ROYAL SOCIETY OPEN SCIENCE

rsos.royalsocietypublishing.org

Research

Cite this article: Lukas JAY, Jourdan J, Kalinkat G, Emde S, Miesen FW, Jüngling H, Cocchiararo B, Bierbach D. 2017 On the occurrence of three non-native cichlid species including the first record of a feral population of *Pelmatolapia* (*Tilapia*) *mariae* (Boulenger, 1899) in Europe. *R. Soc. open sci.* **4**: 170160. http://dx.doi.org/10.1098/rsos.170160

Received: 24 February 2017
Accepted: 24 May 2017

Subject Category:
Biology (whole organism)

Subject Areas:
ecology/genetics/taxonomy and systematics

Keywords:
biological invasion, non-native species, thermally influenced freshwater systems, thermally polluted, invasion biology, tilapia

On the occurrence of three non-native cichlid species including the first record of a feral population of *Pelmatolapia* (*Tilapia*) *mariae* (Boulenger, 1899) in Europe

Juliane A. Y. Lukas[1,2], Jonas Jourdan[3], Gregor Kalinkat[1], Sebastian Emde[4,5], Friedrich Wilhelm Miesen[6], Hannah Jüngling[7], Berardino Cocchiararo[7] and David Bierbach[1]

[1]Department of Biology and Ecology of Fishes, Leibniz Institute of Freshwater Ecology and Inland Fisheries, Mueggelseedamm 310, 12587 Berlin, Germany
[2]Faculty of Life Sciences, Humboldt University of Berlin, Invalidenstrasse 42, 10115 Berlin, Germany
[3]Department of River Ecology and Conservation, Senckenberg Research Institute and Natural History Museum Frankfurt, Clamecystrasse 12, 63571 Gelnhausen, Germany
[4]Institute for Ecology, Evolution and Diversity, Goethe-University, Max-von-Laue-Str. 13, 60438 Frankfurt/M, Germany
[5]Senckenberg Biodiversity and Climate Research Centre, Senckenberg Gesellschaft für Naturforschung, Senckenberganlage 25, 60325 Frankfurt/M, Germany
[6]Zoologisches Forschungsmuseum Alexander Koenig, Sektion Ichthyologie, Adenauerallee 160, 53113 Bonn, Germany
[7]Senckenberg Research Institute and Natural History Museum Frankfurt, Conservation Genetics Group, Clamecystrasse 12, 63571 Gelnhausen, Germany

JAYL, 0000-0003-3336-847X

Author for correspondence:
Juliane A. Y. Lukas
e-mail: contact@julianelukas.com

Thermally influenced freshwater systems provide suitable conditions for non-native species of tropical and subtropical origin to survive and form proliferating populations beyond their native ranges. In Germany, non-native convict cichlids (*Amatitlania nigrofasciata*) and tilapia (*Oreochromis* sp.) have established populations in the Gillbach, a small stream that receives warm water discharge from a local power plant. Here, we report on the discovery of spotted tilapia (*Pelmatolapia mariae*) in the Gillbach, the first record of a reproducing

Electronic supplementary material is available online at https://dx.doi.org/10.6084/m9.figshare.c.3804706.

THE ROYAL SOCIETY PUBLISHING

population of this species in Europe. It has been hypothesized that *Oreochromis* sp. in the Gillbach are descendants of aquaculture escapees and our mtDNA analysis found both *O. mossambicus* and *O. niloticus* maternal lineages, which are commonly used for hybrids in aquaculture. Convict cichlids and spotted tilapia were most probably introduced into the Gillbach by aquarium hobbyists. Despite their high invasiveness worldwide, we argue that all three cichlid species are unlikely to spread and persist permanently beyond the thermally influenced range of the Gillbach river system. However, convict cichlids from the Gillbach are known to host both native and non-native fish parasites and thus, non-native cichlids may constitute threats to the native fish fauna. We therefore strongly recommend continuous monitoring of the Gillbach and similar systems.

1. Introduction

In colder regions of the world, many freshwater systems are thermally influenced, i.e. show water temperatures above the normal range [1]. In addition to sites that receive warm water by geothermal sources (e.g. [2–8]), thermal conditions are often altered by anthropogenic activities such as discharges of heated waters from power plants or the dewatering of mines (e.g. [9–18]). These thermally influenced freshwater (TIF) systems are hotspots for non-native organisms, as they provide suitable habitats for species of tropical and subtropical origin (e.g. [2–4,7–15,17,19–25]). While biological invasions are known to be a major driver of species extinctions and biodiversity loss [26–30], the importance and influence of TIFs remain mostly understudied.

The release of unwanted pets by aquarium hobbyists has been assumed as the main introduction pathway for non-native species into TIFs [17,31–37]. For example, aggressive behaviour, rapid reproduction, large size and illness are factors that increase the likelihood of aquarium fish to be released into the wild [31,38,39]. However, also introductions by aquaculture escapees are documented (e.g. [15,40–42]). The resulting artificial communities in TIFs [43,44] often comprise native as well as non-native species of both invertebrates (e.g. crustaceans; [13,21,45]) and vertebrates (e.g. fish; [4,8,15]).

One of the TIFs in central Europe is the Gillbach near the city of Cologne in Germany. This stream receives warm water effluents year round from a lignite power plant. Near the influx water temperatures rarely drop below 19°C, whereas 2 km downstream a minimum of 13°C was reported (February 2012; [15]). These conditions allowed several non-native tropical and subtropical fish species like *Ancistrus* sp., *Poecilia reticulata* and *Pseudorasbora parva* as well as some invertebrates (*Neocaridina davidi* and *Macrobrachium dayanum*) and tropical plants (*Vallisneria spiralis*) to establish self-sustaining populations [13,15,46]. Most of these are popular ornamental species, making an introduction via aquarium release the most probable invasion pathway and plausible scenario for the Gillbach.

Our current paper focuses on members of another (sub)tropical fish family inhabiting the Gillbach: Cichlidae. All members of this taxonomic family stem from the tropics or subtropics and many of them have been dispersed worldwide over the past century as a result of intentional introductions. Larger predatory cichlids (e.g. *Cichla ocellaris, Cichlasoma managuense, Serranochromis robustus;* [47]) are selected for stock enhancement, whereas certain omnivorous and herbivorous species are used as agents in the control of aquatic weeds (e.g. *O. aureus* [48], *Coptodon zillii* [49]), disease vector insects (e.g. *O. mossambicus* [50]) or nuisance molluscs (*Astatoreochromis alluaudi* [51], *Coptodon rendalli* [52]).

The most famous representatives of this family are commonly known and collectively referred to as 'tilapia' (genera *Sarotherodon, Oreochromis* and *Tilapia*; *sensu* Trewavas [53]) (e.g. [54–56]). According to Canonico *et al*. [55] most introductions of these genera have occurred due to aquaculture activities. In fact, the farming of tilapia (*Oreochromis* spp.) is currently the most widespread type of aquaculture in the world and only second to carp by volume of production [57]. In 1998, first specimens of a tilapia hybrid have been reported for the Gillbach and were identified as *O. niloticus* × *mossambicus* based on live coloration [41]. As most of today's tilapia culture is based on hybrids (most often between *O. niloticus, O. aureus* and *O. mossambicus;* [58]), we used DNA analysis, alongside classical morphological analysis, for species identification in our current study.

Besides their use in aquaculture, cichlids are also very popular with aquarists as they show a rich array of coloration and behavioural displays. Some species (e.g. *Aequidens pulcher, Amatitlania nigrofasciata, Astronotus ocellatus, Cichlasoma* spp., *Geophagus brasiliensis, Hemichromis letourneauxi, Sarotherodon melanotheron*; [59]) have been transported widely around the world via the aquarium trade and many introductions have been the result of occasional releases from home aquaria or stock disposal from dedicated breeding facilities of the aquarium trade. One of the most popular species within the

