RESEARCH ARTICLE

Small but tough: What can ecophysiology of croaking gourami *Trichopsis vittata* (Cuvier, 1831) tell us about invasiveness of non-native fishes in Florida?

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Abstract

Trichopsis vittata (Cuvier, 1831) is a small, freshwater gourami (Fam: Osphronemidae) native to southeast Asia. It was first detected in Florida in the 1970s and seems to have persisted for decades in a small area. In this study, we documented *T. vittata*'s ecophysiological tolerances (salinity and low-temperature) and qualitatively compared them to published values for other sympatric non-native species that have successfully invaded much of the Florida peninsula. *Trichopsis vittata* survived acute salinity shifts to 16 psu and was able to survive up to 20 psu when salinity was raised more slowly (5 psu per week). In a cold-tolerance experiment, temperature was lowered from 24 °C at 1 °C hr^{-1} until fish died. Mean temperature at death (i.e., lower lethal limit) was 7.2 °C. *Trichopsis vittata* seems as tolerant or more tolerant than many other sympatric non-native fishes for the variables we examined. However, *T. vittata* is the only species that has not dispersed since its introduction. Species other than *T. vittata* have broadly invaded ranges, many of which include the entire lower third of the Florida peninsula. It is possible that tolerance to environmental parameters serves as a filter for establishment, wherein candidate species must possess the ability to survive abiotic extremes as a first step. However, a species' ability to expand its geographic range may ultimately rely on a secondary set of criteria including biotic interactions and life-history variables.

Keywords

Trichopsis, ecophysiology, low-temperature tolerance, salinity tolerance, invasiveness

Introduction

Destructive (sometimes catastrophic) ecological impacts have been attributed to the introduction and establishment of non-native fishes across the globe (Canonico et al. 2005; Pelicice and Agostinho 2009; Vitule et al. 2009). However, the severity of negative consequences of non-native fish invasions varies greatly amongst taxa. Variation in the ability of species to establish and spread (i.e., 'invasiveness' *sensu* Rejmánek et al. 2002) has provided clues to underlying ecological attributes correlated with invasiveness (García-Berthou 2007). Understanding the characteristics associated with invasiveness is especially important in predicting potential establishment and spread of newly introduced species or those considered a threat for introduction. Most studies aimed at discriminating ecological features of invasive species quantify, collate and report life-history, ecophysiological, and other data for species that have become invasive (Kolar and Lodge 2002; García-Berthou 2007). Less abundant are data on species that were introduced and died out over time, or those that were introduced and established but did not become invasive. Data on those non-invasive species can be difficult to obtain when species were not intentionally introduced (e.g., via stocking). Population dynamics of fishes that were not introduced intentionally (e.g., aquaculture escapes) may not be closely monitored. Nonetheless, the fate of these populations is important as they may provide clues to allow researchers to be able to identify characteristics unique to invasive fishes from those shared between invasive and non-invasive species.

