on the native biodiversity. The success of invasive species has been largely attributed to propagule pressure and their escape from natural controls such as predators, parasites or competitors that would regulate their population size in their native habitats<sup>3</sup>.

There have been several instances where deliberate introductions for biocontrol have gone wrong. In 1935, invasive cane toads (*Rhinella marina*) were introduced to northeastern Queensland, Australia, from Hawaii to control populations of grey-backed cane beetles (*Dermolepida albobirtum*) and French beetle (*Lepidiota frenchi*). This rapidly turned into a disastrous event as cane toads became a major pest in Australia, causing negative ecological impacts on ants, snails, fishes, anurans, lizards, birds and even young crocodiles<sup>7</sup>. Another interesting case is the introduction of the Rosy wolfsnail (*Euglandina rosea*) in Hawaii<sup>8</sup> to control the giant African snail (*Achatina* spp). In an unexpected turn of events, the wolfsnails preferentially fed upon native molluscs of Hawaii<sup>8</sup> and became a major pest<sup>9</sup>. Introducing an alien species for biocontrol can negatively influence the native diversity and can cause serious impact on people's livelihoods<sup>10</sup>.

Introduction of the western mosquitofish *Gambusia affinis* (Baird & Girard 1853) and the eastern mosquitofish *Gambusia holbrooki* (Girard 1859) in freshwater ecosystems around the world has led to the widespread distribution of these species outside their native range<sup>11</sup>. *G. affinis* and *G. holbrooki* are freshwater fishes that were recommended for biocontrol of mosquitoes globally in malaria control programmes<sup>12</sup>. Native to USA, these fishes are now global residents. The *Gambusia* genus consists of 45 different species<sup>13</sup>. Mosquitofish are now well known to cause negative ecological impact, such as the extinction of native fishes, amphibians and several other freshwater communities by outcompeting and preying on native fauna<sup>14</sup>. *Gambusia* species are some of the most widespread freshwater fishes as they are hardy with high tolerance towards changing environmental conditions<sup>12</sup>. *Gambusia* are small-sized (4–7 cm), diurnal, viviparous surface-feeder fish that feed on copepods, zooplankton, Cladocera, surface insects, dipteran larvae, coleoptera, small benthos, molluscs, vegetation, algae, frog tadpoles and fish eggs<sup>12,15,16</sup>.

The life-history traits of *Gambusia* make them well-suited to establishing self-sustaining populations in new environments. Male *Gambusia* reach sexual maturity in a month and female in six weeks<sup>16</sup>. The gestation period lasts for 24 days. The brood size varies among females with different age classes. Female *Gambusia* can retain sperm from the male and can impregnate themselves at different times, thus having the ability to colonize new locations<sup>12</sup>. In addition, *Gambusia* can adapt to new environments and climate quite effectively<sup>12</sup>, and can be opportunistic at individual as well at population level,

where they can attain early maturity, have a long reproductive season, switch to ovoviviparity and have multiple brood sessions with a high survival rate<sup>14</sup>. Due to such adaptability, they have been successful across various freshwater ecosystems around the world.

### The origins of *Gambusia* in India

The global journey of *Gambusia* started in the early 20th century, when they were first introduced in the vicinity of Camden, England in 1905 (ref. 16). In 1921, *G. affinis* was reported to have been introduced in Italy and other parts of Europe<sup>17</sup>. However, it is not clear as to when it was introduced to India. From the available literature, there seems to be a major contradiction with respect to the species introduced and the time of introduction. *G. affinis* appears to have been introduced in India in 1914 from Italy and in 1930 from Siam<sup>18</sup>. However, there are also contrary reports of this species being introduced from Italy in 1928, by B. A. Rao, to control malarial mosquitoes as a part of the Urban Malaria Scheme (UMS) and the National Malaria Eradication Programme (NMEP)<sup>16,19</sup>.

Adding to the confusion, a large-scale study only showed occurrences of *G. holbrooki* in Europe<sup>20</sup>. Pyke<sup>12</sup> has mentioned that the chance of two *Gambusia* species coexisting in the same water body is rare due to interspecific competition. In India, it is not clear which of the two species was introduced, as there has been no systematic molecular taxonomy work.