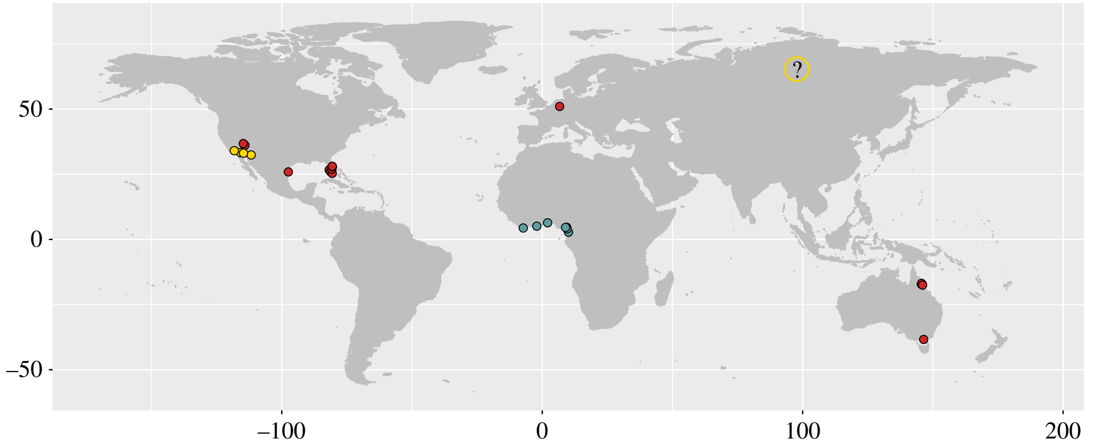

Figure 1. Distribution of *Pelmatolapia mariae*. *P. mariae* has been introduced beyond its natural range in West Africa (blue), with established populations in Australia, USA and Germany (red). Note that some records of *P. mariae* are location unspecific (indicated by question mark) or are suspected of having been subject to misidentification (yellow). For more detailed information on specific introduction sites of *P. mariae* refer to Bradford *et al*. [60] and Nico & Neilson [61].

ornamental trade is the convict cichlid (*Amatitlania nigrofasciata*), originally stemming from Central America. So far, the only stable population of *A. nigrofasciata* in Germany seems to be established in the Gillbach and was first described in 1998 [17,41].

In the current paper, we first report on the occurrence of another reproducing cichlid species in the Gillbach, which we identified as the spotted tilapia, *Pelmatolapia mariae.* With the new record of *P. mariae* (figure 1), the Gillbach now seems to harbour stable populations of at least two large African cichlids (*Oreochromis* sp. and *P. mariae*) and one Central American cichlid (*A. nigrofasciata*)—all of which have a long invasive history all over the world [55].

2. Material and methods

2.1. Study system

The Gillbach is a 28 km long stream within the Erft drainage, part of the Rhine basin of central Europe (figure 2*a*). The river flows through the North Rhine Lignite field in Germany, a hub for opencast mining and electrical energy industries. Its original headwaters being destroyed, it is now fed solely by the warm water discharge of the coal-fired power plant 'Niederaußem' (50°59′46.82′′ N, 6°39′50.56′′ E, RWE Power Inc.; figure 2*b*) located west of Cologne. At the site near Hüchelhoven/Rheidt (51°00′39.5′′ N, 6°41′02.1′′ E), the stream has been straightened to accommodate agriculture and developmental needs. The streambed of the Gillbach (approx. 3 m wide and 30–80 cm deep) consists almost entirely of artificially placed rocks as well as sand and mud. Owing to the coverage by bushes and trees, submerged vegetation is mostly absent (figure 2*c*).

2.2. Sampling

The water temperature at the stream's inlet was recorded every 4 h using an Onset HOBO data logger for the period of 19 March 2016 until 4 May 2017. Two kilometres downstream of this site, voucher specimens were caught using seine-netting (mesh size 6 mm) in September 2016. All specimens were immediately euthanized with an overdose of clove oil and preserved in 99% ethanol for further genetic analysis. Morphological species determination of all African cichlids was performed using standard keys (table 1; [53,62–64]). The identification of the Central American cichlid *A. nigrofasciata* followed Schmitter-Soto [65,66]. All native fish species were identified according to Kottelat & Freyhof [67]. Afterwards, all specimens were integrated into the ichthyologic collection at the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK) in Bonn, Germany under the project numbers Lukas_Gillbach2016_01 to Lukas_Gillbach2016_15.

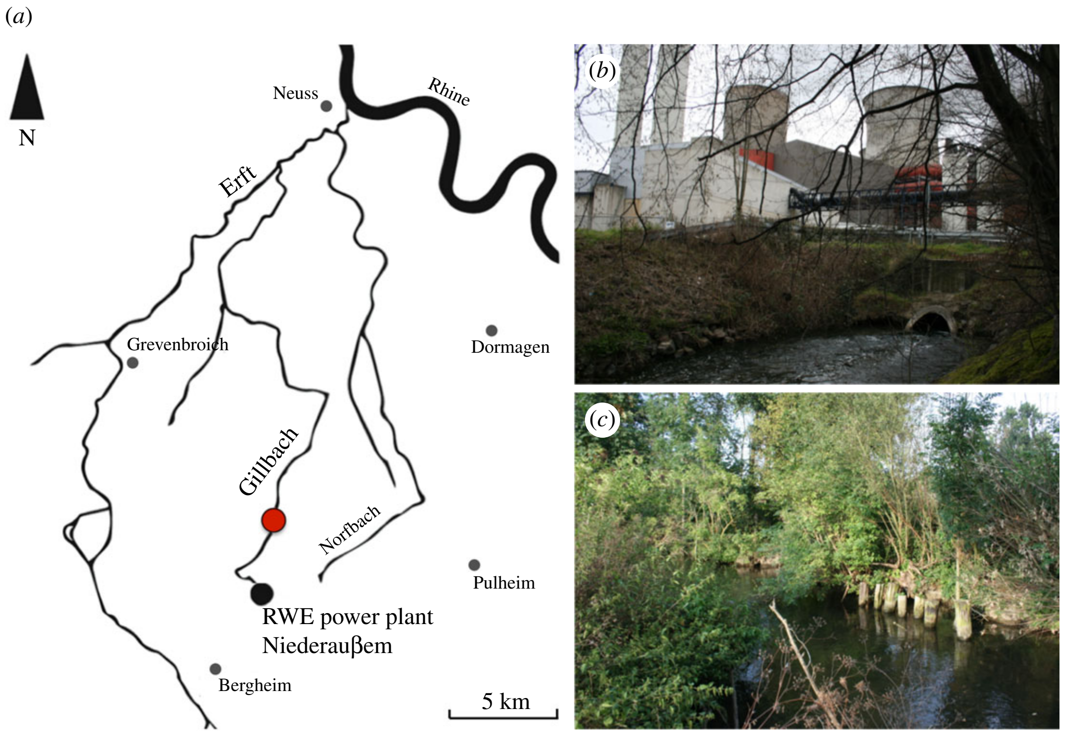

Figure 2. (*a*) Map of the Gillbach and its position within the Rhine catchment. Both the locations of the temperature logger placed by the inlet of the RWE power plant Niederaußem (black; *b*) and the sampling site near Rheidt (red; *c*) are indicated.

2.3. DNA extraction and mitochondrial DNA analysis

We extracted DNA from fin-clips of 14 fish specimens using the QIAGEN Blood and Tissue Kit (QIAGEN GmbH, Germany) as recommended by manufacturer's instructions. All extracts were measured with the NanoDrop™ Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and normalized to a concentration of approximately 10 ng µl^{-1} for further analysis. We analysed partial sequences of two mitochondrial DNA genes using the following primer pairs (for/rev): 1. Cytochrome b (Cyt *b*) L14734 (5′-AACCACCGTTGTTATTCAACT-3′) and H15557 (5′-GGCAAATAGGAARTATCAYTC-3′); 2. Cytochrome c oxidase subunit 1 (COI) L6199 (5′-GCCTTCCCWCGAATAAATAA-3′) and H6855 (5′-AGTCAGCTGAAKACTTTTAC -3′) [68]. PCR reactions were carried out in a total reaction volume of 15 µl, including 3 µl template, 3 mM MgCl2, 1X standard Taq (Mg-free) reaction buffer, 0.2 mM of each dNTP, 0.3 µM of each primer and 0.66 units Taq polymerase (New England BioLabs GmbH, Germany). We used the following thermal cycling parameters: 3 min initial denaturation at 95°C followed by 35 cycles (30 s at 94°C, 30 s at 54°C and 60 s at 72°C) plus a final extension step of 30 min at 72°C. PCR products were purified with a mixture of five units Exonuclease I (Thermo Scientific, Waltham, MA, USA) and 16 units of FastAP™ Thermosensitive Alkaline Phosphatase (Thermo Scientific). Sequencing was performed in both directions with PCR primers using the BigDye Terminator 3.1 sequencing kit (Life Technologies GmbH, part of Thermo Fisher Scientific) with an initial denaturation step of 60 s at 95°C, followed by 30 cycles of 10 s at 96°C, 10 s at 50°C and 120 s at 60°C. Products were purified with ABI-XTerminator beads (Life Technologies GmbH, part of Thermo Fisher Scientific) and separated on an ABI 3730 DNA Analyzer (Life Technologies GmbH, part of Thermo Fisher Scientific). The obtained sequence data were analysed in GENEIOUS 7.1.9 (Biomatters) and blasted for species determination using default settings in the National Centre for Biotechnology Information (NCBI) GenBank. The resulting sequences were submitted as Blast queries to Genbank. COI sequences were deposited in GenBank under accession numbers KY565238–KY565240 and Cyt *b* sequences can be accessed under KY582461–KY582463 (table 2).

