1 **Mosquitofish avoid thermal stress by moving from open water to the shade of the**

2 **mangrove _Rhizophora mangle_**

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16 Short title: Mosquitofish avoid thermal stress using mangrove shade

17 ABSTRACT: Mangrove trees provide environmental buffering for animals by reducing day

18 time water thermal maxima. Shade from _Rhizophora mangle_ trees reduces thermal stress for

19 the mosquitofish, _Gambusia affinis_. Data were collected from mangrove forests in Quintana

20 Roo, Mexico. Soliman Bay, a mangrove forest decoupled from direct ocean water, and La

21 Lunita with semi-direct access to ocean water. During cooler morning hours at Soliman Bay

22 large numbers of mosquitofish foraged in open channels. At the same time, few mosquitofish

23 utilised shaded areas within the _R. mangle_ prop roots. When channels exceeded water

24 temperatures above 38˚C mosquitofish migrated into the shaded areas provided by _R. mangle_

1   trees. Channel water reached a daytime maximum temperature of 46˚C, while daytime shaded

2   *R. mangle* areas remained 6.2°C cooler. Temperature rise, and abundance shifts were not

3   found in La Lunita, which has water temperatures of 27 to 28°C throughout the day. Size

4   distributions between both localities showed mosquitofish caught in Soliman Bay to be more

5   than 10 per cent smaller than the mosquitofish from La Lunita, which had a higher abundance

6   of mature adults. In Soliman Bay mosquitofish were small, and the thermal stresses may

7   impose serious developmental impacts that would be reduced by occupying water shaded by

8   *R. mangle*. *Rhizophora mangle* provide a refuge of environmental buffering for fish and

9   future increasing water temperatures may reveal a greater use of this little-known mechanism.

10   This study provides an example of a mechanism whereby mangroves support intertidal

11   biodiversity through environmental buffering.

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13   KEY WORDS: Biodiversity mechanism, mangroves, thermal tolerance, fish autecology

14

15   **1. INTRODUCTION**

16   Mangrove forests are important marginal habitats that provide physical and

17   environmental protection for the resident fauna (Laegdsgaard & Johnson 2001, Hendy et al.

18   2014). They provide a range of ecosystem services such as erosion reduction, carbon

19   sequestration and biodiversity maintenance (Donato et al. 2011, Hendy et al. 2014, Duke &

20   Schmitt 2015, Eddy et al. 2016). These ecosystem services are being reduced due to the loss

21   of mangrove forests, with increasing ecological and economic consequences for the fauna

22   and people who rely on them (Alongi 2002, Duke et al. 2007).

23   Impacts from altering the mangrove hydrology by building roads across mangrove

24   habitats has caused some mangrove systems to become decoupled or semi-decoupled,

25   depending on whether connection to open ocean water has been completely or partially lost

1   (Teutli-Hernández & Herrera-Silveira 2018). These same impacts have been imposed on the

2   mangrove sites in this present study. The loss of connection may lead to reduced biodiversity

3   due to raised water temperatures and reduced dissolved oxygen (Lewis et al. 2016).

4   Over the last 30 years increases of the sea surface temperatures (SST) have been

5   observed, with estimates of an average increase of almost 4 °C by the year 2100 (Aral &

6   Guan 2016, Maulvault et al. 2017). Increases of SST may lead to behavioural, physiological

7   and developmental problems for marine fish (Pershing et al. 2015, Jeffries et al. 2016,

8   Maulvault et al. 2017).. The rapid warming in the Gulf of Maine led to a decline in Atlantic

9   cod (*Gadus morhua*) recruitment due to an increase in heat-induced mortalities (Pershing et

10   al. 2015). Californian longfin smelt suffer cellular stress due to an upregulation of heat shock

11   proteins when exposed to temperatures beyond their zone of tolerance (Jeffries et al. 2016)

12   and a range of estuarine fish have been shown to suffer from oxidative stress when thermally

13   impacted (Madeira et al. 2013). Thus, with increasing concern for future fisheries, the

14   inability of marine fauna to adapt or survive to rising temperatures will create significant

15   impacts from species- to population-levels and global scales (Pörtner & Knust 2007).

16   Distributions of some pelagic fish species are migrating poleward to cope with SST rise

17   (Donet et al. 2011, Pershing et al. 2015). Lack of thermal adaptation may be important to

18   tropical species living within narrow thermal ranges (Munday et al. 2017). Coupled with

19   losses of habitat complexity and ecosystem services, the continued loss of mangrove forests

20   (Duke et al. 2007, Teutli-Hernández & Herrera-Silveira 2018) may see dramatic declines of

21   terrestrial fauna (Rog et al. 2016) and fish (Muzaki et al. 2017) leading to declines of future

22   fauna biomass for mangroves and adjacent fisheries. The loss of species and populations

23   driven by a warming climate is a global concern (Wiens 2016). Critical thermal limits can

24   deplete genetic diversity, have cascading effects on other species, facilitate trophic collapse,

25   and alter ecosystem functioning and services (Luck et al. 2003, IPCC 2014). Critical thermal

1 limits will impact many organisms, particularly in the marine environment (Pinsky et al.

2 2019).

3   A key mangrove ecosystem service is its nursery function (Laegdsgaard & Johnson

4 2001, Lee et al. 2014, Duke & Schmitt 2015, Muzaki et al. 2017), which benefits reef fish

5 populations by enhancing development and maturation of juveniles within the complexity of

6 root systems that provide shelter from predators (Laegdsgaard & Johnson 2001, Hendy et al.

7 2013). Mangrove roots also buffer wave energy (Ismail et al. 2012) and oxygenate the

8 sediments (Scholander et al. 1955). But little is known of other key biodiversity maintenance

9 mechanisms in mangrove forests, particularly from environmental buffering. Hendy et al.

10 (2013 & 2014) found a little-known biodiversity mechanism inside fallen wood within

11 Indonesian mangrove forests. They showed that evaporative cooling within abandoned

12 teredinid tunnels in wood was shown to benefit a wide range of terrestrial and aquatic

13 juvenile animals. The environmental buffering delivered by cooling wood benefit many

14 juvenile and vulnerable animals including heat-tolerant fish, octopods, spiders, crabs and

15 polychaetes (Hendy et al. 2013 & 2014) and temperatures inside tunnelled wood were 14˚C

16 cooler than ambient temperatures (Hendy et al. 2013). In this study we report another

17 example of behaviour that exploits the cooler parts of the mangrove ecosystem: mosquitofish

18 move to the shade of *Rhizophora* trees when temperatures in open water reach stressful

19 levels.

20   The mosquitofish (*Gambusia affinis*) is a brackish-water tolerant poecilid fish

21 possessing a native range that stretches across the USA, Mexico and parts of the Caribbean

22 (Lee et al. 2017). Introduced to some temperate regions as a biological control against

23 mosquito larvae, this viviparous fish now has a global distribution (Lee et al. 2017). Known

24 for expressing high phenotypic plasticity, this adaptable fish has been used across a broad

25 range of ecological studies (Winkler 1979, Wurtsbaugh & Cech 1983, Benoît et al. 2000) and

is known for its ability to thrive in extreme environmental conditions such as low dissolved

oxygen, high salinities and high temperatures (Wurtsbaugh & Cech 1983). Organisms will

have limits to tolerance of extremes of each of these environmental variables. In the case of

water temperature, the limits are expressed as critical temperature, above which the

organisms start to exhibit a loss of equilibrium and become moribund (Hupfeld et al. 2015).

In this study we investigated how shade from the canopy of *Rhizophora mangle* may

offer environmental buffering for resident mosquitofish. Observations were made in two

Caribbean mangrove forests on the south-western coast of the Yukatan Peninsula (Fig.1), one

(Soliman Bay) with limited water exchange and consequently marked temperature fluctuation

and the other (La Lunita) with a semi-direct connection to the local microtidal regime and

thus much more buffered water conditions. These forests both maintain populations of

Gambusia, but under distinctly different regimes of solar illumination and temperature

fluctuation.