Various Government and private institutions in India stock *Gambusia* in their local waterbodies to fight malaria. The Indian Council of Medical Research (ICMR), New Delhi, National Institute of Malaria Research (NIMR), New Delhi, municipal corporations; district administrations; fisheries departments; district health departments; tribal development agencies; local aquaculturists and the public have all played a major role in introducing mosquitofish (see Supplementary Material 1). *Gambusia* and other guppy fishes were successfully introduced by Government institutions in various landscapes in Assam, Gujarat, Karnataka, Maharashtra, Rajasthan, Tamil Nadu and Uttar Pradesh<sup>21–23</sup>.

The UMS uses *Gambusia* and guppy (*Poecilia reticulata*) for its mosquito control programmes. Using larvivorous fish has been 'an important step' in the Enhanced Malaria Control Project (EMCP) and the Malaria Action Plan<sup>24</sup>. ICMR has even introduced *Gambusia* in the Nicobar Islands<sup>25,26</sup> which are known to be a gold mine of endemic flora and fauna in India. According to the Island Susceptibility Hypothesis, islands are more susceptible to biological invasion than are continents<sup>27</sup>. It has been stated in the ICMR Annual Report 2003–04 that several *G. affinis* hatcheries were established and introduced into the creeks in the Nicobar Islands.

In India, *Gambusia* spp. have been surviving for almost a century across various landscapes and seasons<sup>16</sup>. According to the unified framework of biological invasion<sup>28</sup>, *Gambusia* can be considered as a full-fledged invasive species. Unintentional contamination of *Gambusia* in the aquaculture trade is possible as juvenile *Gambusia* strongly resemble other ornamentally important fishes of the family Poeciliidae. Anthropogenic activities along with natural dispersal are the main reasons for the invasion success of the species in countries outside their home range<sup>12</sup>. However, we did not come across enough evidence in India to support natural dispersal due to a lack of research in this area.

### ***Gambusia*: Are they effective in mosquito control?**

Early 19th century experiments to evaluate the larvicidal efficiency of *Gambusia* were subjective with small sample sizes and observational experiments showing a drastic reduction in mosquito larvae in the presence of mosquitofish<sup>12</sup>. Conversely, some studies reported that *Gambusia* are ineffective as a larvicide<sup>29</sup>. In fact, Pyke<sup>12</sup> reported that *Gambusia* have resulted in increased population of mosquito larvae, as they prey on other larvivorous predators. Multiple studies have reported that although *Gambusia* are quite effective in maintaining low larval densities, they can never succeed in completely replacing rapid-acting chemicals<sup>30</sup>. Guppies also have been introduced into ponds to control mosquito larvae population, but seem less efficient than *Gambusia* and have poor survival rates<sup>31</sup>. However, few studies show that guppies have better efficiency as a larvicide than *Gambusia*<sup>32</sup>. Despite conflicting data, however, it is generally agreed upon that the larvicidal potential of the so-called *G. affinis* in India is considerably higher than that of certain native fishes<sup>33</sup>.

Ghosh *et al.*<sup>19</sup> reported significant reduction in malaria cases in districts of Karnataka where *Gambusia* and *Poecilia* were introduced and provided evidence that *Gambusia* function effectively in open waters such as streams. However, Reddy and Pandian<sup>34</sup> showed that running waters affect the predatory efficacy of mosquitofish. In streams or ponds with agricultural run-off, *Gambusia* may not perform well due to elevated pesticide levels. *G. affinis* is highly vulnerable to insecticides such as profenofos and chlorpyrifos<sup>35–37</sup>. The larvicidal efficiency of *Gambusia* has also been shown to decline in the presence of thick vegetation<sup>38</sup>.