In Florida, there are dozens of non-native fish species that have established and spread widely within the state, especially in the southern half of the peninsula. However, a few species have established but remain localised (Shafland et al. 2008; USGS-NAS 2014). Croaking gourami *Trichopsis vittata* (Cuvier, 1831) was first collected in 1978 and was considered extirpated in the 1990s; however, a localised population was rediscovered in 2013 (Schofield and Pecora 2013; Fig. 1). The species may have persisted in a relatively small area for several decades where it escaped detection. Failure of a species to spread widely after establishment may be due to many factors, such as ecophysiological intolerance or biotic interactions with predators and/or competitors. Little is known regarding the ecophysiology of *T. vittata*, other than the fact that it is an air-breather, making it capable of living in anoxic waters. Other ecophysiological attributes (e.g., tolerance to salinity, extreme temperature) were unknown before this report. Herein, we investigate two ecophysiological parameters for *T. vittata* thought to be conducive to invasiveness in Florida (cold- and salinity-tolerance). We compare those (along with hypoxia-tolerance) to published reports for other non-native fishes with much larger geographic ranges within the State. We ask: Can *T. vittata*'s small geographic range be explained by its relative lack of ecophysiological 'toughness' (i.e., ability to withstand environmental extremes)? In other words, are fishes more tolerant to cold temperatures, low oxygen and salinity predicted to have larger invasive geographic ranges? We hypothesised that *T. vittata*'s small non-native range could be related to a lack of tolerance of ecophysiological variables, and expected it to be less tolerant to environmental variables than sympatric non-native fishes with large ranges.
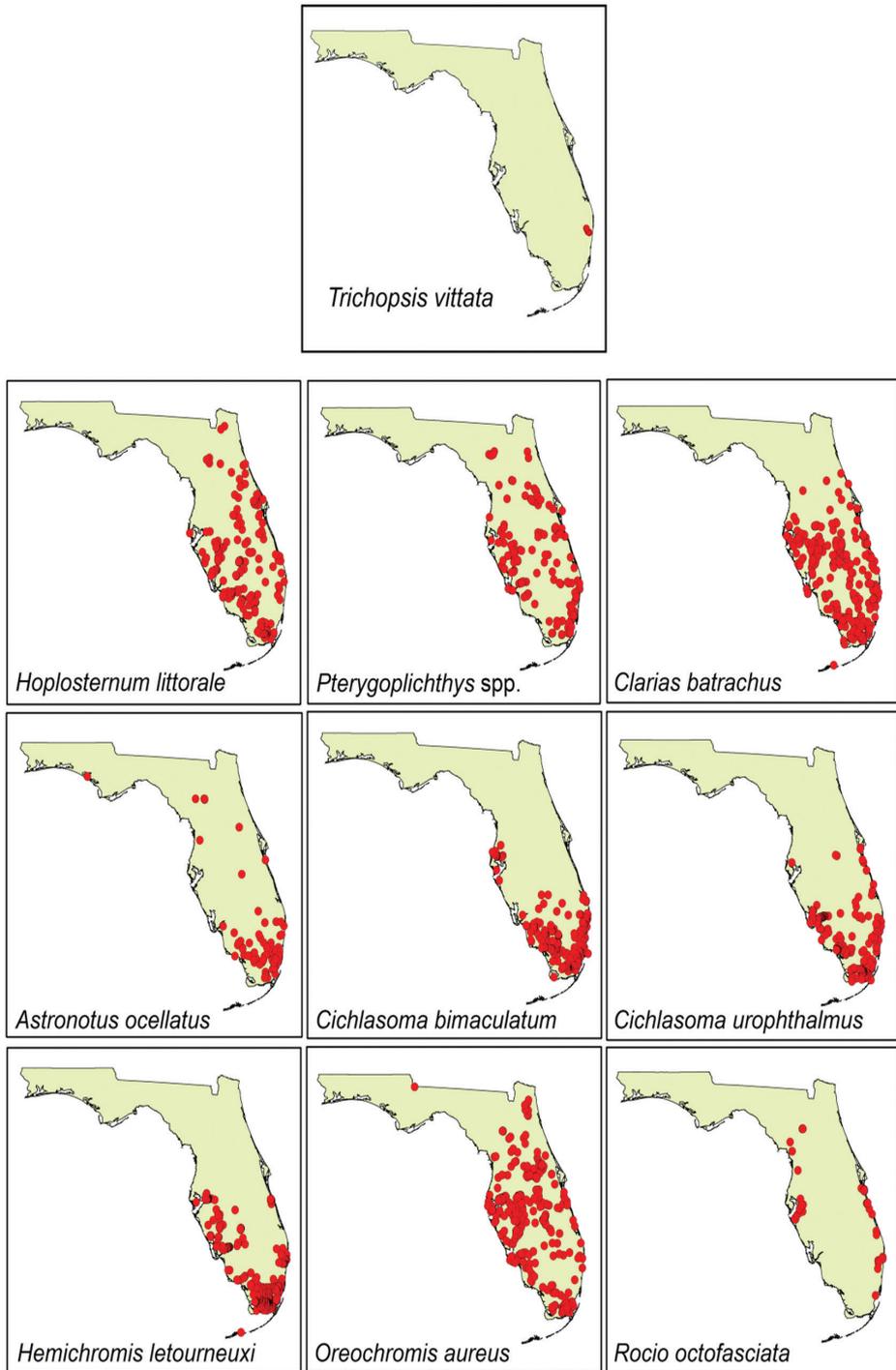
Figure 1. Geographic range of selected non-native fishes in Florida. Occurrence data (red dots) are from USGS-NAS (2014).