We hypothesised that within *R. mangle* stands:

1. Water temperature is a key diurnally-varying environmental variable for mosquitofish
   behaviour

2. Mosquitofish avoid dangerous water temperatures by shade-seeking behaviour

3. Light levels and food availability affect mosquitofish distribution

4. The combination of diurnally fluctuating environmental factors in the more
   environmentally-stressing study site will affect mosquitofish population size
   distribution

The objectives of this study were to:

1. Assess mosquitofish abundance in open water channels and in the shade of *R. mangle*
   trees throughout the day across two mangrove systems

1   2. Characterise behaviour of mosquitofish and evaluate the significance of this

2   behaviour for fish autecology

3   3. Assess food availability in both mangrove forests at morning and afternoon hours

4   using plankton tows

5   4. Quantify mosquitofish size distributions across two mangrove forests

6   **2. MATERIALS AND METHODS**

7

8   *2.1.Sites used in the investigation*

9   Observations of fish behaviour were made in two *Rhizophora mangle*-dominated

10 mangrove forests in Quintana Roo, Mexico. The Soliman Bay dwarf mangrove forest (Fig.1,

11 380 ha, 20˚16'44.18'N, 87˚22'55.32'W) has a canopy height generally less than 1.5m, but at

12 the strandline, which was identified by the accumulation of buoyant plant detritus at the

13 upper limit of tidal reach, has stands of *Conocarpus erectus*, *Laguncularia racemosa* and

14 *Avicennia germinans*. From the strandline mono-specific stands of dwarf *R. mangle* extend

15 across the whole 380 hectares of mangrove forest area. which is only connected to the sea via

16 seepage through a sand bar and possibly via subterranean cenote connections. The other site

17 is a higher canopied mangrove forest at La Lunita (Fig. 1, 2.9 ha, 20˚24'26.5'N,

18 87˚18'27.5'W), which has connections to the sea via a tidal creek that enters Yal Kul lagoon

19 and via cenote-connected subterranean channels.

20   The lack of a channel connecting the Soliman Bay mangrove forest with the sea

21 means that the fish population there is usually unable to move beyond the forest. The

22 contained water there is micro-tidal (± 0.5m) (Teutli-Hernández & Herrera-Silveira 2018)

23 with limited water flow and input via seawater permeating the sand bar and from rainwater,

24 resulting in brackish water, ranging from 10 to 17 practical salinity units (psu). A channel

25 runs through the forest with water depth ranging from 15cm to 40cm. Water temperature can

1   reach up to 46˚C and fluctuate by up to 20˚C in a 24-hour period. Dissolved oxygen (DO_2) is

2   low.

3   During morning hours in the Soliman Bay mangrove, channels were populated by

4   large numbers of mosquitofish, *Gambusia affinis* Baird & Girard, 1853 (Fig. 2) which were

5   observed foraging for food. During afternoon hours almost all fish had vanished from those

6   channels. We believe this sharp shift in behaviour is prompted by an extreme temperature

7   change and that mosquitofish populations in the Soliman Bay mangrove forest seek the shade

8   offered by *R. mangle* stands to avoid thermal stress.

9   At the La Lunita mangrove forest strandline in the Yal Ku lagoon, *Conocarpus*

10   *erectus*, *Laguncularia racemosa*, *Avicennia germinans*, and *R. mangle* trees were found, but

11   most of the forest consists of *R. mangle* reaching heights of up to 20 metres (Fig. 3). The Yal

12   Ku lagoon has a semi-decoupled water system with subterranean cenote connections (Gabriel

13   et al. 2008). The tidal regime is also microtidal. Cenote water in La Lunita is deep, reaching

14   approximately four to five metres. Salinity was low, ranging from four to six psu. Water

15   temperature was consistent throughout the day, ranging from 27 to 28 °C. A cut path that on

16   the landward side of the strandline of the forest gave access to cenote openings above which

17   mosquitofish were sampled to determine the population size distribution. This path was also

18   used for observing fish behavior and measuring water temperature.

19   The semi-open system of La Lunita means that aquatic animals are less restricted in

20   their ability to migrate out of the forest and the freer water circulation. Consequently, the

21   environmental pressures found in Soliman bay are not replicated in La Lunita. Large numbers

22   of mosquitofish were also found here.

23   *2.2. Behaviour and water variables measurements*

24

25   At Soliman Bay, mosquitofish first occurred at 20 metres from the strand line, and

26   their abundance was estimated in open channels and in shaded areas of the *R. mangle* stands

1   at stations between 20 and 200 meters from the strandline. Surveys were conducted during

2   morning (08:00 to 10:00) and afternoon (13:00 to 15:00) hours using 1 m^2 quadrats every

3   three to five metres along transect lines. After positioning quadrats, a five-minute period was

4   left to pass prior to estimating fish numbers, allowing fish to acclimatise to the disturbance.

5   Measurements were then made every 10 minutes.

6   In La Lunita, estimates of mosquitofish abundances were made in the open channel

7   and in areas of the *R. mangle* prop-root stands at 0 to 70 metres from the strandline. Surveys

8   were also conducted during morning (8:00 to 10:00) and afternoon (13:00 to 15:00) hours

9   every three to five metres along the transect lines. Due to practical constraints of water depth

10 estimates of mosquitofish abundance per m^2 were made by observing 1m^2 areas of water

11 surface.

12   Water temperatures at Soliman Bay were measured at three stations placed at 50, 100

13 and 150 metres from the strandline. At each station measurements were made in the open

14 water and in the shade of the *R. mangle* canopy (± 0.5°C, 30-minute intervals). A total of six

15 HOBO Pendant® Temperature/Light 64K Data Loggers were deployed for these

16 measurements.

17   Water temperature recordings at La Lunita were also made in open water (exposed)

18 and shaded areas, using LCD digital thermometers with submersible probes

19 (https://www.tester.co.uk). Recordings were taken every five-metres from the strandline out

20 to 70m over a six-day period in the morning (08:00 to 10:00) and afternoon (13:00 to 15:00)

21 hours.

22 *2.3. Fish behaviour in shaded and unshaded areas*

23   Mosquitofish abundance was monitored at three stations in Soliman Bay from 10:30

24 hrs. Over a six-day period, observations were made at 50, 100 and 150 metres along the

25 strandline. Each day before recordings took place a 10-minute interval was left to allow for

1 any disturbed fish to acclimatise and for any disturbed sediment to settle. To establish

2 mosquitofish distributions in the mangrove forests water temperature (°C) in open channels

3 was measured with an LCD digital thermometer with submersible probe and mosquitofish

4 abundance (per m^2) and in-channel water temperatures were concurrently recorded. Water

5 temperature measurements and fish observations were recorded over a four-and-a-half-hour

6 period or until channel water temperature failed to increase for two or more readings.

7 To establish whether mosquitofish at Soliman Bay were actively seeking cooler water

8 provided by the *R. mangle* shade or simply seeking 'dark' cover, we positioned three parasols

9 (https://www.ikea-parasol) at 50, 100 and 150 metres from the strandline in the open water

10 channel during afternoon hours (12:00 to 14:30). Parasols were secured by driving their poles

11 into channel sediment. Mosquitofish abundance was recorded every 10-minutes in the shade

12 of the adjacent *R. mangle* prop-stands and in the cover of the parasol shaded area. Concurrent

13 water temperatures measurements were also taken from within the open channel and in the

14 cover of the parasol shaded areas.

15 Salinity was measured in practical salinity units (psu) using a Bellingham and Stanley

16 refractometer (https://refractometershop.com/) and dissolved oxygen was measured using a

17 Tetra Oxygen Testing kit (https://www.tetra.net/en/en) measuring in milligrams per litre

18 (DO$_2$ per mg/L). Concentrations of psu and DO$_2$ were measured at 10 locations in Soliman

19 Bay situated every 20 metres from the strandline and 10 locations in La Lunita every seven

20 metres from the strandline.

21 ### *2.4. Food Availability*

22 Mosquitofish diets contain large concentrations of zooplankton (Hurlbert & Mulla

23 1981). Crivelli & Boy (1987) found that some mosquitofish stomach contents consisted of

24 more than 80 per cent of planktonic crustaceans Thus, plankton tows were used to assess food

25 availability within open water channels at both localities during morning and afternoon hours.

1   The plankton net had a frame diameter of 300 mm, net length of 880 mm and mesh filter size

2   of 250 μm. Tow-lines were pulled for five-metres in open water channels at three locations

3   from the strandline (Soliman Bay: 50, 100 and 150 metres and La Lunita: 0, 35 and 70

4   metres). In each locality ~1 m^3 of water was sampled for plankton diversity and abundance.