3. Results

We first observed individuals of *Pelmatolapia mariae* near Rheidt in August 2016 (video observations, see the electronic supplementary material). In September 2016, we captured six specimens of *P. mariae* including two juveniles (figure 3*b*) and four adults (figure 3*a*). These ranged in size from 56 to

154 mm standard length (SL; table 1). All showed typical morphological features (table 1) and the distinctive coloration of *P. mariae* (e.g. ventral bars in juveniles (figure 3*b*) and dark caudal spots in adult specimens (figure 3*a*)). Furthermore, we counted a maximum of 15 gill rakers on the lower limb of the first branchial arch (figure 4*a*). Analysis of the lower pharyngeal jaw (LPJ; figure 4*b*) showed that its ventral keel was shorter than the toothed section. Both features are in accordance with the species' description by Teugels & Thys van den Audenaerde [62,63]. Molecular analysis of the mitochondrial COI gene confirmed the identity of *P. mariae* (100% matching with accession number KJ669646.1).

We caught eight specimens of the genus *Oreochromis*. Our morphological analysis found all individuals to show overlapping morphological characteristics of both *O. mossambicus* and *O. niloticus* (table 1) and a more *O. mossambicus*-like coloration and body shape (figure 3*c*,*d*; reddish fins, elongated snout, no bars at caudal fin typical for *O. niloticus*). However, our molecular analysis found both mitochondrial lineages of *O. mossambicus* (species O1, O3, O5–O8, matching with 99% identity in all cases; table 2) as well as *O. niloticus* (species O2 and O4, matching with greater than or equal to 96% in all cases; table 2), indicating that both maternal lineages were once introduced (*O. mossambicus* and *O. niloticus* and/or their hybrids). The wide range of sizes indicates that the *Oreochromis* population is breeding in the Gillbach. In fact, an adult female was mouthbrooding newly hatched fry at the point of capture.

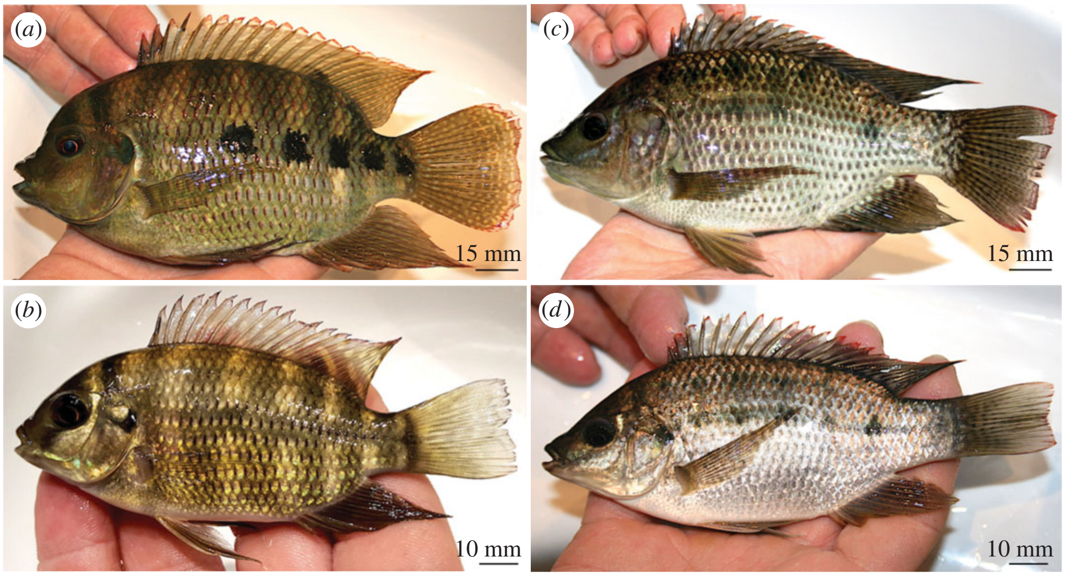

Figure 3. Live coloration of caught African cichlids. (*a*) *Pelmatolapia mariae*, adult male SL 154 mm; (*b*) *Pelmatolapia mariae*, juvenile SL 56 mm; (*c*) *Oreochromis* sp., adult female SL 160 mm; (*d*) *Oreochromis* sp., juvenile SL 92 mm.

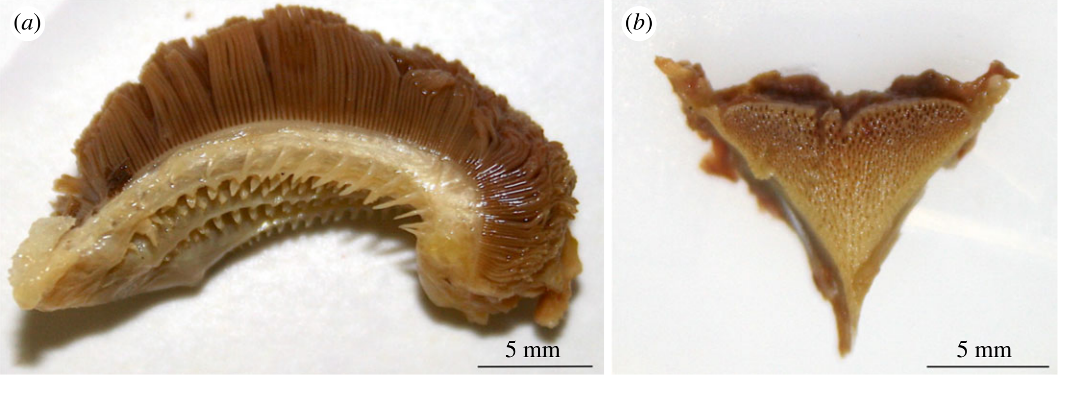

Figure 4. Anatomical features of *Pelmatolapia mariae*. A maximum of 15 gill rakers on lower limb of isolated first gill arch (*a*). The LPJ (*b*) is triangular with blade shorter than toothed section.

Table 1. Basic data on specimens of African cichlid species collected in the Gillbach stream, including numbers of individuals captured, sex, size (standard length, SL) and meristic information. Reference values follow Teugels & Thys van den Audenaerde [62] for *P. mariae* and Trewavas [53] for *Oreochromis* spp. Note that fin ray counts can differ between localities [60].

				spines (no.)		soft rays (no.)		
specimen ID	sex	SL (mm)	ratio head : SL (% of SL)	dorsal	anal	dorsal	anal	scales lateral line (no.)
Pelmatolapia mariae		>323	29.7–34.7	15–17	3	13–15	10–11	29–31
Oreochromis niloticus		>600	31.5–40.5	15–18	3	11–13	9–11	30–34
Oreochromis mossambicus		>390	32.3–39.0	15–18	3	10–13	7–12	27–34
P1	M	154	32.5	16	3	13	10	29
P2	M	145	25.5	15	3	13	11	29
P3	F	107	29.0	17	3	13	11	29
P4	J	85	24.7	17	3	13	11	28
P5	J	79	30.4	15	3	13	12	28
P6	J	56	21.4	16	3	13	11	28
O1	M	177	32.2	17	3	12	11	31
O2	F	160	34.4	16	3	10	10	31
O3	J	118	31.4	17	3	11	9	30
O4	J	116	31.9	16	3	12	9	29
O5	J	92	33.7	17	3	12	11	31
O6	J	97	32.0	17	3	11	10	30
O7	J	110	31.8	15	3	12	9	29
O8	J	60	33.3	17	3	11	10	30

Table 2. Molecular species identification. Processed samples of 'tilapia' species included in this study and top matches from GenBank database. Maximum identity percentage of the sequences refers to pair-wise alignments with the closest match (n.a. identifies no match greater than 90%).