Methods

Specimens of *T. vittata* were collected with dip nets in March and April 2014, from Loxahatchee National Wildlife Refuge, Florida, USA. Fish were transported to the USGS laboratory in Gainesville, Florida within 48 hours of capture. Upon arrival, fish were treated with Pond Rid-Ich® Plus™ (Kordon LLC, Hayward, CA, USA) and erythromycin antibiotic. In the laboratory, fish were held indoors in 380 L fiberglass tanks with aerated well water (0.2 psu, hereafter termed "0" psu, 21-25 ℃) and were fed daily with commercial flake food. Individuals were held in these conditions for about one month before experiments began. Before each experiment, fish were measured (± 0.1 cm total length [TL]), weighed (± 0.1 g) and placed into individual plastic bins (17 × 14 × 11 cm) filled with 8 cm of well water. Bins were equipped with small plastic plants, lids to prevent escape and were blinded on three sides to prevent fish from seeing each other. Because gourami are obligate air breathers, no air was provided except for the low-temperature tolerance experiment, where an airstone was placed in each bin to aid in mixing of the water for even temperature distribution throughout the bin. For both salinity experiments, fish were kept in individual bins inside a temperature-controlled room set at 26 C for the duration of the experiments. Salt water was pre-mixed to various salinities using well water and aquarium salt (Crystal Sea® Marinemix, Marine Enterprises International, Baltimore, MD, USA) before water changes using YSI Professional Plus Multiparameter meter (YSI Inc., Yellow Springs, Ohio, USA; ± 0.05 psu). For all experiments, when death was confirmed, water temperature was measured using a hand-held digital thermometer (EXTECH® waterproof thermometer model #39240, EXTECH Instruments®, Nashua, NH, USA). Individual fish were used only once in one experiment (low-temperature tolerance, chronic salinity-tolerance or acute salinity-tolerance).

Low temperature tolerance

The low-temperature tolerance experiment was conducted in April 2014 inside an environmental chamber in which temperature could be controlled by continuously decreasing the air temperature at a constant (i.e., linear) rate. Two endpoints were determined: loss of equilibrium and death (i.e., lower lethal limit). Loss of equilibrium (LOE) was defined as the fish's inability to right itself after being gently prodded, and death was defined as the extended lack of movement by the fish after it was gently prodded while in the water. Twenty-five fish were used and were not fed during the duration of the experiment. Fish were placed in the environmental chamber in individual bins and left undisturbed for 72 hours at 24 °C to acclimate. The experiment began by decreasing the air temperature by 1 °C hr^{-1} to produce an equivalent decline in water temperature. Control fish (n = 5) were immediately moved from the environmental chamber to a stable "warm room" at 24 °C (± 1 °C) until the end of the testing period, when all experimental fish (n = 20) had succumbed to death. Each hour, air temperature in the chamber was manually adjusted to produce a constant decrease of water

temperature at the rate of 1 °C per hour. Temperature of each bin was measured with a hand-held digital thermometer every 20 minutes. All fish were checked for LOE and death every 20 minutes; time and temperature were recorded when LOE and death were confirmed.

Chronic salinity tolerance

An initial pilot study was conducted on n = 20 individuals to establish a general range of salinity tolerance and determine what experimental salinity levels would be used for the experiment. For the chronic salinity tolerance experiment, fish were allocated randomly to one of five treatments (0 [control] = 8 replicates, 20 psu = 11 replicates, 22.5 psu = 12 replicates, 25 psu = 12 replicates, 27.5 psu = 13 replicates). Fish were held initially for 48 hours in well water after which salinities were gradually increased at a rate of 2.5 psu every 2-3 days (5 psu per week) until fish reached the predetermined target salinity. Once the last experimental fish reached its target salinity, all fish remained in their respective salinities for an additional 30 days or until death. Fish reached their target salinities in a staggered (time-wise) fashion; however, each time the salinities were changed in one or more of the treatments, water changes were performed for all of the fish (including controls) to maintain similarity of handling across treatments. Fish were fed twice per week with a mixture of flake food and pellets on days before water changes. Fish were checked 1-2 times per day, seven days per week for death.

Acute salinity tolerance

To determine how *T. vittata* responded to acute salinity changes, fish were transferred directly from well water (0 psu) into various salinity treatments: 0 [control] = 8 replicates, 14, 16, 18, 20 psu = 10 replicates each. Similar to the chronic-salinity tolerance experiment, values for salinity treatments were derived from a pilot study. After being transferred to their respective treatments, fish were left in bins for seven days or until death. Fish were checked for mortalities every hour for the first six hours and then once per day for the remainder of the experiment.

Analyses

Cold-tolerance of *T. vittata* was compared to published values for other previously tested non-native fishes. We statistically compared four species that are sympatric with *T. vittata* (e.g., are found in Loxahatchee NWR) and are widely distributed across south Florida (*Cichlasoma bimaculatum* [Linnaeus 1758], *Cichlasoma urophthalmus* [Günther 1862], *Hoplosternum littorale* [Hancock 1828], *Hemichromis letourneuxi* Sauvage 1880; Fig. 1). These species were tested in our laboratory using the same technique, acclima-

tion temperature, experimental equipment and rate of temperature decrease used here for *T. vittata* (Schofield et al. 2010; Schofield and Huge 2011; Schofield unpub. data). We only compared data for individuals tested in freshwater and acclimated to 24 °C. Mean temperature at death (lower lethal limit) for these species was compared with one-way analysis of variance (ANOVA), and Dunnett's T3 *post-hoc* test was used to discriminate homogeneous subsets. Levene's test was used to test for heteroscedasticity.