5   A total of 6 samples were collected from each location and stored in 100 ml vials with water

6   from the study site. Each sample was gently shaken, and 10 ml of sample water was extracted

7   from each 100 ml vial using a Stempel pipette and analysed. Numbers were extrapolated to

8   represent abundance of plankton per 1 m^3 of mangrove water between localities. Planktonic

9   communities in the samples were examined at a magnification of x40 using a stereo

10   microscope.

11   *2.5. Size Distribution*

12   The seine-net haul technique was used in random locations from the strandline and

13   further out at both localities to determine mosquitofish size distributions (±0.1 mm; standard

14   length from the anterior tip to the caudal peduncle). The seine-nets used had a mesh size smaller

15   than the diameters of juvenile mosquitofish. Four seine-net hauls were undertaken to capture

16   mosquitofish at Soliman Bay, and five seine-net hauls were undertaken to capture mosquitofish

17   at La Lunita. Photographs of each of the hauls were taken using a Nikon Coolpix A100

18   (https://store.nikon.co.uk/). Images were analysed using the digital analysis package ImageJ.

19   Pooled sample sizes of 391 fish (391 fish were caught at one site and 391 were selected at

20   random from the other site) from each study site were used to distinguish mosquitofish size

21   distributions (https://imagej.nih.gov/ij/).

22   *2.6. Statistical analyses*

23   Temporal and spatial differences of fish distributions, and temperature (˚C) differences from

24   the quadrat data were examined using a General Linear Model (GLM) with time of day and

1 habitat (between roots or out in open channel) used as factors. Differences of temperature and

2 fish abundances in Soliman Bay were examined using a GLM, with distance from the strandline

3 and time as factors between sites and parasol cover compared to open channels. Paired t-tests

4 were used to examine differences of temperatures measured in the open channels with

5 temperatures measured within the shade of the *R. mangle* trees; mean morning and afternoon

6 temperatures and mean morning and afternoon fish abundance in both study locations; and,

7 dissolved O_2 and salinity measurements on the dimensionless practical salinity scale from both

8 study locations. Two-Way Analysis of Variance (ANOVA) was used to analyse variations in

9 food availability in both study locations during morning and afternoon hours with site and time

10 of day (morning and afternoon) as factors. A one-way ANOVA was also used to examine

11 relationships before and after temperatures reach their critical thermal limit for the

12 mosquitofish in the Soliman Bay mangrove open-water channels and shaded areas. All count

13 data were square root transformed and all measurement data were Log transformed with

14 suitability of the transformations scrutinized by examining residuals. Post-hoc, Descriptive

15 Statistics and Tukey's pairwise comparison tests were used to separate values into statistically

16 distinct subsets in all ANOVAs. All statistical analyses were performed using MINITAB

17 (MINITAB Inc, version 13.20).

18 **3. RESULTS**

19 *3.1. Fish Abundance*

20 During morning hours in Soliman Bay the highest abundance of mosquitofish where

21 found in the open water channel. In the afternoon, mosquitofish abundances were significantly

22 greater in the shade amongst the roots of the *R. mangle* trees (Fig. 4 A: GLM, area (channel &

23 roots) and time (am & pm) vs. fish abundance: area vs. fish abundance: $F_{1,87} = 8.5$, $p \leq 0.01$;

24 time vs. fish abundance: $F_{1,87} = 39.1$, $p \leq 0.001$; and area*time vs. fish abundance: $F_{1,87} = 177.2$,

25 $p \leq 0.001$). Water temperature (℃) in the open channel was found to be significantly warmer

1   during the afternoon (12:00 to 18:00) when compared to water temperatures recorded from

2   within the adjacent *R. mangle* tree shaded areas at the same time.

3   Water temperatures in Soliman Bay during afternoon hours were on average > 6 ˚C

4   cooler in the *R. mangle* tree stands, than in open water channel temperatures. Afternoon water

5   temperature measurements within *R. mangle* shaded areas were only 1.7℃ warmer than

6   temperatures measured in the adjacent channels during morning hours (06:00 to 12:00) (Fig. 4

7   B: GLM, area vs. temperature: $F_{1,87} = 799.2$, $p \leq 0.001$; time vs. temperature: $F_{1,87} = 606.2$, p

8   ≤ 0.001; and area*time vs. temperature: $F_{1,87} = 28.5$, $p \leq 0.001$).

9   Mosquitofish abundance in La Lunita remained high in open water channels throughout

10   both morning and afternoon hours. Abundance of mosquitofish increased in the open water

11   channels and among the *R. mangle* prop-root stands from morning hours (GLM, area vs. fish

12   abundance: $F_{1,176} = 170.3$, $p \leq 0.001$; time vs. fish abundance: $F_{1,176} = 7.9$, $p \leq 0.01$; and

13   area*time vs. fish abundance: $F_{1,176} = 1$, $p \geq 0.5$). Water temperatures in La Lunita remained

14   the same in the open channels and between *R. mangle* prop-roots. Morning and afternoon water

15   temperatures remained constant, only changing by an increase of 0.3˚C ± 0.9˚C (mean ± SE)

16   from morning to afternoon hours (GLM, area vs. temperature: $F_{1,176} = 0$, $p \geq 0.05$; time vs.

17   temperature: $F_{1,176} = 79.7$, $p \leq 0.001$; and area*time vs. temperature: $F_{1,176} = 0$, $p \geq 0.05$).

18   *3.2. Mosquitofish Behaviour*

19   The open-water mosquitofish abundance in Soliman Bay significantly dropped across

20   all three monitoring stations on six different sampling days. Areas further away from the

21   strandline in Soliman Bay became warmer later in the day when compared to shallower

22   waters closer to the strandline. Mosquitofish avoided the extreme water temperatures by

23   swimming to the cooler areas of the open channels further out. This resulted in areas closer to

1   the strandline being less abundant of mosquitofish compared to areas further out from the

2   strandline as time passed.

3   In the open channels of Soliman Bay mosquitofish abundance dropped by $> 90\%$

4   above an average water temperature of 38.8˚C $\pm$ 0.1˚C (Fig. 5 A, mean $\pm$ SE). Thus, when

5   channel water temperatures exceeded 38 ˚C during afternoon hours, a large shift in

6   mosquitofish abundance was recorded, with 44.1 $\pm$ 2.8 fish m^{-2} (mean $\pm$ SE) before water

7   temperatures reached critical limits reducing to 10.4 $\pm$ 1.1 fish m^{-2} (mean $\pm$ SE) after critical

8   water temperatures were reached. Declines above the critical water temperature in open

9   channels occurred at all stations on all days (ANOVA, temperature before and after critical

10   thermal temperature vs. mosquitofish abundance in open water channels: $F_{1,327} = 107.1$, $p \leq$

11   0.001).

12   After 14:00 hrs mean water temperatures in the open water channel where almost

13   10˚C hotter compared to morning water temperatures. Water temperatures were highest at

14   the 50-metre station (GLM, time vs. temperature: $F_{2,135} = 170.7$, $p \leq 0.001$; distance from the

15   strandline vs. temperature: $F_{2,135} = 45.2$, $p \leq 0.001$; and time*distance from the strandline vs.

16   temperature: $F_{4,135} = 1.02$, $p \geq 0.05$). All open-water Soliman Bay mangrove forest areas

17   showed similar temperature increases between days. Mosquitofish abundance in the shade of

18   the *Rhizophora* trees was significantly greater after open water reached critical temperatures

19   (Fig. 5 B), 35.1 $\pm$ 2.6 fish m^{-2} (mean $\pm$ SE) compared with mosquitofish abundance in the

20   shade of the trees before open water temperatures reached critical temperatures, 13.3 $\pm$ 2.5

21   fish m^{-2} (mean $\pm$ SE, ANOVA, temperature before and after critical thermal temperature vs.

22   mosquitofish abundance in shaded areas: $F_{1,52} = 28$, $p \leq 0.001$). Burying behaviour and an

23   increase of air-gulping behaviour by the mosquitofish in Soliman Bay was observed when

24   water temperatures reached more than 40˚C.

