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Research article

The occurrence and reproductive status of Yucatan molly *Poecilia velifera* (Regan, 1914) (Poeciliidae; Cyprinodontiformes): an alien fish invading the Songkhla Lake Basin, Thailand

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Abstract

A non-native fish, the Yucatan molly *Poecilia velifera* (Regan, 1914), has recently become established in the Songkhla Lake Basin, south Thailand. Based on our field surveys in December 2007, we conclude that this species is present only in the Haad-kaew Lagoon and the southernmost part of the Songkhla Lagoon, the Thale Sap Songkhla. We sampled *P. velifera* monthly for 13 months (January 2007-January 2008), using beach seine and cast net, to obtain information on its population structure, morphology and reproduction. It was found that male and female *P. velifera* became sexually mature at 16.8 and 17.1 mm standard length, respectively. The overall sex ratio of males to females was 1.0:1.8. However, the proportion of males was less within specimens belonging to larger size classes. *P. velifera* reproduced continuously throughout the year but with two peaks, one in March-May and another in August-December.

Key words: *Poecilia velifera*, alien, reproductive traits, Songkhla Lake Basin, Thailand

Introduction

The Yucatan molly, *Poecilia velifera* (Regan, 1914) (Figure 1), is a member of the Poeciliidae, a large family of small-bodied fishes native to fresh and brackish waters of the New World. *P. velifera* is from Central America and native to the Yucatan Peninsula, Mexico (Hankison et al. 2006), where it typically occurs in coastal brackish waters of Campeche, Yucatan and Quintana Roo City, and the nearby islands of Mujeres and Cozumel (Miller 1983). It has been introduced to many countries around the world via the aquarium fish trade and also as a biological control agent for insects, especially mosquitoes (Courtenay and Meffe 1989; Lever 1996). There are confirmed and unconfirmed reports of non-native *P. velifera*, or possible hybrids, as collected or observed at one or more sites in North America: Florida; South America: Brazil, Colombia and Peru; Asia: Israel, Singapore, Taiwan, Vietnam and Thailand (Welcomme 1988; Ng et al. 1993; Fuller et al. 1999; Shen 1993; Kuo et al. 1999; Golani 2000; Magalhães et al. 2002; Vidthayanon and Premcharoen 2002; Welcomme and Vidthayanon 2003; Ortega et al. 2007). Some, not all, represent established populations (Appendix 1).

Poecilia velifera was reportedly first imported into Thailand in *ca.* 1960 via the aquarium trade (Welcomme and Vidthayanon 2003). Additional stocks were brought into Thailand from Taiwan in 1987 for controlling algal flocs in shrimp ponds in the estuary mouth of the Chao Phraya River (Welcomme and Vidthayanon 2003). As a result, a flourishing wild population was reported in 2002 (as *Poecilia sphenops* Valenciennes, 1846) in a brackish water system of the Chao Phraya River, central Thailand (Vidthayanon and Premcharoen 2002; Nico et al. 2007).

Although *Poecilia velifera* has been widely introduced, there is no information on the reproductive biology of the introduced populations (e.g., sex ratio, size at maturity, fecundity) and little is known even about native populations. This information is important because poeciliids usually exhibit great phenotypic plasticity, the environment having a big influence on its phenotype (Reznick and Miles 1989a; Trexler 1989). Therefore, the life history traits of this fish are expected to differ from place to place.