One-way ANOVA was used to compare fish mass among salinity treatments, and Levene's test was used to check for heteroscedasticity. Life expectancy was estimated with the Kaplan-Meier product-limit estimator (Kaplan and Meier 1958) and the log-rank test was used to compare survivorship curves (Savage 1956; Cox and Oakes 1984). For the acute-salinity challenge, all treatments began at the same time (time = 0). However, for the chronic-salinity experiment, fish reached their target salinities sequentially (i.e., staggered over time). Thus, for the chronic-salinity experiment the day the fish reached their target salinity was designated as time = 0 for that treatment. We set our alpha level for statistical significance at 0.05. All data were analysed using SPSS version 13.0.

Results

Environmental variables measured while collecting *T. vittata* on several occasions (including fish used in this experiment) are provided in Table 1. *Trichopsis vittata* used in the cold-tolerance study averaged 0.73 g (+ 0.67 standard deviation [SD]; range 0.20–3.00 g; n = 25), and 3.7 cm TL (+ 0.99 SD; range 2.5–5.9 cm). Fish lost equilibrium at 10.2 °C (+ 0.68 SD; range 8.2–11.2 °C) and died at 7.2 °C (+ 0.68 SD; range 6.4–8.8 °C). *Trichopsis vittata* was the second-most cold-tolerant species tested (after *H. littorale*), and exhibited greater tolerance to low temperatures than all cichlids (one-way ANOVA F = 49.46, df = 4, P <0.001, Fig. 2).

For the chronic salinity-tolerance experiment, fish mass averaged 0.93 g (+ 0.28 SD; range 0.30–1.5 g; n = 56) and mean TL was 4.2 cm (+ 0.55 SD; range 3.0–5.1 cm; n = 56). Fish mass did not vary significantly by treatment (one-way ANOVA F = 0.11, df = 4, *P* = 0.58). At the end of the experiment, survival was 100% at the control salinity (0 psu), 63% at 20 psu, 25% at 22.5 psu, and 8% at 25 psu (Fig. 3a). All fish at 27.5 psu died by the 24th day after reaching their 27.5 psu salinity goal. Because the majority of the data for the 20 psu treatment was censored (i.e., the majority of fish in this treatment survived the challenge), it was not possible to compute a survival estimate. Mean (95% Confidence Interval [CI]) survival estimates for other treatments are: 18 days (11–25) at 22.5 psu, 10 days (4–16) at 25 psu, 7 days (3–11) at 27.5 psu. All treatments were significantly different from the control except 20 psu (although the *P*-value was close to significance; log-rank statistic = 3.41; *P* = 0.065)

The mean mass of fish used in the acute salinity-tolerance experiment was 0.81 g (+ 0.31 SD; range 0.30–1.6 g; n = 48) and mean length was 4.1 cm TL (+ 0.54 SD; range 3.0–5.2 cm). Fish mass did not vary significantly by treatment (one-way ANOVA F =

0.98, df = 4, *P* = 0.43). After the acute salinity change, *T. vittata* at 20 psu exhibited 60% mortality within the first four hours and 100% mortality within the first six hours (mean survival estimate = 4.5 hrs; 4.1–4.9 hrs 95% CI). The 18 psu treatment group displayed 70% mortality after 24 hours, with no fish surviving longer than 48 hours (mean survival estimate = 30 hrs; 21.5–38.5 95% CI; Fig. 3b). At salinities of 0 and 14, survival was 100% and at 16 psu, it was 90% at the end of the experiment. No survival estimates were calculated for these three treatments as survival was so high (and subsequently most of the data were censored). Survival was equivalent for 0 and 14 psu (100%) and did not differ significantly between 0 and 16 psu (log-rank statistic = 0.80; *P* = 0.37) nor 14 and 16 (log-rank statistic = 1.00; *P* = 0.32).

Table 1. Environmental variables measured while collecting *Trichopsis vittata* on several occasions from Loxahatchee National Wildlife Refuge. Fish for experiments in this report were collected in March and April 2014. N/A = Not Available.