1   No large shifts of mosquitofish abundance in La Lunita was found in the open water

2   channels throughout morning and afternoon hours. Mosquitofish abundance increased by

3   ~20% from morning to afternoon hours within the open water channels (GLM, time vs. fish

4   abundance: $F_{1,84} = 4.8$, $p < 0.05$; distance from the strandline vs. fish abundance: $F_{2,8} = 1.6$, p

5   ≥ 0.05; and time*distance from the strandline vs. fish abundance: $F_{2,84} = 1.5$, $p \geq 0.05$). No

6   difference of fish abundance was found between the three stations.

7   *3.3. Parasol Shaded Area*

8   Mosquitofish abundance was significantly higher in the open channels at Soliman Bay

9   (17.2 fish $\pm$ 1.8 fish m^{-2}, mean $\pm$ SE) when compared to mosquitofish abundance under shading

10   provided by parasols (1.9 fish $\pm$ 0.6 fish m^{-2}, mean $\pm$ SE) during afternoon hours (Fig. 6 A and

11   B, Paired t-test: $P \leq 0.001$, $n = 30$. Water temperature in the open channels, 38.6 ˚C $\pm$ 0.1 ˚C

12   (mean $\pm$ SE) compared to water temperatures recorded in the parasol shaded areas during the

13   same time, 38.4 ˚C $\pm$ 0.1˚C (mean $\pm$ SE) were not significantly different ($n = 30$). Water

14   temperatures in the parasol shaded areas matched those of the ambient open water channel

15   temperatures.

16   Salinity (psu) concentrations at Soliman Bay were 11.5 psu $\pm$ 0.8 psu (mean $\pm$ SE) and

17   were higher when compared with La Lunita, 5.8 psu $\pm$ 0.2 psu (mean $\pm$ SE, Paired t-test: $P \leq$

18   0.001, $n = 10$). Dissolved O_2 (mg/L) readings were significantly lower in the Soliman Bay

19   water channels, 5.4 mg/L $\pm$ 0.7 mg/L (mean $\pm$ SE) when compared with La Lunita water

20   channels, 11 mg/L $\pm$ 0 mg/L (mean $\pm$ SE, Paired t-test: $P \leq 0.001$, $n = 10$).

21   *3.4. Food Availability*

22   Zooplankton abundance in the open water channels was similar between both localities

23   during the morning and afternoon hours (Fig. 7, two-way ANOVA, site*time vs. zooplankton

1   abundance: $F_{1,8} = 0.3$, $p \geq 0.05$). During the morning in Soliman Bay there were 301 ± 33.9

2   (mean ± SE) zooplankton individuals per 1 m³, and in the afternoon, there were 415 ± 65.9

3   (mean ± SE) zooplankton individuals per 1 m³. In the La Lunita mangrove forest there were

4   301 ± 98.5 (mean ± SE) zooplankton individuals per 1 m³ in the morning compared with 320.5

5   ± 80.6 individuals per 1 m³ in the afternoon.

6   *3.5. Size distribution*

7   Mosquitofish from Soliman Bay were on average > 10% smaller (Fig. 8 A) when

8   compared with mosquitofish from La Lunita, with 18.7 mm ± 0.2 mm (mean ± SE) and 21 mm

9   ± 0.2 mm (mean ± SE) respectively (Paired t-test, $P \leq 0.001$). A greater abundance of mature

10   mosquitofish was found in La Lunita (Fig. 8 B). The largest mosquitofish measured in Soliman

11   Bay was 36 mm, and in La Lunita the largest mosquitofish measured was 47 mm.

12   *3.6. Predators*

13   In Soliman Bay, large numbers of predatory birds were witnessed feeding on thermally-

14   stressed mosquitofish showing a loss of equilibrium. During this time, water levels were low,

15   and water temperatures were more than 43˚C (Fig. 9 A and B).

16   4. **DISCUSSION**

17   Sites were not replicated during this study, so it was not possible to draw firm

18   conclusions about site-specific environmental factors. But, our data reveal that when channel

19   water was warmest in Soliman Bay, the mean water temperature shaded by the *R. mangle* trees

20   was more than 6˚C cooler - a significant temperature difference for mosquitofish, especially if

21   reduced dissolved oxygen in the open water impacts feeding rates (Chipps & Wahl 2004),

22   reduces growth rates (Wurtsbaugh & Cech 1983) and increases mortalities (Otto 1973). Water

23   temperatures at the La Lunita mangrove forest was driven by daily flushing from inshore

24   coastal water and the temperature remained constant throughout the day with little change and

1   with a maximum water temperature reaching 28˚C. At times, water temperature in Soliman

2   Bay was ~18˚C hotter. Such an extreme water temperature will place very high physiological

3   demands on the flora (Alongi 2018) and fauna.

4   The high abundance of mosquitofish foraging within open water channels at both

5   localities during day time hours are consistent with fish behaviours observed from other studies

6   (Ling & Willis 2005, Pyke 2005). However, dramatic declines in abundance of mosquitofish

7   within the open channels at Soliman Bay during afternoon hours did not mirror the distribution

8   patterns of mosquitofish observed in La Lunita during the same time. The high abundance of

9   mosquitofish in Soliman Bay shifted from the open channels during morning hours to cooler

10 *R. mangle* prop-root shaded areas in the afternoon. During the same time mosquitofish

11 abundance in La Lunita remained the same in open water channels from morning to afternoon

12 hours and no significant change in mosquitofish abundance was observed. The sharp rise in

13 water temperature observed in Soliman Bay exceeded the critical thermal tolerance for the

14 mosquitofish, which triggered their rapid migrations to seek environmental buffering.

15   Benoît et al. (2000) report that mosquitofish prefer habitats with lots of cover to avoid

16 predation, but Casterlin & Reynolds (1977) report that mosquitofish do not favour such

17 habitats. Our study highlights that mosquitofish behaviour is driven by water temperature and

18 availability of a mangrove root refuge from predation. Our data are similar to Winkler (1979),

19 who found that mosquitofish prefer water temperatures of 31 to 35˚C, avoiding a critical water

20 temperature limit of 39˚C (Cherry et al. 1976) and an upper lethal limit of 38˚C and above

21 (Otto 1973). Despite the availability of many vegetated areas and complex habitats in Soliman

22 Bay, mosquitofish only appeared to show preference to these habitats when water temperatures

23 reached 38˚C and above during afternoon hours. We found that mosquitofish in Soliman Bay

24 preferred channel water temperature ranges of 35 to 37˚C with a maximum thermal tolerance

25 of 38 to 39˚C, which agrees with previous studies of mosquitofish (Otto 1973, Cherry et al.

1 1976, Winkler 1979). Our results demonstrate that mosquitofish are actively seeking areas

2 provided by *R. mangle* prop-roots when open channel water temperatures reach lethal limits of

3 38˚C and above to avoid thermal stress. Prop roots also provide greater in-water complexity at

4 the same time as the canopy provides shade. In addition, antipredator responses could also play

5 a role as predatory birds were often observed.

6 Mosquitofish are opportunistic omnivores, consuming algae and zooplankton (Crivelli

7 & Boy 1987). They are known to have extremely high feeding rates (up to 83 per cent of their

8 total body weight per day) when exposed to water temperatures of 10℃ to 35℃ (Wurtsbaugh

9 & Cech 1983, Chipps & Wahl 2004), very similar to the water temperatures at Soliman Bay

10 from 10:00 to 12:30. Mosquitofish metabolic rates increase with temperature until DO_2

11 availability becomes too low (Cech et al. 1985). Thus, this may explain the high abundance of

12 mosquitofish in the open channels at the Soliman Bay as the perfect feeding environment for

13 mosquitofish would be between these times. Their appetites were corroborated by the fact that

14 mosquitofish were observed in high abundance in open areas throughout the day continuously

15 foraging for food, particularly on the water surface and occasionally on the surface of the

16 sediment. The optimum feeding temperatures may also explain why mosquitofish abundance

17 in the open water channels at La Lunita increased by almost 20 per cent during afternoon hours

18 when water temperatures reached the ideal conditions for feeding. In addition, mosquitofish in

19 Soliman Bay were not as numerous in the shade of the parasols compared to the open channels

20 and shade of the mangrove trees. Unlike the mangrove prop roots, the parasols provided no

21 additional underwater structure and did not provide any water temperature buffering.