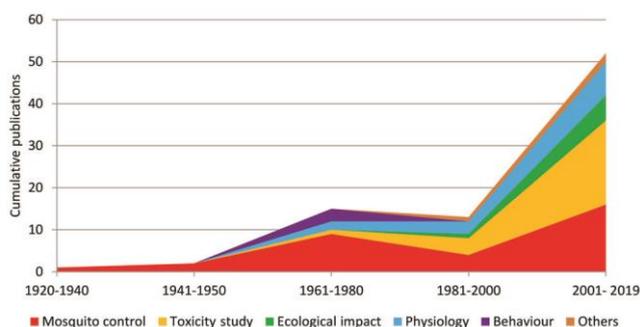
As discussed above, studies have not necessarily been conclusive about the effectiveness of *Gambusia* as a viable mosquito control. *G. holbrooki* also has been suggested to be ineffective for mosquito control<sup>39,40</sup>. This species feeds mostly on zooplankton, insects and zoobenthos, and mosquito larvae are negligible in its diet<sup>39</sup>. Mass reduction of zooplanktons further leads to algal

bloom, thus reducing the water quality<sup>41</sup>. In fact, *G. affinis* faces heavy mortality when it feeds only on mosquito larvae and in order to survive, requires a mixed diet.

### **Threats of *Gambusia***

Seven decades after the first introduction outside its native environment, the negative impacts of *Gambusia* on native biodiversity are becoming clear<sup>12</sup>. Several experimental studies showed a reduction in rotifers, crustaceans, backswimmers, water beetles and odonate larvae in the presence of *Gambusia*. The negative impacts of *G. holbrooki* on native species include competition, predation and aggressive interactions which can lead to trophic cascades in the invaded ecosystem<sup>41,42</sup>. *Gambusia* are also known to devour amphibian tadpoles. They have been shown to feed on *Microhyla* tadpoles and can significantly reduce the tadpole density<sup>43–45</sup>. Tadpoles of bullfrog (*Lithobates catesbeianus*) and American toad (*Bufo americanus*) often fail to recognize the presence of alien predators like *G. affinis*<sup>46</sup>.

In India, there have been few studies on the interactions of *Gambusia* with the native freshwater communities. In fact, the major focus of prior research has been to evaluate *Gambusia* as an effective player in mosquito control (Figure 1). Only few studies have evaluated the potential threats from frequent introductions of *Gambusia* into new ecosystems. In Pune, the native population of striped panchax (*Aplocheilichthys lineatus*) was affected by mosquitofish<sup>47</sup>. *Gambusia* species are more resistant to pollution compared to other native fishes, thus outcompeting the latter<sup>48,49</sup>. Increase in *Gambusia* population



**Figure 1.** Number of publications with respect to subject area from 1920 to 2019. The term 'Gambusia India' was searched in Google. The 500 foremost search results were accessed, and only the appropriate publications ( $n = 83$ ) were considered and separated based on five major subject areas (mosquito control, toxicity study, ecological impacts, physiology, others (including studies on reproductive biology, infection and survival)). (We recorded the impacts of these two species on local habitats on the basis of literature review. We collated 500 articles based on the purpose of the study, collecting a total of 83 reports focusing on the genus in India. The purpose of these studies was categorized into mosquito control, toxicity study, ecological impacts, physiological studies, and others (including studies on reproductive biology, infection and survival). Using these data, we plotted a graph with time of publication as the base (see Supplementary Material 2).

correlates with a decline in other fishes, as they might compete for common resources<sup>12,50</sup>. *Gambusia* express dominant behaviour such as chasing other fishes and fin-nipping<sup>42</sup>. Injured native fishes are prone to bacterial infections and may eventually die<sup>50</sup>. Additionally, *Gambusia* feed on zooplankton and aquatic insects, which may increase the phytoplankton population<sup>12</sup>. Researchers suggested eradicating *G. holbrooki* from Nainital Lake, as it was found to alter the planktonic community<sup>51</sup>. Apart from affecting aquatic ecology, there is a possibility of *Gambusia* acting as a vector for diseases<sup>12,52</sup>. They may also carry alien parasites that may infect native species<sup>41</sup>.