	GenBank accession number		best BLAST hit with GenBank accession number and maximum identity percentage		
specimen ID	COI	Cyt b	COI	Cyt b	identified species
P1	KY565240	KY582463	KJ669646.1 (100%)	n.a.	*P. mariae*
P2	KY565240	KY582463	KJ669646.1 (100%)	n.a.	*P. mariae*
P3	KY565240	KY582463	KJ669646.1 (100%)	n.a.	*P. mariae*
P4	KY565240	KY582463	KJ669646.1 (100%)	n.a.	*P. mariae*
P5	KY565240	KY582463	KJ669646.1 (100%)	n.a.	*P. mariae*
P6	KY565240	KY582463	KJ669646.1 (100%)	n.a.	*P. mariae*
O1	KY565238	KY582461	AY597335.1 (99%)	AY597335.1 (99%)	*O. mossambicus*
O2	KY565239	KY582462	GU370126.1 (98%)	GU477628.1 (97%)	*O. niloticus*
O3	KY565238	KY582461	AY597335.1 (99%)	AY597335.1 (99%)	*O. mossambicus*
O4	KY565239	KY582462	GU370126.1 (98%)	GU477628.1 (97%)	*O. niloticus*
O5	KY565238	KY582461	AY597335.1 (99%)	AY597335.1 (99%)	*O. mossambicus*
O6	KY565238	KY582461	AY597335.1 (99%)	AY597335.1 (99%)	*O. mossambicus*
O7	KY565238	KY582461	AY597335.1 (99%)	AY597335.1 (99%)	*O. mossambicus*
O8	KY565238	KY582461	AY597335.1 (99%)	AY597335.1 (99%)	*O. mossambicus*

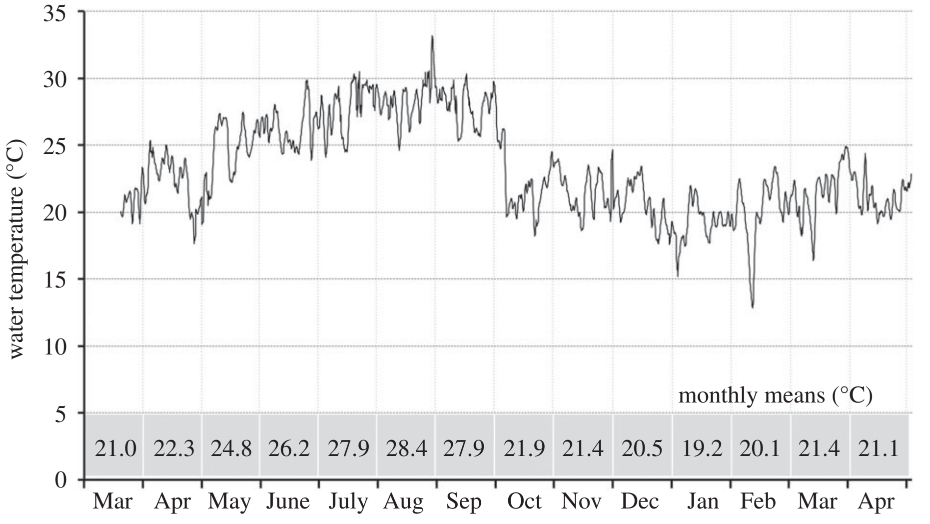

Figure 5. Daily water temperatures in the Gillbach in 2016/2017. Fluctuations in the daily means (six data points per day) were recorded for the source of the Gillbach from March 2016 to May 2017 and monthly means were calculated (bottom panel).

A third cichlid species, the convict cichlid (*Amatitlania nigrofasciata*) was also caught and we observed many breeding pairs in shallow areas along the stream. In addition, two native European chub (*Squalius cephalus*), one native barbel (*Barbus barbus*), as well as one specimen of the tropical *Ancistrus* sp. were caught. Assignment of armoured catfish specimens further than the genus *Ancistrus* remains tentative until systematics are further resolved [69].

Our temperature measures largely confirmed previous measures [15] and water temperatures never dropped below 8.38°C (only three readings were below 12.5°C). Monthly means at the Gillbach (figure 5, bottom panel) ranged from 19.2°C (January 2017) to 28.4°C (August 2016). Reference measurements taken at the sampling site in Hüchelhoven revealed an average temperature difference of ±2 K compared with the stream's source.

4. Discussion

The Gillbach near Cologne represents a thermal refuge, which provides suitable conditions for a variety of introduced non-native species. In fact, the cichlids *O. niloticus* × *O. mossambicus*, *A. nigrofasciata* and *Maylandia* (*Pseudotropheus*) *aurora* have been reported for the Gillbach/Erft system during previous samplings [15,17,41]. The occurrence of *M. aurora* could not be confirmed after 1998, but our sampling now adds another species to that list: the West African spotted tilapia *P. mariae*.

Our mtDNA analysis found both *O. mossambicus* and *O. niloticus* maternal lineages. Sequencing of the Cyt *b* and COI gene, however, does not enable us to clearly detect hybrids. Nevertheless, found specimens from the Gillbach largely share the same morphological phenotype corroborating the presence of a hybrid population. *Oreochromis* species hybridize well when occurring syntopically [58] and the use of hybrids is common practice in aquaculture. The Gillbach's *Oreochromis* sp. are assumed to be (descendants of) escapees from a closed aquaculture facility that employed the power plant's warm water discharge for its production [41]. We do not know whether both species (*O. niloticus* and *O. mossambicus*) were initially introduced and hybridized afterwards in the Gillbach or whether hybrids were initially released. Samplings in 2012 [15] and 2016 (present study) found both adults and juveniles, thus, *Oreochromis* sp. can be considered to have established a reproducing population within the Gillbach.

Amatitlania nigrofasciata have successfully persisted in the Gillbach for more than 18 years now (first record by Höfer & Staas [41]). Individuals of different size classes were plentiful during samplings in 1998 [41], 2012 [15], 2014 [17] and 2016 (present study). Established wild populations are known for Asia, the Middle East, North and Central America and Australia [59,70–73]. So far the only other introduction sites within Europe are two thermal refugia in Italy [4] and Austria [3], both of which are very similar to the Gillbach system in their habitat characteristics and species assemblage.

To this day, *P. mariae* has been introduced to at least three continents (figure 1; [59–61]). Records from Australia show an established population in the cooling waters of a power station [74] very similar to the Gillbach system. The Gillbach, with its sandy streambed and shallow littoral zones, allows for conditions that correspond to *P. mariae*'s natural habitats in Nigeria [75]. Submerged vegetation is mostly absent in this section of the Gillbach; however, artificially placed rocks may be suitable as spawning substrate [76] and provide shelter during the larval and juvenile stages, which are most prone to predation [77]. Mature individuals have virtually no predators in the Gillbach, but eggs and larvae are most probably cannibalized or preyed on by bigger *Oreochromis* sp., as well as native species, such as European chub and Common barbel. The dental and gill morphology of the spotted tilapia allows for foraging behaviour that includes both plankton-filtering and grazing [78,79] and thus *P. mariae* finds suitable conditions in the Gillbach for a diet dominated by plant material [17]. Moreover, the species' documented tolerance to a wide range of temperatures, salinity and dissolved oxygen concentrations [60], not only fosters its dispersal, but also makes it a potential candidate for aquaculture [80,81]. However, the spotted tilapia is an available aquarium fish and its first occurrence in the Gillbach almost two decades later than the closure of the aquaculture facility renders a release by aquarists the most likely reason for the introduction of the species. Similar introduction pathways are known from certain locations in Australia [82–84] and North America ([85,86]; figure 1).

As all cichlid species currently present in the Gillbach stem from the tropics and subtropics, they cannot cope with water temperatures commonly encountered during harsh German winters. Several studies report on the temperature ranges in their natural habitats (17°C–35°C for *O. mossambicus* and 13.5°C–33°C for *O. niloticus* [87]; 20°C–36°C for *A. nigrofasciata* [88]; 20°C–25°C for *P. mariae* [89]). However, extended temperature ranges have been shown for introduced populations of *Oreochromis* spp. [87] and *P. mariae* [90,91] with lethal limits of all three species being reported to be below 11.5°C [92–94]. While average monthly temperatures in the Gillbach never dropped below 19°C (figure 5), we did record temperature spikes (less than 4 h) down to 9.5°C (26 April 2016) and even 8.4°C (30 April 2016, 2 January 2017). However, fish originating from the ornamental trade are often more cold-tolerant than their wild-type counterparts (e.g. [15] for *Poecilia reticulata*; [14] for *Xiphophorus variatus*). In fact, several studies showed certain tilapias (*Oreochromis* spp.) are capable of surviving in rapid temperature fluctuations down to 10°C with seemingly no detrimental effect [9,95,96].

The Gillbach drains into the Erft river, which is equally influenced by the effluents of nearby power plants (e.g. RWE power plant 'Frimmersdorf'; [97,98]) and mine dewatering (Lignite mining area 'Garzweiler'). Temperatures in 2016 never dropped below 10°C (e.g. February near Glesch; [99]) and tropical non-natives such as *Poecilia reticulata* and even piranhas have been found here regularly (Udo Rose 2007, personal communication). The Erft drains into the Rhine, which is currently the most thermally polluted river in the world [1] with a high richness and abundance of non-native species [100,101]. Nevertheless, temperatures in the Rhine sometimes drop to below 4°C (e.g. January 2017 near Düsseldorf-Flehe; [99]) and thus most non-natives with tropical or subtropical origin would not survive outside the areas affected by warm water influx. In fact, both Deacon *et al*. [102] and Jourdan *et al*. [15] suggested that an expansion of the tropical guppy into adjacent, not artificially heated streams is unlikely.