22 Plankton tows from this study demonstrated that food availability remained constant

23 throughout morning and afternoon hours at both localities and consisted of gastropods and

24 crustaceans. High concentrations of zooplankton were found in all samples providing further

1 evidence that mosquitofish shifting abundance from open channels to cooler *R. mangle* shaded

2 areas during afternoon hours at Soliman Bay were not driven by food availability but by

3 avoiding extreme thermal stress. The lack of thermal stress and extreme water temperatures in

4 La Lunita may explain why mosquitofish remained foraging in open channels throughout the

5 day, as food was plentiful.

6   At birth mosquitofish are between 6 to 8 mm in length and grow at a rate of

7 approximately 1 to 2 mm per week (Stearns 1983). This means that the greatest proportion of

8 mosquitofish caught from both localities were aged between 3.5 and 6.5 weeks old. However,

9 mosquitofish populations caught in La Lunita possessed higher ratios of fish aged 6.5 weeks

10 and older. Male mosquitofish mature after 8 weeks and females mature after 10 weeks (Pyke

11 2005). This means that sexually mature individuals are more abundant in La Lunita. The size

12 of mosquitofish caught in Soliman Bay were on average more than 10 per cent smaller than

13 fish measured from La Lunita. Different predators and predation pressures coupled with

14 extreme water temperatures in Soliman Bay may have caused changes with the *Gambusia* fish

15 communities (Phenix et al. 2019). Stressful temperatures may have created developmental

16 consequences for juvenile mosquitofish. Studies found that when tropical shallow-water fish

17 are exposed to extreme water temperatures they exhibit energetic trade-offs to survive the heat

18 stress, such as smaller body sizes, reduced energy reserves and increased rate of protein and

19 lipid depletion (Madeira et al. 2017). Under stressful environments juvenile and vulnerable

20 individuals divert more energy into dealing with stress instead of gonad and somatic

21 development (Shahjahan et al. 2017, Wang et al. 2017). Such stress can also lead to higher

22 mortalities (Madeira et al. 2017). Without cooler water provided by the *R. mangle* shade,

23 populations of mosquitofish in Soliman Bay would suffer serious thermal stresses combined

24 with increases of mortalities.

1   Improved fitness levels in mosquitofish are reported to occur at water temperatures of

2   30˚C with female mosquitofish investing higher proportions of energy into reproduction at

3   average water temperatures of 25˚C (Wurtsbaugh & Cech 1983). A similar water temperature

4   environment was found in La Lunita and may explain why the abundance and size distribution

5   of mosquitofish were greater at this locality. Mosquitofish growth can be reduced by lower

6   food availability and by water temperatures above 35˚C (Wurtsbaugh & Cech 1983). Due to

7   the extreme water temperatures at Soliman Bay mosquitofish sacrifice feeding in open water

8   channels in favour of reduced environmental stressors provided by the *R. mangle* stands.

9   Air-gulping behaviour expressed by mosquitofish was observed in Soliman Bay. This

10   behaviour was previously documented with fish known to survive in DO_2 concentrations as

11   low 0.28 mg/l, providing they can breathe atmospheric air from surface waters (Pyke 2005).

12   As dissolved oxygen in the general water column approaches zero, mosquitofish survival

13   depends on their ability to gain access to the surface water (Homski et al. 1994). They either

14   take in water that is relatively oxygen-rich at the atmosphere-water interface (Lewis 1970) or

15   gulp air from the atmosphere. Their dorsally oriented mouth and dorso-ventrally flattened head

16   is the ideal morphology for breathing at the surface-water interface (Lewis 1970).

17   The mosquitofish burying behaviour observed in this study has not previously been

18   reported. Further studies would be needed to confirm this novel behaviour, as our study

19   indicates that mosquitofish are also utilising cooler temperatures provided within sediments

20   shaded by *R. mangle* trees.

21   5. **Conclusion**

22   Ecosystem services derived from mangrove roots in the form of the nursery function have been

23   well-documented (Beck et al. 2001, Nagelkerken 2009, Alongi 2014). However, the role of

24   environmental buffering is less understood. This study gives an example of how *R. mangle*

prop-roots and canopy may reduce thermal stress, provide environmental buffering and protection for animals in shallow, stagnant water in a Caribbean mangrove forest. The ecological function of thermal buffering may also benefit many other mangrove fauna (Hendy et al. 2013 & 2014). The importance of mangrove hydrology and subsequent maintenance of thermoregulation (Teutli-Hernández & Herrera-Silveira 2018) is also highlighted in this study. Water temperatures in La Lunita remained at optimum levels for mosquitofish fitness and reproduction (Wurtsbaugh & Cech 1983). In Soliman Bay however, thermal extremes impacted mosquitofish ecology, which may include impacts on their size and feeding activity. This study highlights the importance of environmental buffering provided by the *R. mangle* stands. Conservation and protection of these important ecosystems will serve to maintain fish populations in a changing climate. Biodiversity maintenance offered by mangrove forests to juvenile and vulnerable marine animals demonstrates the need for their protection and restoration to maintain ecosystem-level biomass and productivity. Management planning of mangrove ecosystems, especially where major harvesting is involved should take extreme care to ensure that *Rhizophora* stands are retained within the ecosystem to protect important ecological functions.

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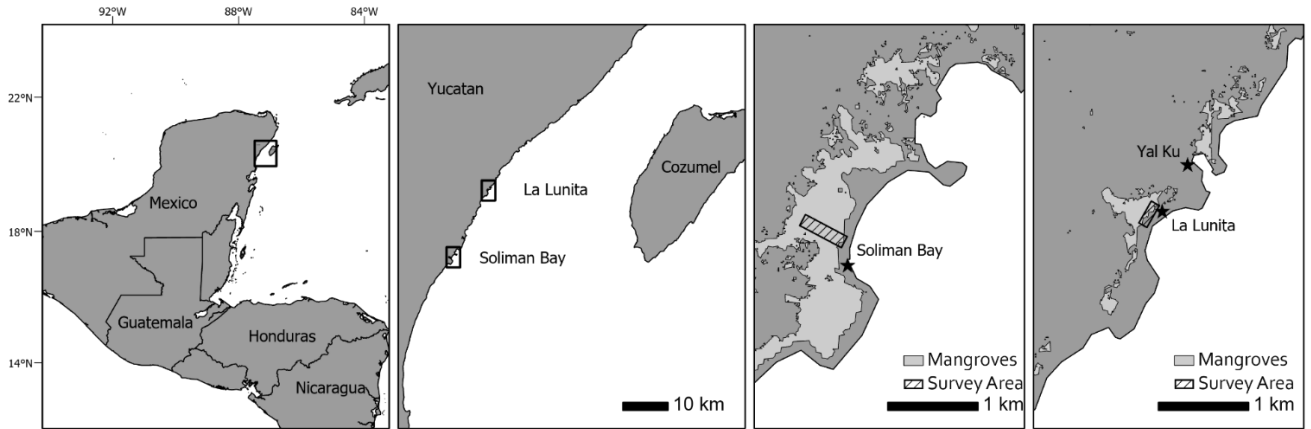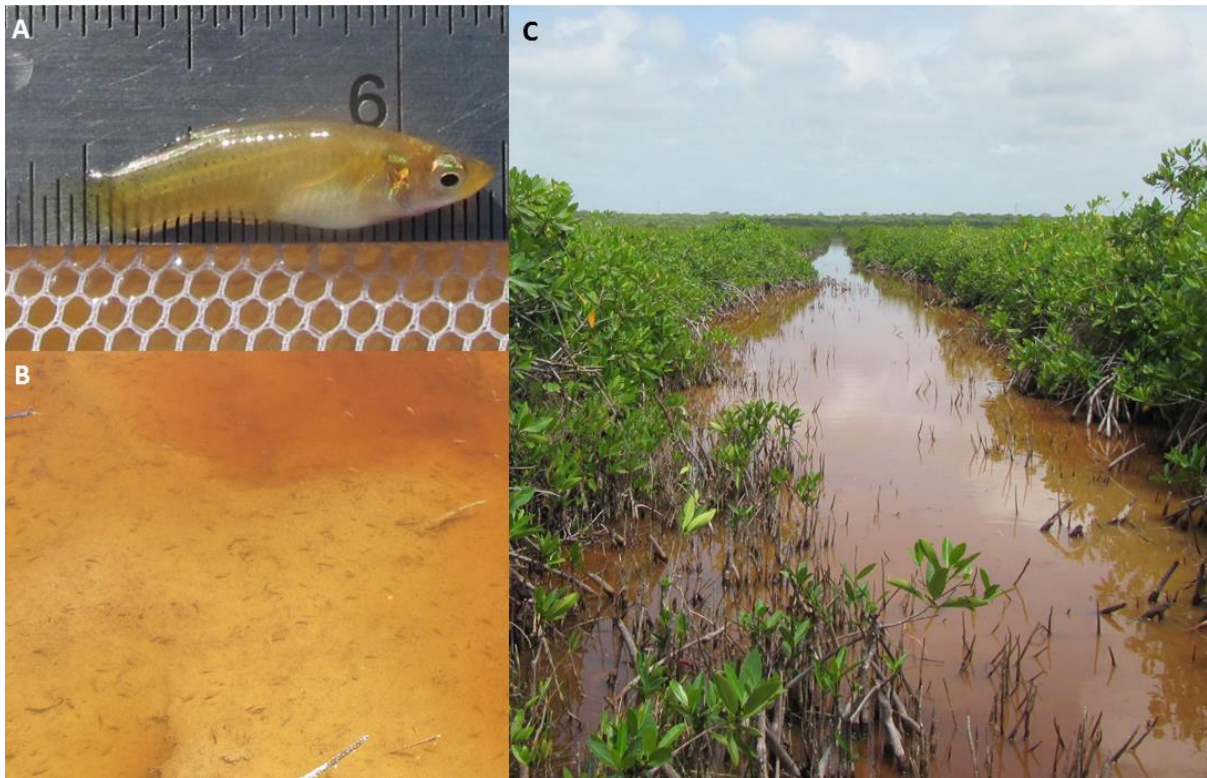
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Figures