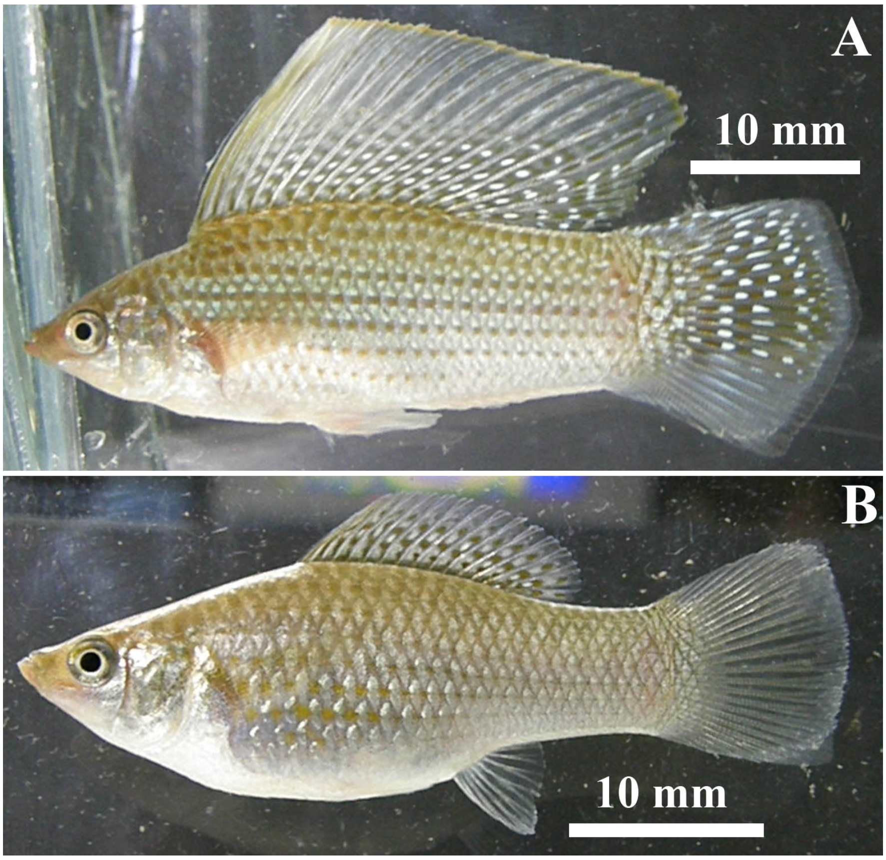

Figure 1. Yucatan molly *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin; A: male, B: female (photographs taken by S. Sa-nguansil).

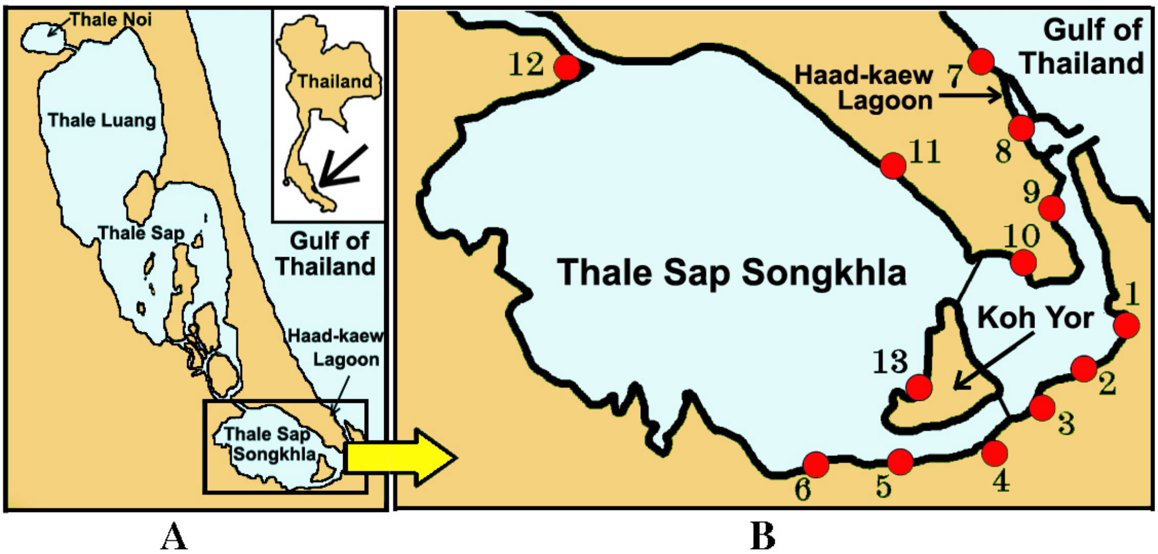

Figure 2. Distribution of *Poecilia velifera* (Regan, 1914) in the Songkhla Lake Basin, south Thailand; A: map of the Songkhla Lagoon and the Haad-kaew Lagoon, B: the dots show location of sites where *P. velifera* was detected during field surveys conducted in December, 2007 (see Appendix 2 for additional information).