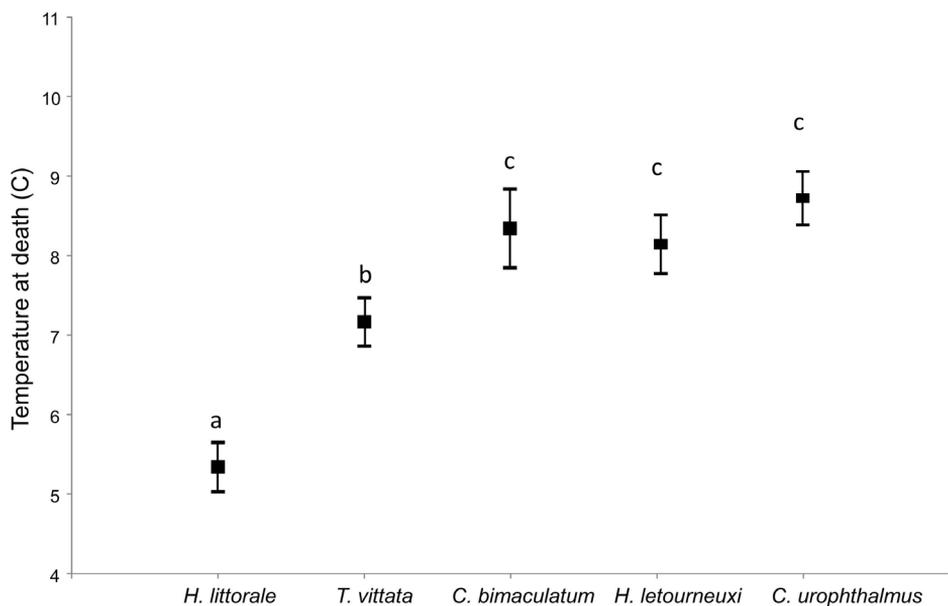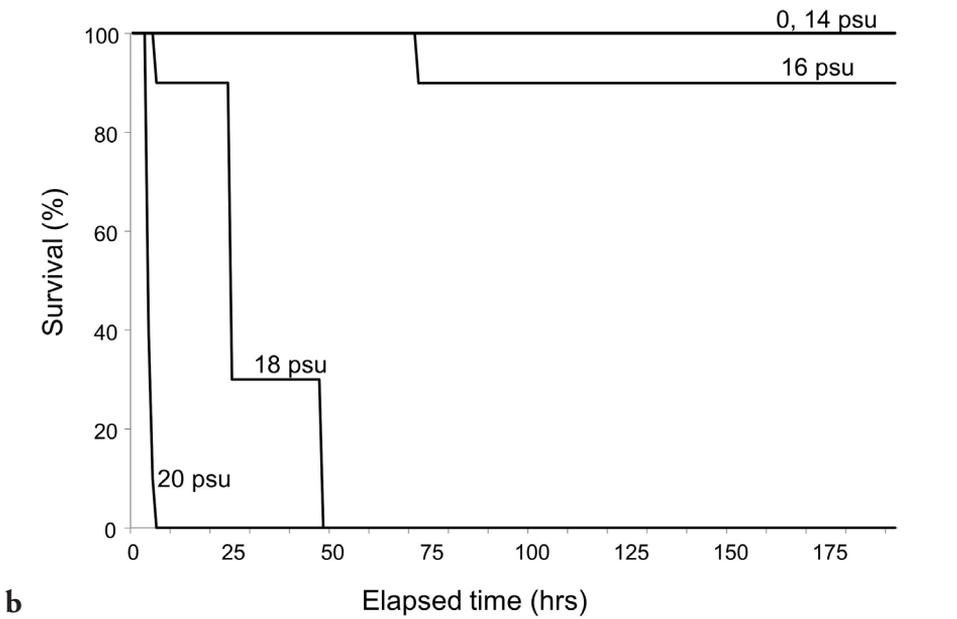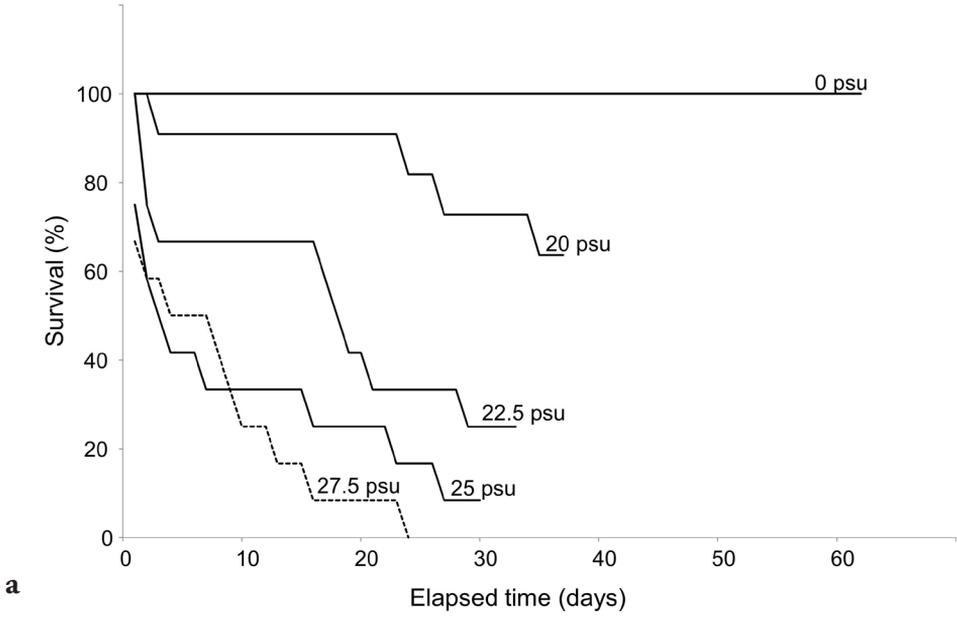| Collection date	Temperature (°C)	Salinity (psu)	Dissolved Oxygen (mg L⁻¹)	pH
7 March 2014	20.6	0.07	0.87	N/A
23 April 2014	25.1	0.21	0.74	7.18
24 April 2014	21.8	0.22	0.67	7.12
31 March 2015	21.2	0.17	3.14	7.27