One often neglected risk emanating from non-native species is their ability to distribute non-native pathogens [103,104]. Emde *et al*. [17] demonstrated that TIFs may function as reservoirs for non-native pathogens and parasites. The authors found convict cichlids from the Gillbach to serve as both intermediate and final host for one native (*A. anguillae*) and three introduced fish parasite species (*A. crassus*, *B. acheilognathi*, *C. cotti*), thereby increasing the risk of spread of these parasites beyond their current distribution. First samples of *Oreochromis* sp. indicate that this species plays no significant role in the spread of parasites within the Gillbach system due to its mainly plant-based diet (Sebastian Emde 2016, personal communication). Whether *P. mariae* constitutes a greater threat in this regard should be in the focus of future investigations as its diet differs from that of *Oreochromis* sp. In its native range, *P. mariae* carries heavy parasite loads with a large proportion of the population being infected (greater than 50%; [105–107]).

The Gillbach exemplifies that TIFs can accumulate more and more non-native species over time [13,15,41,46]. Observations from the Warmbach near Villach (Austria) provide similar results: each consecutive sampling found new non-native species (2001: *Hemichromis letourneauxi*; 2002: *Hemichromis fasciatus*; 2005: *Procambarus clarkia*, *Lepomis gibbosus*, *Poecilia reticulata*, *Xiphophorus maculatus*, *Xiphophorus hellerii*; 2007: *Amatitlania nigrofasciata*, *Oreochromis mossambicus*, *Ancistrus dolichopterus*, *Maylandia aurora*, *Hyphessobrycon erythrostigma* [3,108]). Overall, greater effort in prevention of the release of non-native species is required to stop the spread outside their native range through a raising of awareness in fish keepers and society alike.

5. Conclusion

(i) The Gillbach—a TIF that has accumulated non-native species over time—is now harbouring stable populations of three cichlid species. We confirm the occurrence of *A. nigrofasciata* and *Oreochromis* sp., both of which have been previously described for this system. In fact, molecular analyses of *Oreochromis* specimens identified the existence of mitochondrial lineages of *O. mossambicus* and *O. niloticus*. We further report on the occurrence of *P. mariae*, which is the first record of this species in Europe.

(ii) Cichlids in TIFs may play a role in disease and parasite transmission. It has been shown that convict cichlids from the Gillbach serve as hosts for both non-native and native parasites. Thus, we strongly recommend further investigations on the potential transmitter role of *Oreochromis* sp. and *P. mariae* in the Gillbach system.

(iii) We urgently call for an inclusion of TIFs into continuous monitoring programmes. The Gillbach provides a fruitful system to study invasion processes in detail and improve our understanding of potential impacts on native species and ecosystems. We further prompt that raising public awareness is much needed. While there are several scientific publications on the Gillbach's non-native fish fauna, alien species databases either show outdated records [109,110] or no records of any introduced cichlids in Germany [111,112] as of May 2017. Furthermore, we urge fish keepers to refrain from releasing their pets into 'suitable' habitats (which is already prohibited by the German Animal Welfare Act [113]; §3 Abs. 3, 4 TierSchG).

Ethics. Permission to collect specimens of the family Cichlidae from the Gillbach was granted to the authors by the Erftfischereigenossenschaft (Bergheim, Germany) through Dr Udo Rose. No further collecting permits or approvals were needed. No animal care protocol was required for our research.

Data accessibility. Our data are deposited at Dryad (http://dx.doi.org/10.5061/dryad.sd7vh; [114]). mtDNA sequences are available at the NCBI GenBank under the following accession codes: KY565238–KY565240 and KY582461–KY582463. All specimens were integrated into the ichthyologic collection at the ZFMK in Bonn, Germany under the project numbers Lukas_Gillbach2016_01 to Lukas_Gillbach2016_15.

Authors' contributions. J.A.Y.L., J.J., G.K., S.E., F.W.M. and D.B. jointly collected the fish. J.A.Y.L. performed the morphological identification and prepared DNA samples, which were further analysed by J.J., B.C. and H.J. J.A.Y.L., J.J. and D.B. interpreted the results. J.A.Y.L. wrote the manuscript. All authors gave final approval for publication.

Competing interests. We declare we have no competing interests.

Funding. Financial support was given by the Gesellschaft für Ichthyologie e.V. (German Ichthyological Society).

Acknowledgements. The authors thank Dr Udo Rose—biologist at the Erftverband and member of the Erftfischereigenossenschaft (Bergheim, Germany)—for the permission to do research at the Gillbach. Further, we would like to express our gratitude to the Gesellschaft für Ichthyology e.V. (GfI) for financially supporting the project. We were further supported by several fish enthusiasts: Geoffrey P. F. Mazué, Vivek Hari Sridhar, Bernd Neu, Roman De Giorgi, Judith Kochmann, as well as Petr Zajicek and Marcus Ebert from IGB Berlin. We want to express our sincere appreciation for their lively participation during numerous samplings. Lastly, we would like to thank the anonymous reviewers for their valuable comments and suggestions during the revision process.

References

1. Raptis CE, van Vliet MT, Pfister S. 2016 Global thermal pollution of rivers from thermoelectric power plants. *Environ. Res. Lett.* **11**, 104011. (doi:10.1088/1748-9326/11/10/104011)
2. Specziár A. 2004 Life history pattern and feeding ecology of the introduced eastern mosquitofish, *Gambusia holbrooki*, in a thermal spa under temperate climate, of Lake Hévíz, Hungary. *Hydrobiologia* **522**, 249–260. (doi:10.1023/B:HYDR.0000029978.46013.d1)
3. Petutschnig J, Honsig-Erlenburg W, Pekny R. 2008 Zum aktuellen Flusskrebs- und Fischvorkommen des Warmbaches in Villach. *Carinthia II* **198**, 95–102 [In German].
4. Piazzini S, Lori E, Favilli L, Cianfanelli S, Vanni S, Manganelli G. 2010 A tropical fish community in thermal waters of southern Tuscany. *Biol. Invasions* **12**, 2959. (doi:10.1007/s10530-010-9695-x)
5. O'Gorman EJ *et al.* 2012 Impacts of warming on the structure and functioning of aquatic communities: individual- to ecosystem-level responses. *Adv. Ecol. Res.* **47**, 81–176. (doi:10.1016/B978-0-12-398315-2.00002-8)
6. O'Gorman EJ, Benstead JP, Cross WF, Friberg N, Hood JM, Johnson PW, Sigurdsson BD, Woodward G. 2014 Climate change and geothermal ecosystems: natural laboratories, sentinel systems, and future refugia. *Glob. Change Biol.* **20**, 3291–3299. (doi:10.1111/gcb.12602)
7. Milenković M, Žikić V, Stanković SS, Marić S. 2013 First study of the guppy fish (*Poecilia reticulata* Peters, 1859) occurring in natural thermal waters of Serbia. *J. Appl. Ichthyol.* **30**, 160–163. (doi:10.1111/jai.12218)
8. Sas-Kovács I, Telcean IC, Covaciu-Marcov SD. 2015 A non-native fish assemblage in geothermal waters of Romania. *J. Appl. Ichthyol.* **31**, 211–213. (doi:10.1111/jai.12652)
9. Langford TE, Aston RJ. 1972 The ecology of some British rivers in relation to warm water discharges from power stations. *Proc. R. Soc. Lond. B* **180**, 407–419. (doi:10.1098/rspb.1972.0027)
10. Langford T. 1990 *Ecological effects of thermal discharges*. Heidelberg, Germany: Springer Science & Business Media.
11. Fuller PL, Nico LG, Williams JD. 1999 Nonindigenous fishes introduced into inland waters of the United States. *Am. Fish. Soc.* **27**, 613.
12. Simard MA, Paquet A, Jutras C, Robitaille Y, Blier PU, Courtois R, Martel AL. 2012 North American range extension of the invasive Asian clam in a St. Lawrence River power station thermal plume. *Aquat. Invasions* **7**, 81–89. (doi:10.3391/ai.