This paper provides evidence of a newly established, non-native population of *Poecilia velifera* in Thailand, in the Songkhla Lake Basin (SLB), south Thailand. We also report information on aspects of the population's reproductive biology.

Methods

Study site

There are two lagoon systems in the SLB: the Songkhla Lagoon and the Haad-kaew Lagoon. These lagoons are strongly influenced by tropical monsoons. There are 3 seasons in a year according to the seasonal wind direction: an intermediate rainy period resulting from the strong south-west monsoon (May-September), a rainy period resulting from the north-east monsoon (October-December) and a relatively dry season influenced by south-east predominant wind (January-April) (Evenson 1983).

The Songkhla Lagoon is a large shallow water body, covering approximately 1082 km^2 (ILEC 2010), lying between 7°08'N and 7°48'N, and between 100°07'E and 100°35' E. The lagoon is connected to the Gulf of Thailand at its southern end and exhibits three water regimes: fresh, brackish and salt water. The lagoon can be divided into four distinct parts: Thale Noi, Thale Luang, Thale Sap and Thale Sap Songkhla, arranged respectively from north to south (Figure 2A). The average water depth is 1.2 m, with the deepest parts approximately 8.0-8.8 m in connecting channels and in the mouth of the lagoon (Angsupanich and Rakkheaw 1997). The average annual water temperature is 29.7°C (Angsupanich 1997). Salinity ranges from 0-34 psu in the Thale Sap Songkhla (the part of the Songkhla Lagoon connecting to the sea), and 0 psu or almost nil in Thale Noi (the innermost part). On the shores of Songkhla Lagoon are mixtures of urban areas, fishing villages, shrimp ponds, seasonally flooded forests, paddy fields and mangroves.

The Haad-kaew Lagoon is a much smaller water body (0.37 km^2), about 2.7 km long with a maximum width of 300 m (Figure 2B). It is located between 7°14'N and 7°15'N, and between 100°32'E and 100°34'E. This lagoon is connected to the mouth of the Songkhla Lagoon and is divided by a sand bar that effectively splits the lagoon into two parts: a seasonally-closed part in the north and an open part in the south. The water in the Haad-kaew Lagoon is mostly shallow, with an average water depth of about 1.9 m, except the main channel where the depths range from 2 to 5 m. The lagoon shores have a couple of fishing villages, small degraded mangroves and at its mouth the Songkhla Deep Sea Port.

Preliminary field survey

To determine the approximate geographic distribution of *Poecilia velifera*, we surveyed SLB in December 2007, traveling around Songkhla and Haad-kaew lagoons by both land and boat. Presence/absence of *P. velifera* was assessed at multiple sites, conducted by visual scans of areas where the water was clear or by use of sampling gear in turbid water. During preliminary surveys, gear included a beach seine (4.2 m×1.3 m with 0.5 cm mesh size) and a cast-net (2.4 m diameter with 2.5 cm mesh size). *P. velifera* is a surface-dwelling fish that tends to congregate in shoals, consequently, the species is easily detected in clear water sites, especially if the fish is present in high numbers. In sites deeper than 1 m, a cast net was used to confirm the existence of the fish.

Sampling method and data collection

To obtain specimens for further study, we selected 6 sites (Stations 2,3,4,7,10 and 11 in Figure 2B) where *Poecilia velifera* was abundant and in habitats, coastal shallow water near vegetated areas, where our collecting gear would be most effective. In these sites we collected fish using bag beach seines (5.0 m×1.2 m with 0.5 cm mesh size). The sites were sampled monthly for 13 months (January 2007 to January 2008). Captured fish were kept in an ice box while traveling and later frozen in a freezer in the laboratory.

In the laboratory, each specimen was measured for standard length (SL) to the nearest 0.1 mm. Gender and stage of maturity were determined. Only males with completely developed gonopodium were considered mature. Pregnant mature females were individuals containing eggs or embryos in an enlarged ovary. Non-pregnant adult females were considered to be any females 30.0 mm SL or longer but with no evidence of embryos in the ovary. The genders of immature specimens were determined by gonad examination. In this study, gravid females were defined by the presence of embryos in the ovary (*sensu* Snelson et al. 1980). To estimate batch size of gravid females, we counted the number of embryos present.