Figure 2. Mean temperature (+ 2 SE) at which fishes died in cold-temperature tolerance experiments (i.e., lower lethal limit). Letters denote significant differences (one-way ANOVA with Dunnett's T3 *post-hoc* test; see text for details). References for data sources are given in Table 2.

Figure 3. Salinity tolerance of *T. vittata*. Results from **a** chronic and **b** acute salinity-tolerance trials.

Discussion

Trichopsis vittata has been known from Florida since the 1970s, when an established population was discovered within 10 km of its current range (Courtenay et al. 1984, 1986; Schofield and Pecora 2013). Its introduction source is unknown; however, at the time of discovery it was speculated that it had escaped from nearby aquarium fish farms (Courtenay et al. 1984, 1986). Over time, the species was thought to have been extirpated (Shafland 1996; Shafland et al. 2008) until its recent rediscovery (Schofield and Pecora 2013). No fish-monitoring programmes cover urban areas in this region of Florida, so it is unclear how long the fish was established before its recent collection at Loxahatchee NWR. Furthermore, it is unclear whether the species had died out and was subsequently re-introduced or whether this is a remnant population. Nevertheless, its ability to persist in this small range for many decades makes it an interesting candidate for study. The purpose of this investigation was to document ecophysiological attributes of the species and qualitatively compare them to sympatric species, to see if perhaps reduced ecophysiological tolerance might be related to the lack of geographic expansion.

In general, our hypothesis (low ecophysiological toughness ≈ small geographic range) was not supported. Ecophysiological traits of *T. vittata* and nine sympatric non-native fishes known from Florida freshwaters were tabulated (Table 2). Sympatric non-native fishes include ones that have been established since the 1950s (*Pterygoplichthys* spp., *Astronotus ocellatus* [Agassiz 1831], *Cichlasoma bimaculatum*), 1960s (*Clarias batrachus* [Linnaeus 1758], *Hemichromis letourneuxi*, *Oreochromis aureus* [Steindachner 1864]), 1970s (*Rocio octofasciata* [Regan 1903]), 1980s (*C. urophthalmus*) and 1990s (*H. littorale*; see Shafland et al. 2008, Schofield and Loftus 2014 for establishment timelines; Table 2, Fig. 1). Tolerance of these species to hypoxia and low-temperatures was graphically compared (Fig. 4). *Trichopsis vittata* was more tolerant of cold than many sympatric non-native fishes, leading us to believe it could tolerate habitats north of its current range; however, it has not expanded its range in any direction. Furthermore, its ability to breathe atmospheric air (via a labyrinth organ) imparts an ability to live indefinitely in water devoid of oxygen. It should be able to tolerate a variety of marginal habitats such as shallow pools, vegetation-choked swamps, and habitats with low light levels as it does at Loxahatchee NWR and in its native range (Rainboth 1996). As for salinity, we documented herein that *T. vittata* was tolerant to acute shifts in salinity to 16 psu and gradual shifts to 20 psu. This level of tolerance is lower than published values for most cichlids, but greater than many non-cichlid invasive fishes (Table 2). Nonetheless, it is a species that is probably tolerant enough to occupy freshwater tidal or low-salinity estuarine areas, or use them as salt bridges for dispersal. Yet it has not moved into coastal areas even though the current population is less than 20 km from the Atlantic coast. In summary, while *T. vittata* seems as tough or tougher than other sympatric non-native fishes (in terms of ecophysiology; Table 2, Fig. 4), it has not been able to capitalise on these advantages and expand its geographic range as the others have. It is possible that tolerances to environmental parameters are not directly correlated with geographic range for this group of species, but instead serve as