2012.7.1.009) The path is (http://www.aquaticinvasions.net/2012/AI_2012_1_Simard_etal.pdf)
13. Klotz W, Miesen FW, Hüllen S, Herder F. 2013 Two Asian fresh water shrimp species found in a thermally polluted stream system in North Rhine-Westphalia, Germany. *Aquat. Invasions* **8**, 333–339. (doi:10.3391/ai.2013.8.3.09) The path is (http://www.aquaticinvasions.net/2013/AI_2013_3_Klotz_etal.pdf)
14. Cohen AE, Dugan LE, Hendrickson DA, Martin FD, Huynh J, Labay BJ, Casarez MJ. 2014 Population of variable platyfish (*Xiphophorus variatus*) established in Waller Creek, Travis County, Texas. *Southwest. Nat.* **59**, 413–419. (doi:10.1894/MP-10.1)
15. Jourdan J, Miesen FW, Zimmer C, Gasch K, Herder F, Schleucher E, Plath M, Bierbach D. 2014 On the natural history of an introduced population of guppies (*Poecilia reticulata* Peters, 1859) in Germany. *BioInvasions Rec.* **3**, 175–184. (doi:10.3391/bir.2014.3.3.07) The path is (http://www.reabic.net/journals/bir/2014/3/BIR_2014_Jourdan_etal.pdf)
16. Worthington TA, Shaw PJ, Daffern JR, Langford TEL. 2015 The effects of a thermal discharge on the macroinvertebrate community of a large British river: implications for climate change. *Hydrobiologia* **753**, 81–95. (doi:10.1007/s10750-015-2197-1)
17. Emde S, Kochmann J, Kuhn T, Dörge DD, Plath M, Miesen FW, Klimpel S. 2016 Cooling water of power plant creates 'hot spots' for tropical fishes and parasites. *Parasitol. Res.* **115**, 85–98. (doi:10.1007/s00436-015-4724-4)
18. Mulhollem JJ, Colombo RE, Wahl DH. 2016 Effects of heated effluent on Midwestern US lakes: implications for future climate change. *Aquat. Sci.* **78**, 743–753. (doi:10.1007/s00027-016-0466-3)
19. Koschel RH, Gonsiorczyk T, Krienitz L, Padisák J, Scheffler W. 2002 Primary production of phytoplankton and nutrient metabolism during and after thermal pollution in a deep, oligotrophic lowland lake (Lake Stechlin, Germany). *Proc. Int. Assoc. Theor. Appl. Limnol.* **28**, 569–575.
20. Bianco PG, Turin P. 2009 Record of two established populations of Nile tilapia, *Oreochromis niloticus*, in freshwaters of northern Italy. *J. Appl. Ichthyol.* **26**, 140–2. (doi:10.1111/j.1439-0426.2009.01315.x)
21. Jaklič M, Vrezec A. 2011 The first tropical alien crayfish species in European waters: the redclaw *Cherax quadricarinatus* (Von Martens, 1868)(Decapoda, Parastacidae). *Crustaceana* **84**, 651–665. (doi:10.1163/001121611X577936)
22. Burchardt L *et al.* 2014 Spring phytoplankton and periphyton composition: case study from a thermally abnormal lakes in Western Poland. *Biodiv. Res. Conserv.* **36**, 17–24. (doi:10.2478/biorc-2014-0010)
23. Pilecka-Rapacz M, Piasecki W, Czerniawski R, Sługocki Ł, Krepski T, Domagała J. 2015 The effect of warm discharge waters of a power plant on the occurrence of parasitic Metazoa in freshwater bream, *Abramis brama* (L.). *Bull. Eur. Ass. Fish Pathol.* **35**, 94–103.
24. Yanygina LV. 2015 Spatial distribution of *Gmelinoides fasciatus* Steb. in thermally polluted water (Belovo Reservoir, Southwest Siberia). *Int. J. Environ. Res.* **9**, 877–884.
25. Lipták B, Mrugała A, Pekárik L, Mutkovič A, Gruľa D, Petrusek A, Kouba A. 2016 Expansion of the marbled crayfish in Slovakia: beginning of an invasion in the Danube catchment? *J. Limnol.* **75**, 305–312. (doi:10.4081/jlimnol.2016.1313)
26. Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA. 2000 Biotic invasions: causes, epidemiology, global consequences, and control. *Ecol Appl.* **10**, 689–710. (doi:10.1890/1051-0761(2000)010[0689:BICEGC]2.0.CO;2)
27. Clavero M, García-Berthou E. 2005 Invasive species are a leading cause of animal extinctions. *Trends Ecol. Evol.* **20**, 110. (doi:10.1016/j.tree.2005.01.003)
28. Shvidenko A. 2005 *Ecosystems and human well-being: synthesis. Millennium Ecosystem Assessment 2005*. Washington, DC: Island Press.
29. Butchart SH *et al.* 2010 Global biodiversity: indicators of recent declines. *Science* **328**, 1164–1168. (doi:10.1126/science.1187512)
30. Dudgeon D. 2014 Threats to freshwater biodiversity in a changing world. In *Global environmental change* (ed. B Freddman), pp. 243–253. The Netherlands: Springer.
31. Padilla DK, Williams SL. 2004 Beyond ballast water: aquarium and ornamental trades as sources of invasive species in aquatic ecosystems. *Front. Ecol. Environ.* **2**, 131–138. (doi:10.1890/1540-9295(2004)002[0131:BBWAAO]2.0.CO;2)
32. Copp GH *et al.* 2005 To be, or not to be, a non-native freshwater fish? *J. Appl. Ichthyol.* **21**, 242–262. (doi:10.1111/j.1439-0426.2005.00690.x)
33. Gozlan RE, Britton JR, Cowx I, Copp GH. 2010 Current knowledge on non-native freshwater fish introductions. *J. Fish. Biol.* **76**, 751–786. (doi:10.1111/j.1095-8649.2010.02566.x)
34. Strecker AL, Campbell PM, Olden JD. 2011 The aquarium trade as an invasion pathway in the Pacific Northwest. *Fisheries* **36**, 74–85. (doi:10.1577/03632415.2011.10389070)
35. Chucholl C. 2013 Invaders for sale: trade and determinants of introduction of ornamental freshwater crayfish. *Biol. Invasions* **15**, 125–141. (doi:10.1007/s10530-012-0273-2)
36. Maceda-Veiga A, Escribano-Alacid J, de Sostoa A, García-Berthou E. 2013 The aquarium trade as a potential source of fish introductions in southwestern Europe. *Biol. Invasions* **15**, 2707–2716. (doi:10.1007/s10530-013-0485-0)
37. Rabitsch W, Milasowszky N, Nehring S, Wiesner C, Wolter C, Essl F. 2013 The times are changing: temporal shifts in patterns of fish invasions in central European fresh waters. *J. Fish. Biol.* **82**, 17–33. (doi:10.1111/j.1095-8649.2012.03457.x)
38. Duggan IC, Rixon CA, MacIsaac HJ. 2006 Popularity and propagule pressure: determinants of introduction and establishment of aquarium fish. *Biol. Invasions* **8**, 377–382. (doi:10.1007/s10530-004-2310-2)
39. Gertzen E, Familiar O, Leung B. 2008 Quantifying invasion pathways: fish introductions from the aquarium trade. *Can. J. Fish. Aquat. Sci.* **65**, 1265–1273. (doi:10.1139/F08-056)
40. Stauffer Jr JR, Boltz SE, Boltz JM. 1988 Cold shock susceptibility of blue tilapia from Susquehanna River, Pennsylvania. *N. Am. J. Fish. Manage.* **8**, 329–332. (doi:10.1577/1548-8675(1988)008<0329:CSSOBT>2.3.CO;2)
41. Höfer S, Staas S. 1998 Bericht zur fischereibiologischen Untersuchung des Gillbaches im Bereich Bergheim-Auenheim Zoologisches Institut der Universität zu Köln, Abt. Allgemeine Ökologie und Limnologie, Köln [In German].
42. Peterson MS, Slack WT, Woodley CM. 2005 The occurrence of non-indigenous Nile tilapia, *Oreochromis niloticus* (Linnaeus) in coastal Mississippi, USA: ties to aquaculture and thermal effluent. *Wetlands* **25**, 112–121. (doi:10.1672/0277-5212(2005)025[0112:TOONNT]2.0.CO;2)
43. Williams JW, Jackson ST. 2007 Novel climates, no-analog communities, and ecological surprises. *Front. Ecol. Envrion.* **5**, 475–482. (doi:10.1890/070037)
44. Lurgi M, López BC, Montoya JM. 2012 Novel communities from climate change. *Proc. R. Soc. B* **367**, 2913–2922. (doi:10.1098/rstb.2012.0238)
45. Lőkkös A, Müller T, Kovács K, Várkonyi L, Specziár A, Martin P. 2016 The alien, parthenogenetic marbled crayfish (Decapoda: Cambaridae) is entering Kis-Balaton (Hungary), one of Europe's most important wetland biotopes. *Knowl. Manag. Aquat. Ecosyst.* **417**, 1–9. (doi:10.1051/kmae/2016003)
46. Kempkes M, Budesheim F, Rose U. 2009 *Etho-ecological observations of a guppy population (Poecilia reticulata Peters, 1859) in a thermally polluted stream in Germany*. BrehmSpace: Westarp Wissenschaften [In German].
47. Silva SS, Amarasinghe US. 1989 Stunting in *Oreochromis mossambicus* (Peters)(Pisces, Cichlidae): an evaluation of past and recent data from Sri Lankan reservoir populations. *J. Appl. Ichthyol.* **5**, 203–210. (doi:10.1111/j.1439-0426.1989.tb00493.x)
48. Heaton W. 2015 Data from: Evaluation of blue tilapia (*Oreochromis aureus*) for duckweed (*Lemna minor*) control in South Carolina's private waters. Doctoral dissertation, Clemson University TigerPrints.
49. Rickel BW. 1975 Data from: The effectiveness of *Tilapia zillii* in controlling aquatic vegetation in a southwestern pond. MS thesis, Universsity of Arizona Campus Repository. See http://hdl.handle.net/10150/566546 (accessed on 1 February 2017).
50. Hauser WJ, Legner EF, Medved RA, Platt S. 1976 Tilapia—a management tool for biological control of aquatic weeds and insects. *Bull. Am. Fish. Soc.* **1**, 15–16.
51. Slootweg R, Malek EA, McCullough FS. 1994 The biological control of snail intermediate hosts of schistosomiasis by fish. *Rev. Fish. Biol. Fish.* **4**, 67–90. (doi:10.1007/BF00043261)
52. Graber M, Gevrey JP, Euzeby JA. 1981 Biological control of the mollusc vector of Katayama disease: predatory action of *Tilapia rendalli* and *Sarotherodon mossambicus* with regard to *Biomphalaria glabrata*. *Hydrobiologia* **78**, 253–257. (doi:10.1007/BF00008521)
53. Trewavas E. 1983 *Tilapiine fishes of the genera Sarotherodon, Oreochromis and Danakilia*. London, UK: British Museum (Natural History).
54. Bhassu S, Yusoff K, Panandam JM, Embong WK, Oyyan S, Tan SG. 2004 The genetic structure of *Oreochromis* spp. (Tilapia) populations in Malaysia as revealed by microsatellite DNA analysis. *Biochem. Genet.* **42**, 217–229. (doi:10.1023/B:BIGI.0000034426.31105.da)