Data analysis

Size at first maturity was represented by the smallest individual (in terms of SL) of sexually mature males and females. Then the average standard length of sexually mature females was calculated. The sex ratio was calculated from the overall samples and in each 10.0 mm size class. The overall sex ratio and the sex ratio of the size class >10.0-20.0 mm SL were tested for equality by using Yates' Chi-square test. The relation between batch size and body length was assessed by simple linear regression, with independent and dependent variables represented by female SL and batch size, respectively. Then the predicted fecundity, fecundity at the average sexually mature female length, was calculated (Reznick and Miles 1989a). All statistical analyses were performed following the guidance of Zar (1996).

Results

Identification of Poecilia in the Songkhla Lake Basin

We initially identified the SLB poeciliid as *Poecilia velifera* (Regan, 1914), a determination based largely on body color and certain external anatomical characteristics. To confirm the identification, we more carefully examined 31 mature male specimens (31.5 to 56.7 mm SL) taken from the Thale Sap Songkhla in the SLB. These specimens were registered and deposited at Prince of Songkla University Zoological Collection (PSUZC-20100204.01). Meristic and color data from these fish were compared to information provided in a key to the Mexican Poeciliidae and illustrations appearing in Miller et al. (2005). *P. velifera* are distinguished from most other members of the genus by its very large dorsal fin, with high number of dorsal fin rays in males. Dorsal rays numbered 16-19 in our 31 specimens, well within the range reported by Miller et al. (2005). We counted 27-29 scales in the lateral series, whereas Miller et al. stated "lateral-line scales usually 26 or 27". One discrepancy is the absence or near absence of dark blotches on the dorsal fin in the SLB males. It is uncertain if the variation represents some degree of introgressive hybridization.

Other anatomical information taken from the 31 SLB specimens are as follows: body slender, laterally compressed; body depth 2.5-2.7 in SL. Mouth is normal, relatively small, supraterminal and protrusible. The origin of dorsal fin ray is well in advance of pelvic-fin insertion. Length of dorsal fin base is 0.8-1.6 times predorsal distance. Gonopodium, from ventral surface of ray 3, large, fleshy palp usually arising that may envelop ventral half of gonopodium. Subdistal segments of gonopodial ray with spines and processes. Nine predorsal and 20 circum-peduncular scales. Mature males with brilliantly colored fin ornaments and a very large dorsal. Body is marmorated velvet white and grey in color, dorsal and upper half of caudal fins are marked with small, round light dots.

Distribution range

During our survey of the coast of the Haad-kaew Lagoon and the Songkhla Lagoon, we detected *Poecilia velifera* in both parts of the Haad-kaew Lagoon (Figure 2B: Station 7, 8). But for SLB, the fish were only found in the Thale Sap Songkhla.

In there its distribution ranged from Ban Hua Khao Daeng (Station 9: 7°13′04″N, 100°34′ 28″E) to Ban Bor Ang (Station 11: 7°13′01″N, 100°32′03″E), and Ban Tha Sa Arn (Station 1: 7°10′57″N, 100°35′37″E) to Ban Pak Ror (Station 12: 7°15′03″N, 100°26′03″E). The species was also observed around Koh Yor, a large island located in the southeast part of the Thale Sap Songkhla (Figure 2B; Appendix 2).

During our study, *Poecilia velifera* was found to occupy near-shore shallow water, not more than 1 m deep. The preferred habitats were sheltered areas such as mangroves, flooded grassland, canals in shrimp ponds, and shallow lakesides with garbage (Figure 3).

Reproductive traits

The smallest *Poecilia velifera* considered mature consisted of a male 16.8 mm SL and a female 17.1 mm SL. The largest individuals in our samples were a male 62.3 SL mm and a female 69.8 mm SL. Average SL of sexually mature males and females were 34.3 ±0.3 and 39.0 ±0.2 mm (mean ±S.E.; n_{males}=1080; $n_{females}$ = 1655). The overall sex ratio (male: female) was 1.0:1.8, significantly different from 1:1 ratio (χ^2 =3.841; d.f. = 1; p <0.0001; n = 6,033). The sex ratio of the smallest size class (>10.0-20.0 mm SL) was 1.0:1.3, not 1:1 (χ^2 =