Fig. 1. Map illustrating country, region and site-specific scale mangrove localities. The area surveyed within each mangrove forest is highlighted.

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Fig. 2. Soliman Bay mangrove forest, Tulum, Mexico: (A) an adult female mosquitofish. (B) Dense shoals of mosquitofish foraging for food in open channels and (C) The main channel in the Soliman Bay mangrove forest where large numbers of mosquitofish forage for food in the morning.
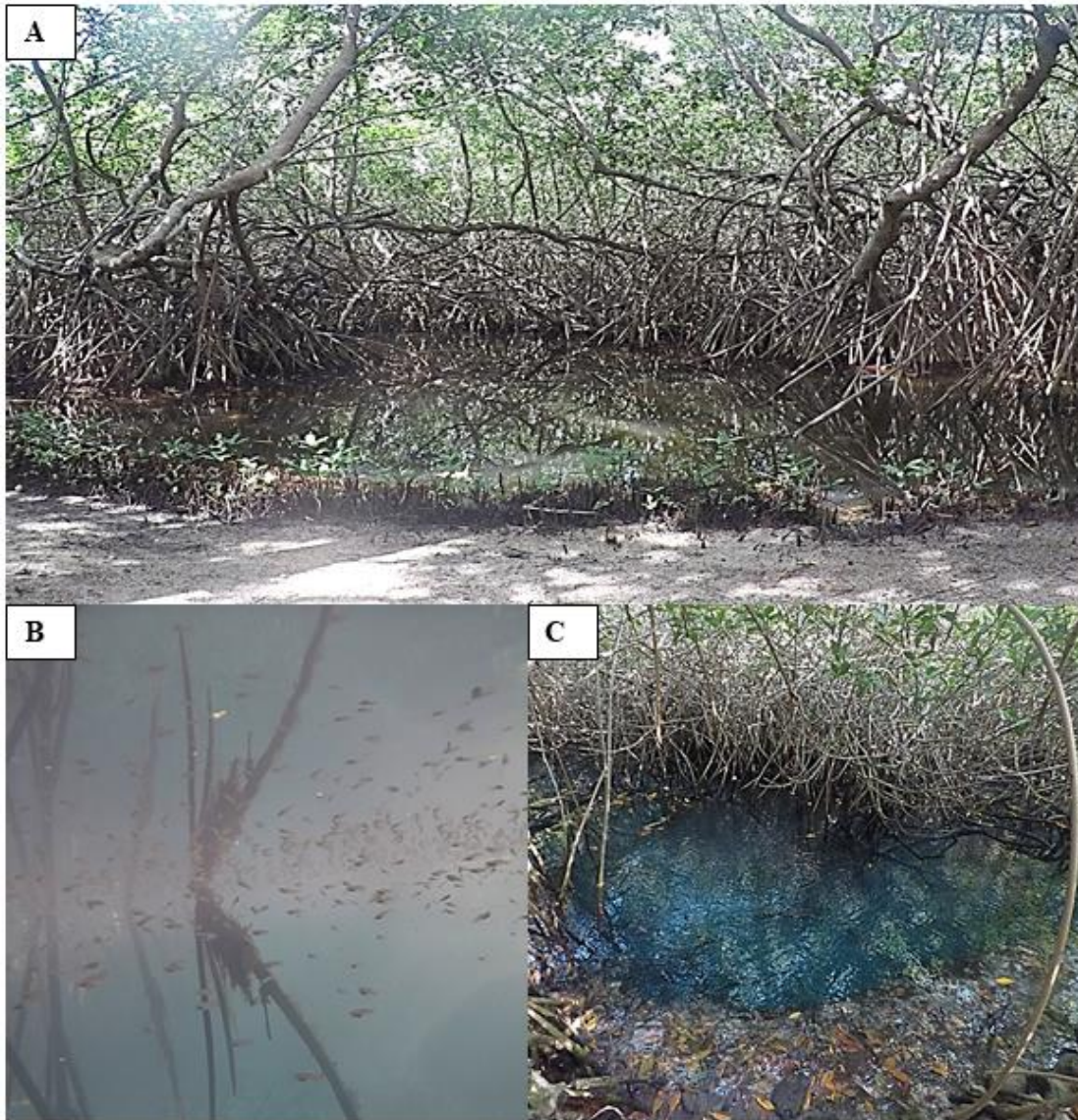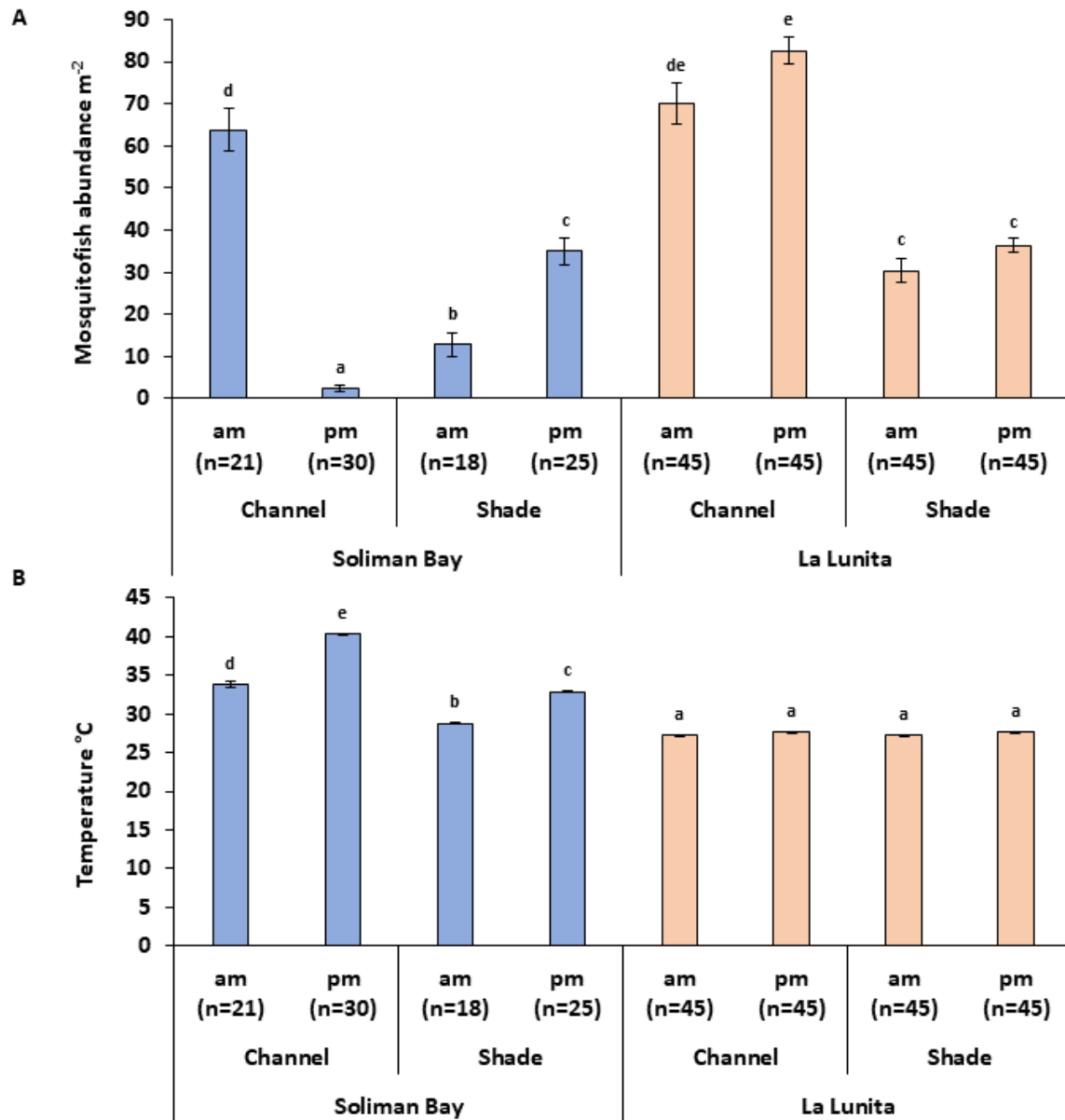
Fig. 3. The La Lunita mangrove forest, Tulum, Mexico: (A) The strand line channel lined with large *R. mangle* trees. (B) Dense shoals of large mosquitofish foraging for food in open channels and *Rhizophora* roots (C) One of many cenote openings with deep flowing water.