55. Canonico GC, Arthington A, McCrary JK, Thieme ML. 2005 The effects of introduced tilapias on native biodiversity. *Aquat. Conserv.* **15**, 463–483. (doi:10.1002/aqc.699)
56. Dunz AR, Schliewen UK. 2013 Molecular phylogeny and revised classification of the haplotilapiine cichlid fishes formerly referred to as 'Tilapia'. *Mol. Phylogenet. Evol.* **68**, 64–80. (doi:10.1016/j.ympev.2013.03.015)
57. FAO (United Nations Food and Agriculture Organization). 2014 *The state of world fisheries and aquaculture*. Rome: FAO.
58. D'Amato ME, Esterhuyse MM, Van Der Waal BC, Brink D, Volckaert FA. 2007 Hybridization and phylogeography of the Mozambique tilapia *Oreochromis mossambicus* in southern Africa evidenced by mitochondrial and microsatellite DNA genotyping. *Conserv. Genet.* **8**, 475–488. (doi:10.1007/s10592-006-9186-x)
59. Welcomme RL. 1988 International introductions of inland aquatic species. FAO Fisheries Technical Paper No. 294, p. 318. Rome, Italy: FAO.
60. Bradford M, Kroon FJ, Russell DJ. 2011 The biology and management of *Tilapia mariae* (Pisces: Cichlidae) as a native and invasive species: a review. *Mar. Freshw. Res.* **62**, 902–917. (doi:10.1071/MF10289)
61. Nico L, Neilson M. 2017 *Tilapia mariae.* Gainsville, FL: Data from: USGS Nonindigenous Aquatic Species Database. See https://nas.er.usgs.gov/queries/factsheet.aspx?SpeciesID=482 (Revision Date: 4/19/2013).
62. Teugels GG, Thys van den Audenaerde DFE. 2003 Cichlidae. In *Faune des poissons d'eaux douces et saumâtres d'Afrique de l'Ouest. Volume 40. Coll. Faune Tropicale n° 28* (eds DC Paugy, A Lévêque, GG Teugels), pp. 521–600. Paris: Musée Royal de l'Afrique Centrale, Tervuren, Belgique and ORSTOM.
63. Teugels GG, Thys van den Audenaerde DFE. 1992 Cichlidae. In *Faune des poissons d'eaux douces et saumâtres d'Afrique de l'Ouest. Volume 2. Coll. Faune Tropicale n° 28* (eds C Lévêque, D Paugy, GG Teugel), pp. 714–779. Paris: Musée Royal de l'Afrique Centrale, Tervuren, Belgique and ORSTOM.
64. Stiassny ML, Lamboj A, De Weirdt D, Teugels GG. 2007 Cichlidae. In *Poissons d'eaux douces et saumâtres de basse Guinée, ouest de l'Afrique central*, vol. 2 (eds ML Stiassny, GG Teugels, CD Hopkins), pp. 269–403. Paris, France: IRD Éditions.
65. Schmitter-Soto JJ. 2007 A systematic revision of the genus *Archocentrus* (Perciformes: Cichlidae), with the description of two new genera and six new species. *Zootaxa* **1603**, 1–78.
66. Schmitter-Soto JJ. 2007 Phylogeny of species formerly assigned to the genus *Archocentrus* (Perciformes: Cichlidae). *Zootaxa* **1618**, 1–50.
67. Kottelat M, Freyhof J. 2007 *Handbook of European freshwater fishes*, 646p. Berlin, Germany: Publications Kottelat.
68. Inoue JG, Miya M, Tsukamoto K, Nishida M. 2000 Complete mitochondrial DNA sequence of the Japanese sardine *Sardinops melanostictus*. *Fisheries Sci.* **66**, 924–932. (doi:10.1046/j.1444-2906.2000.00148.x)
69. Fisch-Muller S. 2003 Loricariidae-Ancistrinae (Armored catfishes). In *Checklist of the freshwater fishes of South and Central America* (eds RE Reis, SO Kullander, CJ Ferraris), pp. 373–400. Brasil: Porto Alegre.
70. Tachihara K, Tokunaga K, Chimura Y. 2002 Alien fishes in Okinawa Island. In *Handbook of alien species in Japan* (ed. Ecological Society of Japan), pp. 248–249. Tokyo: Chijin-Shokan.
71. Roll U, Dayan T, Simberloff D, Goren M. 2007 Characteristics of the introduced fish fauna of Israel. *Biol. Invasion* **9**, 813–824. (doi:10.1007/s10530-006-9083-8)
72. Hovey TE, Swift CC. 2012 First record of an established population of the convict cichlid (*Archocentrus nigrofasciatus*) in California. *Calif. Fish Game* **98**, 125–128.
73. Esmaeili HR, Teimori A, Feridon OW, Abbasi K, Brian WC. 2015 Alien and invasive freshwater fish species in Iran: diversity, environmental impacts and management. *Iran J. Ichthyol.* **1**, 61–72.
74. Cadwallader PL, Backhouse GN, Fallu R. 1980 Occurrence of exotic tropical fish in the cooling pondage of a power station in temperate South-Eastern Australia. *Mar. Freshw. Res.* **31**, 541–546. (doi:10.1071/MF9800541)
75. Ikomi RB, Jessa HO. 2003 Studies on aspects of the biology of *Tilapia mariae* (Boulenger, 1899) (Osteichthyes Cichlidae) in Ethiope River, Niger Delta, Nigeria. *Afr. Zool.* **38**, 255–264. (doi:10.1080/15627020.2003.11407279)
76. Annett CA, Pierotti R, Baylis JR. 1999 Male and female parental roles in the monogamous cichlid, *Tilapia mariae*, introduced in Florida. *Environ. Biol. Fish.* **54**, 283–293. (doi:10.1023/A:1007567028017)
77. Schwanck EJ. 1989 Parental care of *Tilapia mariae* in the field and in aquaria. *Env. Biol. Fish.* **24**, 251–265. (doi:10.1007/BF00001399)
78. Trewavas E. 1974 *The freshwater fishes of rivers Mungo and Meme and lakes Kotto, Mboandong and Soden*, pp. 329–419. West Cameroon: British Museum (Natural History).
79. Anibeze CI. 2001 Stomach length and food preference of three tilapia species (Osteichthyes: Cichlidae) in Agulu Lake Basin, Nigeria. *J. Aquat. Sci.* **16**, 57–60. (doi:10.4314/jas.v16i1.20004)
80. Ajuzie CC. 1996 *Tilapia mariae*: a possible candidate for culture in Nigeria. *Aquacult. Mag.* **22**, 46–53.
81. Vassallo P, Bastianoni S, Beiso I, Ridolfi R, Fabiano M. 2007 Emergy analysis for the environmental sustainability of an inshore fish farming system. *Ecol. Indic.* **7**, 290–298. (doi:10.1016/j.ecolind.2006.02.003)
82. Webb AC. 2007 Status of non-native freshwater fishes in tropical northern Queensland, including establishment success, rates of spread, range and introduction pathways. *J. Proc. R. Soc. New South Wales* **140**, 63–78.
83. Greiner R, Gregg D. 2008 *Tilapia in north Queensland waterways: Risks and potential economic impacts*. Townsville, Australia: River Consulting.
84. Russell DJ, Thuesen PA, Thomson FE. 2012 A review of the biology, ecology, distribution and control of Mozambique tilapia, *Oreochromis mossambicus* (Peters 1852) (Pisces: Cichlidae) with particular emphasis on invasive Australian populations. *Rev. Fish. Biol. Fish.* **22**, 533–554. (doi:10.1111/j.1095-8649.2012.03267.x)
85. Courtenay Jr WR, Hensley DA. 1980 Special problems associated with monitoring exotic species. In *Biological monitoring of fish* (eds CM Hocutt, JR Stauffer Jr), pp. 281–307. Washington, DC: Lexington Books.
86. Brooks WR, Jordan RC. 2010 Enhanced interspecific territoriality and the invasion success of the spotted tilapia (*Tilapia mariae*) in South Florida. *Biol. Invasions* **12**, 865–874. (doi:10.1007/s10530-009-9507-3)
87. Philippart JC, Ruwet JC. 1982 Ecology and distribution of tilapias. In *The biology and culture of tilapias* (eds RSV Pullin, RH Lowe, RH McConnell), pp. 15–60. Manila, Philippines: ICLARM.
88. Bussing WA. 1998 Cichlidae. In *Freshwater fishes of Costa Rica. Volume 2*, pp. 293–383. San José, Costa Rica: Editorial Universidad de Costa Rica.
89. Baensch HA, Riehl R. 1991 Fische. In *Aquarien Atlas. Band 5*. Melle: Mergus Verlag für Natur- und Heimtierkunde, Germany [In German].
90. Courtenay Jr WR, Deacon JE. 1983 Fish introductions in the American Southwest: a case history of Rogers Spring, Nevada. *Southwest. Nat.* **28**, 221–224. (doi:10.2307/3671390)
91. Siemien MJ, Stauffer JR. 1989 Temperature preference and tolerance of the spotted tilapia and Rio Grande cichlid. *Arch. Hydrobiol.* **115**, 287–303.
92. Shafland PL, Pestrak JM. 1982 Lower lethal temperatures for fourteen non-native fishes in Florida. *Env. Biol. Fish.* **7**, 149–156. (doi:10.1007/BF00001785)
93. Smitherman RO. 1988 Cold tolerance and growth of three strains of *Oreochromis niloticus*. In *2nd Int. Symp. on Tilapia in Aquaculture 16–20 March 1987*, vol. 15 (ed. Department of Fisheries), pp. 215–218. Bangkok, Thailand.
94. Charo-Karisa H, Mahmoud AR, Bovenhuis H, Komen H. 2005 Heritability of cold tolerance in Nile tilapia *Oreochromis niloticus* juveniles. *Aquacult.* **249**, 115–123. (doi:10.1016/j.aquaculture.2005.04.029)
95. Ross FF. 1970 *Proc. Inst. Wat. Pollut. Control*.
96. Arthington AH. 1991 Ecological and genetic impacts of introduced and translocated freshwater fishes in Australia. *Can. J. Fish. Aquat. Sci.* **48**, 33–43. (doi:10.1139/f91-302)
97. Friedrich G. 2000 Die untere Erft—ein subtropischer Fluss. Gewässergütebericht: 101–105 [In German].
98. Hussner A, Lösch R. 2005 Alien aquatic plants in a thermally abnormal river and their assembly to neophyte-dominated macrophyte stands (River Erft, Northrhine-Westphalia). *Limnologica* **35**, 18–30. (doi:10.1016/j.limno.2005.01.001)
99. LANUV (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen) Hydrologische Rohdaten Online: Messstation Düsseldorf-Flehe. 2017. See http://luadb.it.nrw.de/LUA/hygon/ (accessed 1 May 2017) [In German].
100. Leuven RS, van der Velde G, Baijens I, Snijders J, van der Zwart C, Lenders HR, bij de Vaate A. 2009 The river Rhine: a global highway for dispersal of aquatic invasive species. *Biol. Invasions* **11**, 1989–2008. (doi:10.1007/s10530-009-9491-7)
101. Panov VE *et al.* 2009 Assessing the risks of aquatic species invasions via European inland waterways: from concepts to environmental indicators. *Integr. Environ. Assess. Manag.* **5**, 110–126. (doi:10.1897/IEAM_2008-034.1)