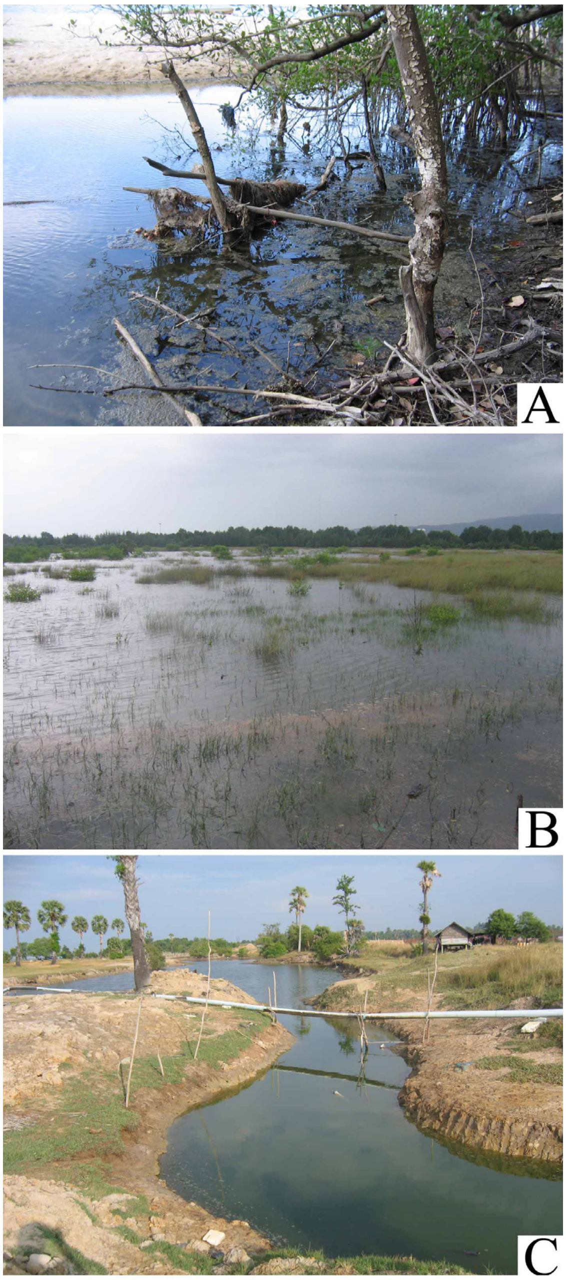

Figure 3. Habitats of *Poecilia velifera* (Regan, 1914) in the Songkhla Lake Basin, Thailand; A: mangrove, B: flooded grassland, C: irrigation canal in shrimp farm (photographs taken by S. Sa-nguansil).

8.2222; d.f. = 1; $p = 0.0041$; $n = 666$). It is interesting to note that the sex ratio changed continuously through the size classes from 1.0:1.3 in the smallest size class (>10-20mm SL) to 1.0:7.7 in the biggest size class (>60-70mm SL) (Figure 4).

Female *Poecilia velifera* produced varying numbers of offspring in each batch, ranging from 5–252 embryos ($n = 876$). The trend of the fecundity appeared to be as shown in (Figure 5):

$$\text{Batch size} = 3.7182e^{0.0531(\text{female body length})}$$

(Length in mm SL, $r^2 = 0.4831$; $n = 876$, $p < 0.05$)

The predicted fecundity of the average sexually mature female length (SL), was 29.5 offspring.

From January 2007 to January 2008, at least 30 percent of all mature females were pregnant each month. Although there was evidence that *Poecilia velifera* were reproducing throughout the year, there were two periods of high reproductive activity (>45% of females were pregnant): March 2007 to May 2007, and August 2007 to October 2007. As might be expected, each peak of reproduction was followed by high proportion of immature fish present in samples: June-August 2007 and November-December 2007 (Figure 6).

Discussion

There is no documentation as to when and how *Poecilia velifera* was introduced to the SLB. Based on interviews with local fishermen, however, it appears that the non-native was introduced to the SLB before at least 2002, the year when fishermen began finding large numbers of *P. velifera* in most fish traps set in the Thale Sap Songkhla. We found no evidence to support the hypothesis that people residing near Thale Sap Songkhla stocked *P. velifera* to control algae in their shrimp ponds or as a biological control for mosquitoes. However, *P. velifera* is a popular aquarium fish in Thailand, and its presence in the Thale Sap Songkhla possibly resulted from release or escape of aquaria specimens.