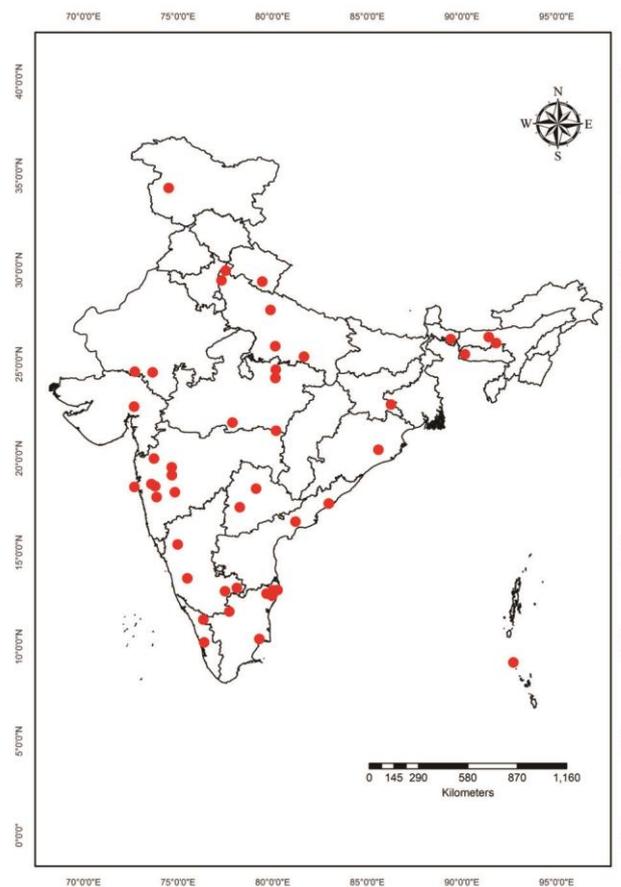
*G. affinis* and *G. holbrooki* have now been identified as invasive species in many countries across six continents, except Antarctica<sup>12</sup>. *Gambusia* have been identified as an impending threat to several freshwater fishes of Australia<sup>41</sup>. *G. holbrooki* was introduced into Australia via Italy in 1925 as a mosquito control agent. It is now known to alter the existence and richness of most wetland species<sup>53</sup>. After their introduction, *Gambusia* started competing and negatively impacted several fishes belonging to the families Pseudomugilinae, Gobiidae, Terapontidae, Percichthyidae, Antherinidae and Ambassidae. *Gambusia* are also reported to prey upon certain native fishes of Australia, such as rainbowfish of the Melanotaeniidae family, black mudfish (*Neochanna diversus*), purple-spotted gudgeon (*Mogurnda adspersa*), native galaxia (*Galaxias gracilis*), garnet minnow (*Aphyocypris lini*) and Japanese rice fish (*Oryzias latipes*)<sup>44,54</sup>. Tadpoles of several native Australian frogs were also impacted due to the presence of *Gambusia*. Several amphibian species belonging to the families Limnodryadidae, Pleodryadidae, Rhacophoridae, etc. were under threat<sup>44,54</sup>. As *Gambusia* are a threat to biodiversity, the afflicted countries are trying to eradicate them from the ecosystem.

## Proposed management of invasive *Gambusia*

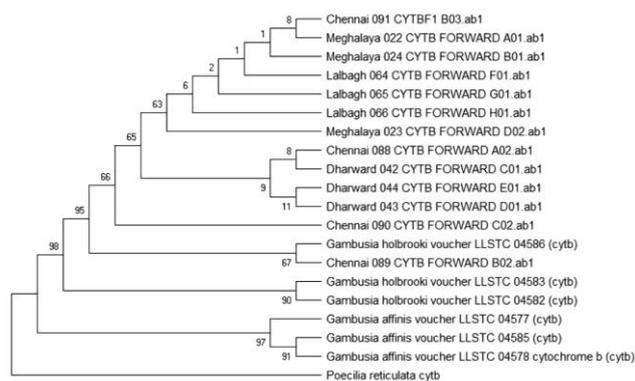
### Identification of the invasive species