Fig. 4: Temporal and spatial variations of mosquitofish abundance and water temperature in open channels and prop root shaded areas in the Soliman Bay and La Lunita localities: (A) mosquitofish abundances (individuals m^{-2}, mean $\pm$ SE) during morning (am) and afternoon (pm) hours in open water channels and shaded *R. mangle* prop-root areas. GLM, area (channel & roots) and time (am & pm) vs. fish abundance: area vs. fish abundance: $F_{1,87} = 8.5$, $p \leq 0.01$; time vs. fish abundance: $F_{1,87} = 39.1$, $p \leq 0.001$; and area*time vs. fish abundance: $F_{1,87} = 177.2$, $p \leq 0.001$) (B) temperature measurements (°C, mean $\pm$ SE) during morning (am) and afternoon (pm) hours in open water channels and shaded *R. mangle* prop-root areas. GLM, area vs. temperature: $F_{1,87} = 799.2$, $p \leq 0.001$; time vs. temperature: $F_{1,87} = 606.2$, $p \leq 0.001$; and area*time vs. temperature: $F_{1,87} = 28.5$, $p \leq 0.001$). Letters above the bars = Tukey's pairwise comparisons tests, *n*-values = number of quadrats.

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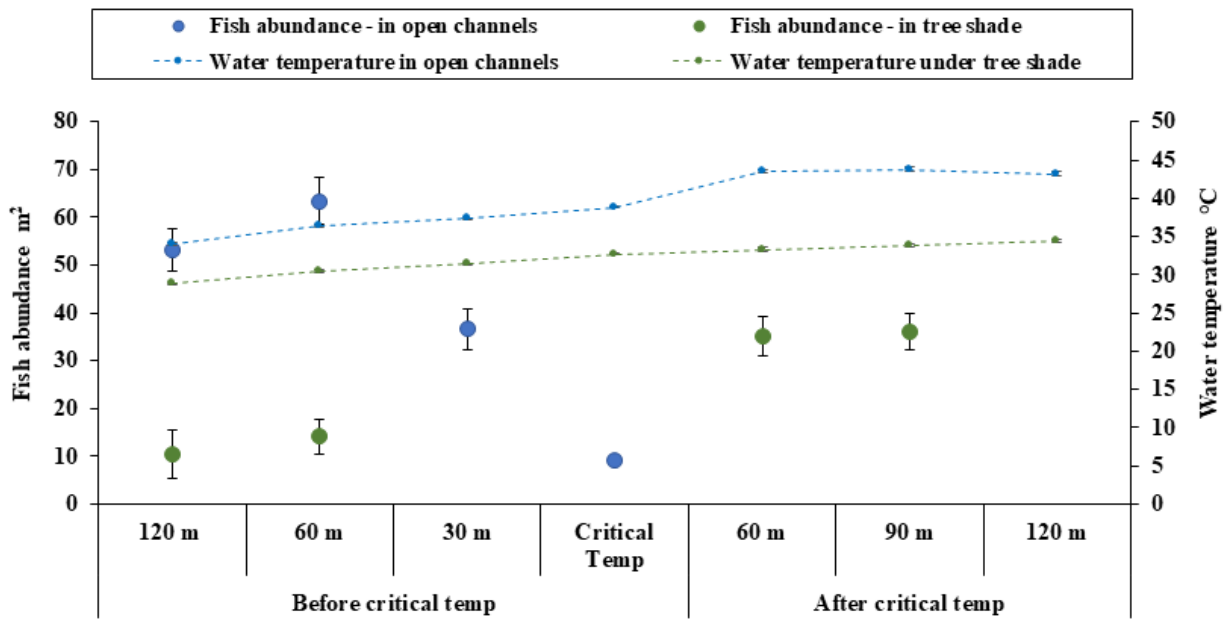
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3 Fig. 5: The abundance of mosquitofish (individuals m^{-2}) and water temperatures estimated in open