The present study found that *Poecilia velifera* prefers near-shore, highly vegetated shallow habitats characterized by little or no water current, similar to conditions described by Courtenay and Meffe (1989) and Hankison et al. (2006). During our survey of the Songkhla Lagoon coast we found these types of habitat to be common throughout the shoreline of the Lagoon, including areas not yet invaded in Thale

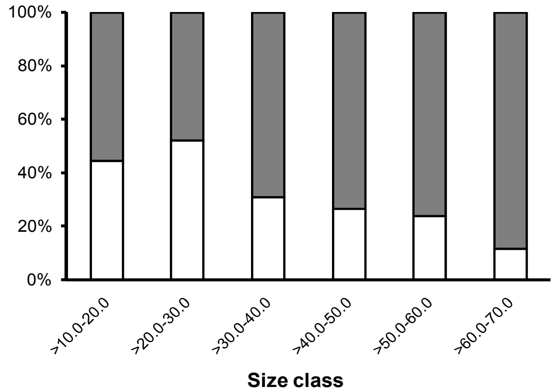

Figure 4. Sex ratios of *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin divided into size classes: open bars – males, filled bars – females.

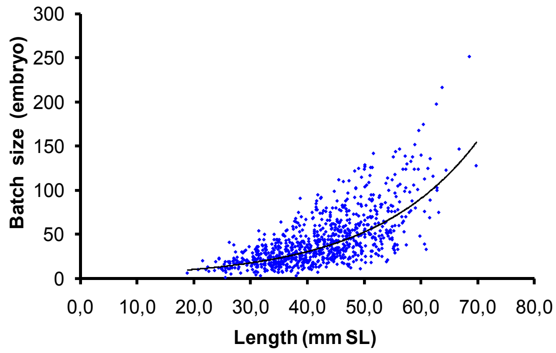

Figure 5. Fecundity of female *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin (Jan. 2007-Jan 2008).

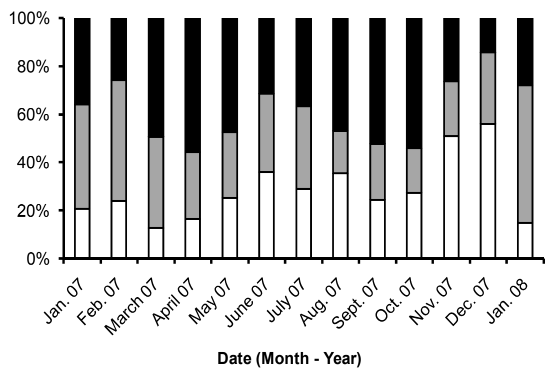

Figure 6. Proportions of female *Poecilia velifera* (Regan, 1914) in each of the 3 reproductive stages collected from the Songkhla Lake Basin (Jan. 2007-Jan 2008): open bars – immature females, grey bars – mature-not pregnant, black bars – mature pregnant.

Sap, Thale Luang and Thale Noi. Indeed, there are many seemingly suitable habitats in those areas that would likely support establishment of this species. In addition, this fish can survive over a wide salinity range, greater than salinity fluctuation in the SLB (Suwiporn, pers. com.). Based on the above, *P. velifera* will probably extend their distribution range and eventually colonize all or most of the shallow water habitats in the SLB.

It was also found that the overall sex ratio of *Poecilia velifera* in the SLB was skewed to females (Figure 4), similarly to most of the other poeciliids (Snelson and Wetherington 1980; Snelson 1989). However, the sex ratio of the smallest size class does not depart much from 1:1, whereas the proportion of males was less within specimens belonging to the larger size classes. This is not supposed to be the result of sexual dimorphism, with females larger than males, because *P. velifera* in the SLB display not different age-length relationships between sexes (Sa-nguansil, unpublished data). The skewness towards females of *P. velifera* in the present study was possibly due to a higher death rate among males, as suggested in Snelson (1989). Mature poeciliid males are more vulnerable to a variety of stressors such as extreme temperature, overcrowding and starvation and probably more vulnerable to predation due to their highly visible dorsal and anal fins (Snelson 1989; MacLaren et al. 2004).