Though the available records mention that *G. affinis* was introduced in India in 1928 from Italy, field assessments have found *G. holbrooki* in local water bodies<sup>16</sup>. Thus, it is unclear as to which of the two species was introduced in India. A large-scale molecular taxonomic study from Europe showed only the presence of *G. holbrooki*<sup>55</sup>. In India, researchers have described the presence of both *G. affinis* and *G. holbrooki*. Since both species have very little phenotypic variation, it is hard to distinguish them morphologically. We have collected *Gambusia* samples from creeks in Tura (Meghalaya), Chennai (Tamil Nadu), Dharwad as well as from Lalbagh, Bengaluru (Karnataka), where they were reportedly first introduced (see Supplementary Material 3) (Figure 2). Using the identification guide devised by Jayaram<sup>56</sup>, we morphologically identi-

fied the sampled fish as *Gambusia* species. DNA was isolated from fish tissue, and the *cytb* gene was amplified and sequenced (by Eurofins Genomics India Pvt Ltd, Bengaluru)<sup>55</sup>. In MEGA X, the sequences were aligned using ClustalW and a cladogram was constructed using maximum likelihood method with 1000 bootstrap replicates. Our preliminary analysis clearly indicated only the presence of *G. holbrooki* in the sampling sites across India (Figure 3). This is an important finding given that *Gambusia* has been used as a model organism in various toxicology and infectious disease studies in India<sup>52</sup>. The pre-existing official documents of malarial control departments and research articles, including taxonomic descriptions claiming the species is *G. affinis* need to be revised. Our molecular study also found the presence of only *G. holbrooki* from Lalbagh Lake in Bengaluru, which was supposed to be the first introduced population of *Gambusia* in India. However, the possibility of separate introduction of *G. affinis* in India cannot be ruled out, unless systematic sampling and identification are done throughout the country.



**Figure 2.** Current distribution of *Gambusia* in India based on the literature (see Annexure 2 for details). (We went through sample collection details of the reviewed articles. Field-visit data points of *Gambusia* were also included in this study. While we reviewed news articles and on-line media, we excluded these data as they were not scientifically confirmed. We used a larger pool of data points (45 points) and structured methodologies to update a previous map of *Gambusia* in India<sup>16</sup>.)



**Figure 3.** Simple cladogram representing the genetic relatedness of *Gambusia* species based on partial Cytb sequences.

### Genetic reconstruction of invasion history

Understanding the invasion history of a species plays a major role in developing strategies and policy measures to eradicate or to control biological invasions. Studying invasion history provides information on the origin, invasion route and the number of introductions. There are direct and indirect methods available to reconstruct a species invasion route<sup>57</sup>. While direct methods rely on historical records and notes, and/or interviews with stakeholders or key informants<sup>58</sup>, indirect methods rely on molecular-based comparisons between native and introduced populations of the species<sup>59–61</sup>. The invasion history of the American bullfrog in Montana was assessed using the genetic differences between native and invaded populations<sup>62</sup>. The study found a broad genetic variability in the population. Based on these studies, the authors suggested legislative action to prevent further introduction, and emphasized the importance of recreating genetic history and its significance in management strategies. Comparing the haplotypic diversity and phylogeographic surveys of an introduced species within and between countries can shed light on species diversity, founding populations and their origin. With this information, the type of vectors, the route of invasion and the number of introductions can be speculated<sup>57</sup>.

Thus rebuilding the invasion history of *Gambusia* is important in India, especially when there is confusion with respect to the origin of source population and the species identity. While the existing literature suggests that the ongoing introductions are from a single source, there is a possibility that multiple introductions might have occurred in the past.

### Risk assessment

Risk assessment is a series of methodologies to measure the potential impacts of non-native species prior to their introduction and their likelihood of establishment in the

non-native environment<sup>63</sup>. Using niche modelling tools and molecular genetics can help assess the risk of introducing a species. Species distribution modelling (SDM) is widely used in invasion ecology to assess the habitat suitability of invasive species in the non-native environment<sup>64</sup>. Using suitable variables, the probable niche shifts of invasive species under future climate change scenarios could also be predicted<sup>64</sup>. Employing genomic tools such as metabarcoding for analysing the gut content of invasive fishes can provide information on their feeding behaviour. Following the modern invasion ecology framework, the Environmental Impact Classification for Alien Taxa (EICAT), alien species can be categorized into various subgroups ranging from minimal to major concern. This will help in the management of invasive species. There are only limited studies that have assessed the impacts of *Gambusia* in freshwater ecosystems (Figure 1). It is important that species-specific risk assessments are done at a wider scale across different habitats before *Gambusia* spreads across India's freshwater ecosystems.