4 channels and among tree shade areas in Soliman Bay (mean ± SE), recorded 120 minutes before and
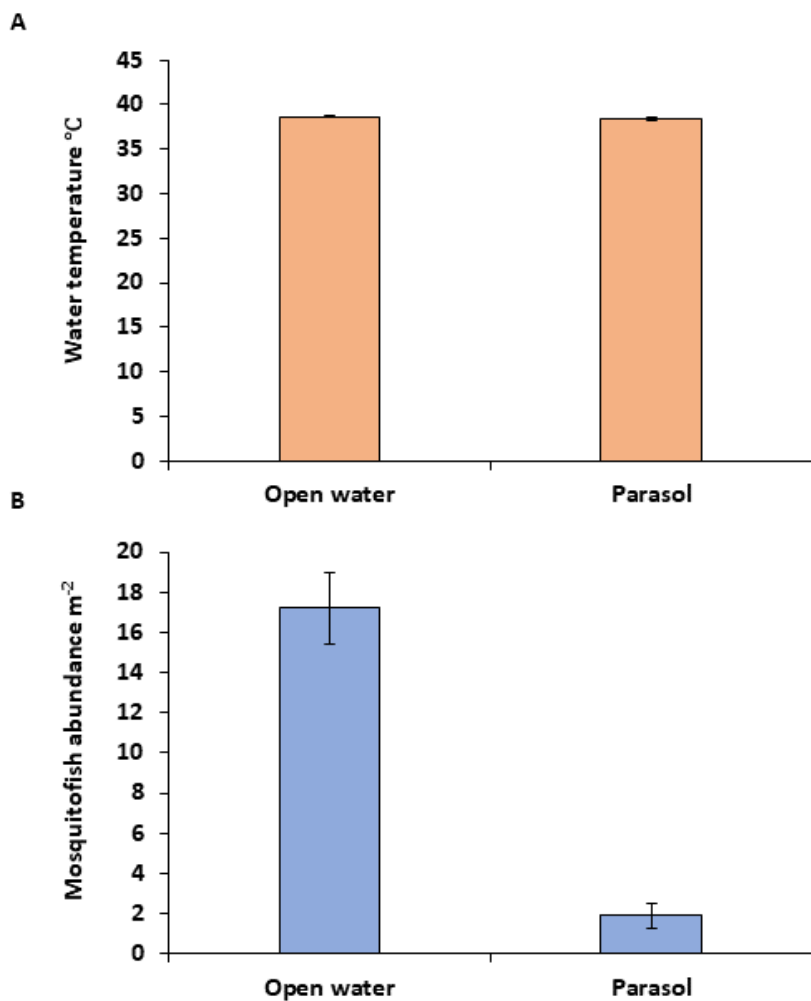
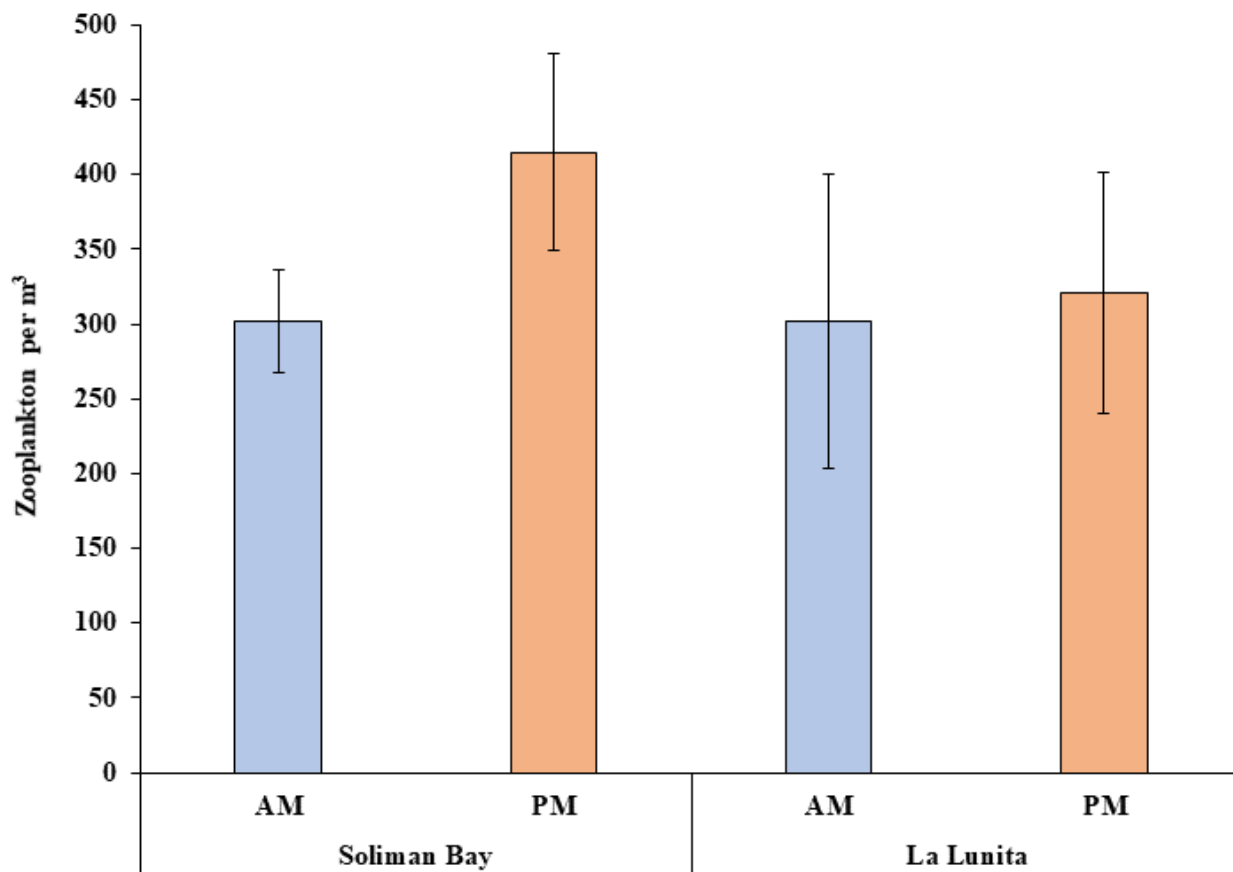5 after critical temperatures were reached.

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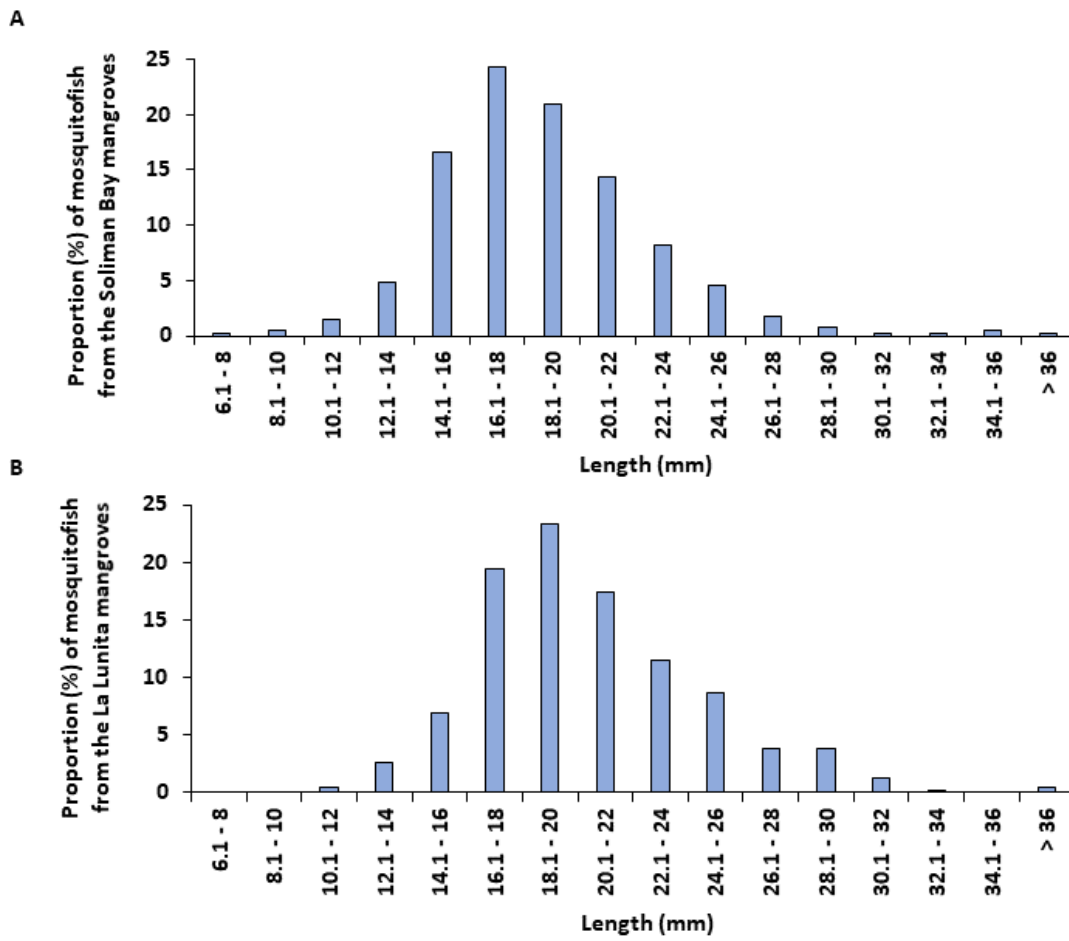

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Fig. 6. A) Water temperatures and B) distributions of mosquitofish (Paired t-test: $P \geq 0.05$ and $P \leq 0.001$, respectively, mean ± SE) in open water and in shaded parasol areas in Soliman Bay observed during afternoon hours.

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Fig. 7. Zooplankton abundance estimated from 6 plankton tows in the Soliman Bay and La Lunita

2 mangrove forests (mean ± SE, two-way ANOVA, $P \geq 0.05$).

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Fig. 8: Estimated lengths (mm) of mosquitofish caught from four nettings within the, (A) Soliman Bay ($n = 391$), and (B) five nettings from La Lunita ($n = 391$) mangrove forests.

Fig. 9. A. Egrets feeding on thermally stressed mosquitofish, B. An attacked and dead mosquitofish, and C. Highlighted in red, mosquitofish burying into sediment when channel water temperatures reached more than 43 ˚C during the afternoon in the Soliman Bay mangrove forest.