In this present study, relatively high fecundity of *Poecilia velifera* caught from the SLB was noted (Figure 5; Reznick and Miles 1989b). We also found gravid females every month between January 2007 to January 2008 (Figure 6), indicating that the *P. velifera* in the SLB reproduces continuously all the year round. These reproductive traits differ from the populations in temperate zones which reproduce seasonally (Billard 1986; Constantz 1989). In a tropical zone, food for poeciliids is likely available throughout the year (Axelrod et al. 1981; Mills and Vevers 1989), possibly related to a longer photoperiod and associated tropical environments may govern a greater per-capita fecundity rate (Travis and Trexler 1987; Reznick and Miles 1989a).

Interestingly, there were two modes of gravid females found in the study period. The first and the second modes of gravid females occurred 1-2 months before the south-west and north-east monsoons respectively (Figure 6). The seasona-lity trend in the present study is approximately that of spawning trends in coastal fish in the tropical Indo-Pacific region, as noted in Tiews et al. (1975). There is a possible relationship between reproduction of *Poecilia velifera* in the SLB and monsoon winds as suggested by Pauly and Navaluna (1983). Given their abundance and wide distribution, *P. velifera* in the SLB have apparently adapted their reproductive tactics to the SLB region's climate and local environmental conditions.

Although the impact of non-native *Poecilia velifera* on an ecosystem is not clearly understood, potential ecological effects may result from predation and competition for food and space. Our findings on *P. velifera* in the SLB provide baseline information important for management of the natural resources of SLB and in documenting invasion rates and other aspects of invasion ecology.

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Appendix 1. Published records of *Poecilia velifera* (Regan, 1914).

Country	Location	Status	Establishment	Reference
Mexico	Yucatan Peninsula	native	yes	Hankison et al. 2006
America	coastal Florida	alien	yes	Ferriter et al. 2006
Colombia	no data	alien	yes	Welcomme 1988
Israel	no data	alien	no data	Golani 2000
Peru	no data	alien	yes	Ortega et al. 2007
Taiwan	no data	alien	no data	Shen 1993
Vietnam	Maekong River Delta	alien	no data	Welcomme and Vidthayanon 2003;
Singapore	no data	alien	no data	Ng et al. 1993
Thailand	Chao Phraya River Delta	alien	yes	Vidthayanon and Premcharoen 2002; Nico et al. 2007
Thailand	Songkhla Lake Basin	alien	yes	present study

Appendix 2. First recorded detection of *Poecilia velifera* (Regan, 1914) in the Songkhla Lake Basin, Thailand.

Record No. (Fig. 2B)	Sampling Station	Record coordinates		Record date	Reference
		Latitude	Longitude		
1	Ban Tha Sa-an	7°13'04"N	100°34'28"E	25 Nov 2007	Present study
2	Ban Kok Rai	7°09'57"N	100°35'02"E	25 Nov 2007	Present study
3	Klong Khwang	7°09'04"N	100°34'03"E	25 Nov 2007	Present study
4	Klong Pa-wong	7°08'31"N	100°33'26"E	25 Nov 2007	Present study
5	Ban Tha Nanghom1	7°08'13"N	100°32'04"E	25 Nov 2007	Present study
6	Ban Tha Nanghom2	7°08'17"N	100°30'41"E	25 Nov 2007	Present study
7	Seasonally-closed Haadkaew Lagoon	7°15'12"N	100°33'00"E	26 Nov 2007	Present study
8	Open Haadkaew Lagoon	7°14'23"N	100°33'36"E	26 Nov 2007	Present study
9	Ban Hua Khao Daeng	7°13'04"N	100°34'28"E	26 Nov 2007	Present study
10	Ban Khao Nui	7°11'49"N	100°33'34"E	26 Nov 2007	Present study
11	Ban Bor Ang	7°13'01"N	100°32'03"E	26 Nov 2007	Present study
12	Ban Pak Ror	7°15'03"N	100°26'03"E	18 Nov 2007	Present study
13	Koh Yor	7°09'20"N	100°31'56"E	13 Sep 2008	Present study