102. Deacon AE, Ramnarine IW, Magurran AE. 2011 How reproductive ecology contributes to the spread of a globally invasive fish. *PLoS ONE* **6**, e24416. (doi:10.1371/journal.pone.0024416)

103. Prenter J, MacNeil C, Dick JT, Dunn AM. 2004 Roles of parasites in animal invasions. *Trends Ecol. Evol.* **19**, 385–390. (doi:10.1016/j.tree.2004.05.002)

104. Kelly DW, Paterson RA, Townsend CR, Poulin R, Tompkins DM. 2009 Parasite spillback: a neglected concept in invasion ecology? *Ecology* **90**, 2047–2056. (doi:10.1890/08-1085.1)

105. Nmor JC, Egwunyenga AO, Ake JE. 2003 Observations on the intestinal helminth parasites of cichlids in the upper reaches of River Orogodo, a freshwater body in Delta State, southern Nigeria. *Trop. Freshwat. Biol.* **12**, 131–136.

106. King RP, Etim L. 2004 Reproduction, growth, mortality and yield of *Tilapia mariae* Boulenger 1899 (Cichlidae) in a Nigerian rainforest wetland stream. *J. Appl. Ichthyol.* **20**, 502–510. (doi:10.1111/J.1439-0426.2004.00545.X)

107. Olurin KB, Somorin CA. 2006 Intestinal helminths of the fishes of Owa stream, South-West Nigeria. *Res. J. Fish. Hydrobiol.* **1**, 6–9.

108. Honsig-Erlenburg W. 2001 Zum Fischbestand des Warmbaches in Villach. *Carinthia II* **191**, 135–140 [In German].

109. NOBANIS (European Network on Invasive Alien Species). 2017. See http://www.nobanis.org (accessed on 1 May 2017).

110. EASIN (European Alien Species Information Network). Version 5.7 of the EASIN Species Catalogue. See http://easin.jrc.ec.europa.eu/ (accessed on 1 May 2017).

111. DAISIE (Delivering Alien Invasive Species Inventories for Europe) European invasive alien species gateway database. 2017 See http://www.europe-aliens.org (accessed on 1 May 2017).

112. FishBase. 2017 In *Fishbase 2000: concepts, design and data sources* (eds R Froese, D Pauly), p. 344. Los Baños, Laguna, Philippines: ICLARM. See www.fishbase.org (accessed on 1 May 2017).

113. Tierschutzgesetz. German Animal Welfare Act in the version of the promulgation of 18 May 2006 (Federal Law Gazette [BGBI.] Part I pp. 1206, 1313), most recently amended by Article 4 (87) of the Act of 18 July 2016 (Federal Law Gazett [BGBI.] Part I p. 1666). Available at: http://www.gesetze-im-internet.de/tierschg/BJNR012770972.html (accessed on 1 May 2017).

114. Lukas JAY, Jourdan J, Kalinkat G, Emde S, Miesen F, Jüngling H, Cocchiararo B, Bierbach D. 2017 Data from: On the occurrence of three non-native cichlid species including the first record of a feral population of *Pelmatolapia ('Tilapia') mariae* (Boulenger, 1899) in Europe. *Dryad Digital Repository.* (doi:10.5061/dryad.sd7vh)