Towards this end, we have carried out an extensive literature survey using Google Scholar to collate scientific articles published on *Gambusia* spp. (Figure 1) between 1920 and 2019. We used the search terms 'Gambusia + mosquito fish + India' to narrow down the results. We manually filtered the initial 500 results and collected information regarding sampling sites. Additionally, we included first-hand field-visit data points from different parts of the country to collect *Gambusia* specimens. The previous map of the spread of *Gambusia* did not explain the methods of collection and utilized 15 data points<sup>16</sup>. Our updated map follows a structured methodology and has more than 45 data points, adding to its accuracy. Updated range and risk maps for invasive species can help managers target susceptible or infected areas, and focus their resources towards monitoring and addressing the spread of *Gambusia* in those regions.

### Monitoring

Early detection of invasive species and monitoring the potential spread to new areas are essential steps to effectively plan management strategies. Compared to a terrestrial set-up, detection and monitoring becomes a complicated process in aquatic ecosystems<sup>14</sup>. Recent advancements in eDNA-based approaches could help in monitoring the population spread as well as their densities in water bodies<sup>65,66</sup>. *Gambusia* can survive in a wide range of freshwater habitats and unless periodic monitoring is carried out, the species could become a major threat to other aquatic fauna<sup>12</sup>. Periodic monitoring should be carried out in the location where the fish has been introduced and also in adjacent freshwater ecosystems.

In Australia, *Gambusia* have escaped into the wild streams causing serious impact<sup>41</sup>. This situation can be avoided in India by periodic monitoring. Such large-scale monitoring can be efficiently carried out using citizen science approach<sup>67</sup>, though this approach for the identification of cryptic species does come with its own challenges. However, using an effective filtering process and obtaining large-scale data could help resolve this issue<sup>68</sup>.

### Policy-level changes

*Gambusia* have been recognized as one of the worst invasive species by the IUCN<sup>69</sup>. *G. holbrooki* is listed under the Invasive Alien Species Act of Japan<sup>70</sup>. *G. affinis* is regulated as a major invasive alien species in China<sup>55</sup>. In Australia, *Gambusia* are listed under the Biosecurity Act of 2014. In India, *G. affinis* and *G. holbrooki* have already been listed as invasive species by the National Biodiversity Authority<sup>71</sup>. Based on the existing data, one possible assumption is that the biological invasion of *Gambusia* has reached the final stage. In the unified framework the final stage, viz. 'symbol E', denotes fully invasive species<sup>28</sup>. *Gambusia* are now an invasive species, with the population surviving and reproducing in various habitats across India. Unfortunately, lack of coordination between various Government institutions has resulted in *Gambusia* still being widely introduced into water bodies<sup>72,73</sup>. Stricter policies and enforcement are required to avoid subsequent introductions and to manage the resultant impact of *Gambusia* in our fragile aquatic ecosystems. Detailed studies on these species should culminate in developing robust policy measures to monitor and, if needed, in the eradication and prevention of possible spread to other water bodies.

The World Health Organization, Geneva, has stopped recommending *Gambusia* as a mosquito control agent nearly four decades ago<sup>41</sup>. However, in India, both Government and nongovernmental agencies alike continue to introduce the species for mosquito control. Each year, numerous species of fish are being described by ichthyologists all over the world, including India<sup>74</sup>. It is highly likely that invasive fishes like *Gambusia* can cause several silent extinctions of native species. In New South Wales, Australia, under the Threatened Species Conservation Act (1995), *Gambusia* are listed as a key threatening species. The same law also urges landowners to avoid additional spread of the fish<sup>41</sup>. India must take a strong stand against further introductions and limit the spread of *Gambusia* in the country in order to preserve our inherent aquatic biodiversity and native species health.

### Conclusion

*Gambusia* is now widespread across India. Despite being listed as an invasive species by the National Biodiversity

Authority (NBA) and lack of conclusive studies on its effectiveness in mosquito control, it continues to be introduced in Indian water bodies. It is critical that further introductions are avoided and existing sites of introduction are actively monitored to limit the negative impacts of this fish on native biodiversity. Efforts should also be made to eradicate this species from freshwater ecosystems in India.

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Received 11 December 2019; revised accepted 4 September 2020

doi: 10.18520/cs/v119/i11/1752-1759