Table 2. Ecophysiological traits for *Trichopsis vittata* and several species of sympatric non-native fishes. For hypoxia tolerance, 'extremely tolerant' = can survive in water with no oxygen (<0.02 mg L^{-1}) for prolonged periods, as long as surface access is allowed.

| Species	Hypoxia tolerance	Chronic salinity tolerance	Acute salinity tolerance	Cold tolerance (°C)	Max body size in FL (cm TL)
Hoplosternum littorale	air-breather	unknown for adults	7 psu (larvae Mol 1994)	5.7 (Schofield and Huge 2011) 10 (Gestring et al. 2009)	26.2 (Gestring et al. 2009)
Pterygoplichthys spp.	air-breather	unknown	10 psu (Capps et al. 2011)	9–11 for *P. multiradiatus*; 4–6 for *P. disjunctivus* (Gestring et al. 2010)	50.4 (Gestring et al. 2010)
Clarias batrachus	air-breather	unknown	Unknown	9.8 (Shafland and Pestrak 1982)	52.9 (Shafland 1996)
Trichopsis vittata	air-breather	20 psu (this study)	16 psu (this study)	7.2 (this study)	6.5 (Schofield and Pecora 2013)
Astronotus ocellatus	extremely tolerant (Almeida-Val et al. 2000)	14 psu (Gutierre et al. in press)	16 psu (Gutierre et al. in press)	12.9 (Shafland and Pestrak 1982)	34.8 (Shafland 1996)
Cichlasoma bimaculatum	unknown	unknown	unknown	8.6 (Schofield and Huge 2011) 8.9 (Shafland and Pestrak 1982)	18.6 (Shafland 1996)
Cichlasoma urophthalmus	extremely tolerant (Schofield et al. 2009)	> 37 psu (Stauffer and Boltz 1994)	unknown	8.7 (Schofield unpub. data) 14–15 (Stauffer and Boltz 1994)	28.8 (Idelberger et al. 2011)
Hemichromis letourneuxi	extremely tolerant (Schofield et al. 2007)	50 psu (Langston et al. 2010)	20 psu (Langston et al. 2010)	8.1 (Schofield unpub. data) 9.5 (Shafland and Pestrak 1982)	11.2 (Shafland 1996)
Oreochromis aureus	unknown	52 psu (Suresh and Lin 1992)	unknown	6.2 (Shafland and Pestrak 1982)	54.3 (Shafland 1996)
Rocio octofasciata	extremely tolerant (Obordo and Chapman 1997)	unknown	unknown	8.0 (Shafland and Pestrak 1982)	13.7 (Jennings 1986)
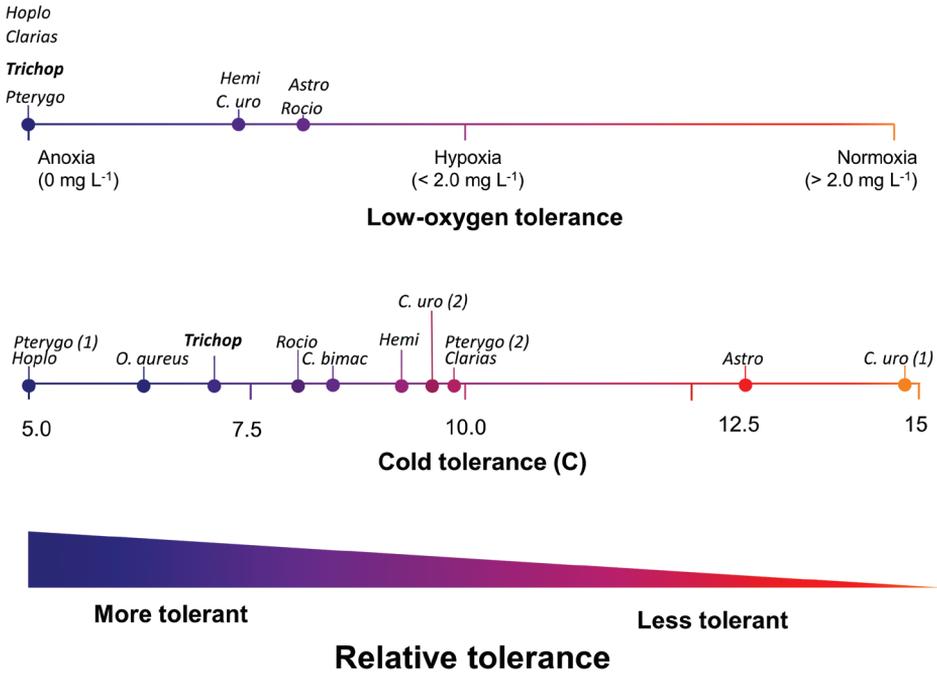
Figure 4. Graphic representation of relative ecophysiological 'toughness' for *T. vittata* and sympatric non-native fishes. References for cold and low-oxygen tolerance are given in Table 2. Two values are presented for cold tolerance of *C. urophthalmus* as two separate reports provided dissimilar data (Table 2). Two values are given for *Pterygoplichthys* spp. corresponding to two different species (Table 2). "*O. aureus*" = *Oreochromis aureus;* "*Pterygo*" = *Pterygoplichthys* spp.; "*Hoplo*" = *Hoplosternum littorale*; "*Clarias*" = *Clarias batrachus*; "*Trichop*" = *Trichopsis vittata*; "*Hemi*" = *Hemichromis letourneuxi*; "*C. uro*" = *Cichlasoma urophthalmus*; "*Astro*" = *Astronotus ocellatus*; "*Rocio*" = *Rocio octofasciata*; "*C. bimac*" = *Cichlasoma bimaculatum*.

a filter for establishment, wherein candidate species must possess the ability to survive abiotic extremes as a first step (Peterson et al. 2004). Once fish have passed through this step, invasiveness (at least in terms of geographic spread) may ultimately rely on a secondary set of criteria including biotic interactions and life-history variables.

The intriguing combination of high abiotic tolerance and low invasiveness in *T. vittata* may support the biotic-abiotic constraining hypothesis (Quist et al. 2003), wherein abiotic environmental variables structure population levels until overridden by biotic ones (e.g., predation, competition). For example, Quist et al. (2003) showed that variation in walleye *Stizostedion vitreum* (now *Sander vitreus* [Mitchill, 1818]) populations in Kansas reservoirs could be explained by environmental variables until a critical threshold for biotic interactions was reached. In that case, once the density of a predator (*Pomoxis annularis* Rafinesque, 1818) was exceeded, then biotic interactions overrode abiotic influences and *S. vitreum* population dynamics were related to *P. annularis* density. Similarly, Weber and Brown (2011) showed that variation in density of native

fish populations were related to environmental variables until a threshold density of *Cyprinus carpio* Linnaeus, 1758 was reached and then biotic interactions overrode abiotic ones. As for *T. vittata*, future research on its co-occurrence with competitors and predators may shed light on the relative influences of abiotic versus biotic constraints.

There are many other factors that could explain the lack of geographic range expansion for *T. vittata.* Some of the most obvious candidates include body size, diet and their interaction. *Trichopsis vittata* is smaller than other sympatric non-native fishes and occupies a relatively low position on the predation spectrum (i.e., primarily consumes small invertebrates; Rainboth 1996). This combination of attributes separates *T. vittata* from the other non-native fishes that are either: 1) large-bodied species that consume benthic algae and detritus (e.g., *Pterygoplichthys* spp., *O. aureus*) or 2) large- to medium-sized fish predators (cichlids, *C. batrachus*). One species that does not fit this pattern is *H. letourneuxi*, which consumes both invertebrates and fish and does not reach a large body size (Table 2), yet is extremely invasive (Kline et al. 2013; Fig. 1). Protection from bony dermal plates may confer an additional advantage to catfishes (*H. littorale*, *Pterygoplichthys* spp.) and bolster their ability to spread geographically. Other factors that could affect invasiveness include biotic resistance (e.g., Thompson et al. 2012), specific requirements for egg/larval development or nesting, multiple introductions (Collins et al. 2002), predation susceptibility (e.g., Rehage et al. 2009) and propagule pressure (Colautti et al. 2006). Application of modern modelling techniques may allow researchers to identify which variables are most important for an invader's success (and spread) and the critical thresholds for those variables (e.g. Kolar and Lodge 2002).

Acknowledgments

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