Mosquitofish avoid thermal stress by moving from open water to the shade of the mangrove *Rhizophora mangle*

Ian W. Hendy[1,2,*], Owen Burt[3], Sarah Clough[4], Laura Young[5], Simon M. Cragg[1]

[1]**Institute of Marine Sciences, School of Biological Sciences, University of Portsmouth, Ferry Road, PO4 9LY, UK**
[2]**Blue Marine Foundation, South Building, Somerset House, The Strand, London, WC2R 1LA, UK**
[3]**Engineering and the Environment, University of Southampton, Highfield, Southampton, Hampshire, SO17 1BJ, UK**
[4]**Marine Science and Technology, Armstrong Building, Newcastle University, Newcastle Upon Tyne, NE1 7RU, UK**
[5]**Geography and Environmental Science, School of Social Sciences, University of Dundee, Nethergate, Dundee, DD1 4HN, Scotland, UK**

ABSTRACT: Mangrove trees provide environmental buffering for animals by reducing daytime water thermal maxima. Shade from *Rhizophora mangle* trees reduces thermal stress for the mosquitofish *Gambusia affinis*. Data were collected from mangrove forests in Quintana Roo, Mexico, at 2 sites: (1) Soliman Bay, a mangrove forest decoupled from direct ocean water, and (2) La Lunita with semi-direct access to ocean water. During cooler morning hours at Soliman Bay, large numbers of mosquitofish foraged in open channels. At the same time, few mosquitofish utilised shaded areas within the *R. mangle* prop roots. When channels exceeded water temperatures above 38°C, mosquitofish migrated into the shaded areas provided by *R. mangle* trees. Channel water reached a daytime maximum temperature of 46°C, while daytime-shaded *R. mangle* areas remained 6.2°C cooler. Temperature rise and abundance shifts were not found in La Lunita, which has water temperatures of 27 to 28°C throughout the day. Size distributions between both localities showed mosquitofish caught in Soliman Bay to be >10% smaller than the mosquitofish from La Lunita, which had a greater abundance of mature adults. In Soliman Bay, mosquitofish were small, and the thermal stresses may impose serious developmental impacts that would be reduced by occupying water shaded by *R. mangle*. Refuge from *R. mangle* provides environmental buffering for fish, and future increasing water temperatures may reveal a greater use of this little-known mechanism. This study provides an example of a mechanism whereby mangroves support intertidal biodiversity through environmental buffering.

KEY WORDS: Biodiversity mechanism · Mangroves · Thermal tolerance · Fish autecology

1. INTRODUCTION

Mangrove forests are important marginal habitats that provide physical and environmental protection for the resident fauna (Laegdsgaard & Johnson 2001, Hendy et al. 2014). They provide a range of ecosystem services such as erosion reduction, carbon sequestration and biodiversity maintenance (Donato et al. 2011, Hendy et al. 2014, Duke & Schmitt 2015, Eddy et al. 2016). These ecosystem services are being reduced due to the loss of mangrove forests, with increasing ecological and economic consequences for the fauna and people who rely on them (Alongi 2002, Duke et al. 2007).

Impacts from altering the mangrove hydrology by building roads across mangrove habitats has caused

some mangrove systems to become decoupled or semi-decoupled, depending on whether connection to open ocean water has been completely or partially lost (Teutli-Hernández & Herrera-Silveira 2018). These same impacts have been imposed on the mangrove sites in this present study. The loss of connection may lead to reduced biodiversity due to raised water temperatures and reduced dissolved oxygen (Lewis et al. 2016).

Over the last 30 yr, increases of the sea surface temperatures (SST) have been observed, with estimates of an average increase of almost 4°C by the year 2100 (Aral & Guan 2016, Maulvault et al. 2017). Increases of SST may lead to behavioural, physiological and developmental problems for marine fish (Pershing et al. 2015, Jeffries et al. 2016, Maulvault et al. 2017). The rapid warming in the Gulf of Maine led to a decline in Atlantic cod *Gadus morhua* recruitment due to an increase in heat-induced mortalities (Pershing et al. 2015). Californian longfin smelt suffer cellular stress due to an upregulation of heat shock proteins when exposed to temperatures beyond their zone of tolerance (Jeffries et al. 2016), and a range of estuarine fish have been shown to suffer from oxidative stress when thermally impacted (Madeira et al. 2013). Thus, with increasing concern for future fisheries, the inability of marine fauna to adapt or survive to rising temperatures will create significant impacts from species to population levels and global scales (Pörtner & Knust 2007). Distributions of some pelagic fish species are migrating poleward to cope with SST rise (Doney et al. 2012, Pershing et al. 2015). Lack of thermal adaptation may be important for tropical species living within narrow thermal ranges (Munday et al. 2017). Coupled with losses of habitat complexity and ecosystem services, the continued loss of mangrove forests (Duke et al. 2007, Teutli-Hernández & Herrera-Silveira 2018) may see dramatic declines of terrestrial fauna (Rog et al. 2017) and fish (Muzaki et al. 2017) leading to declines of future fauna biomass for mangroves and adjacent fisheries. The loss of species and populations driven by a warming climate is a global concern (Wiens 2016). Critical thermal limits can deplete genetic diversity, have cascading effects on other species, facilitate trophic collapse, and alter ecosystem functioning and services (Luck et al. 2003, IPCC 2014). Critical thermal limits will impact many organisms, particularly in the marine environment (Pinsky et al. 2019).

A key mangrove ecosystem service is its nursery function (Laegdsgaard & Johnson 2001, Lee et al. 2014, Duke & Schmitt 2015, Muzaki et al. 2017), which benefits reef fish populations by enhancing development and maturation of juveniles within the complexity of root systems that provide shelter from predators (Laegdsgaard & Johnson 2001, Hendy et al. 2013). Mangrove roots also buffer wave energy (Ismail et al. 2012) and oxygenate the sediments (Scholander et al. 1955). But little is known of other key biodiversity maintenance mechanisms in mangrove forests, particularly from environmental buffering. Hendy et al. (2013, 2014) found a little-known biodiversity mechanism inside fallen wood within Indonesian mangrove forests. They showed that evaporative cooling within abandoned teredinid tunnels in wood benefited a wide range of terrestrial and aquatic juvenile animals. The environmental buffering delivered by cooling wood benefits many juvenile and vulnerable animals including heat-tolerant fish, octopods, spiders, crabs and polychaetes (Hendy et al. 2013, 2014), and temperatures inside tunnelled wood were 14°C cooler than ambient temperatures (Hendy et al. 2013). In this study, we report another example of behaviour that exploits the cooler parts of the mangrove ecosystem: mosquitofish move to the shade of *Rhizophora* trees when temperatures in open water reach stressful levels.

The mosquitofish *Gambusia affinis* is a brackish-water tolerant poecilid fish possessing a native range that stretches across the USA, Mexico and parts of the Caribbean (Lee et al. 2017). Introduced to some temperate regions as a biological control against mosquito larvae, this viviparous fish now has a global distribution (Lee et al. 2017). Known for expressing high phenotypic plasticity, this adaptable fish has been used across a broad 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 2 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
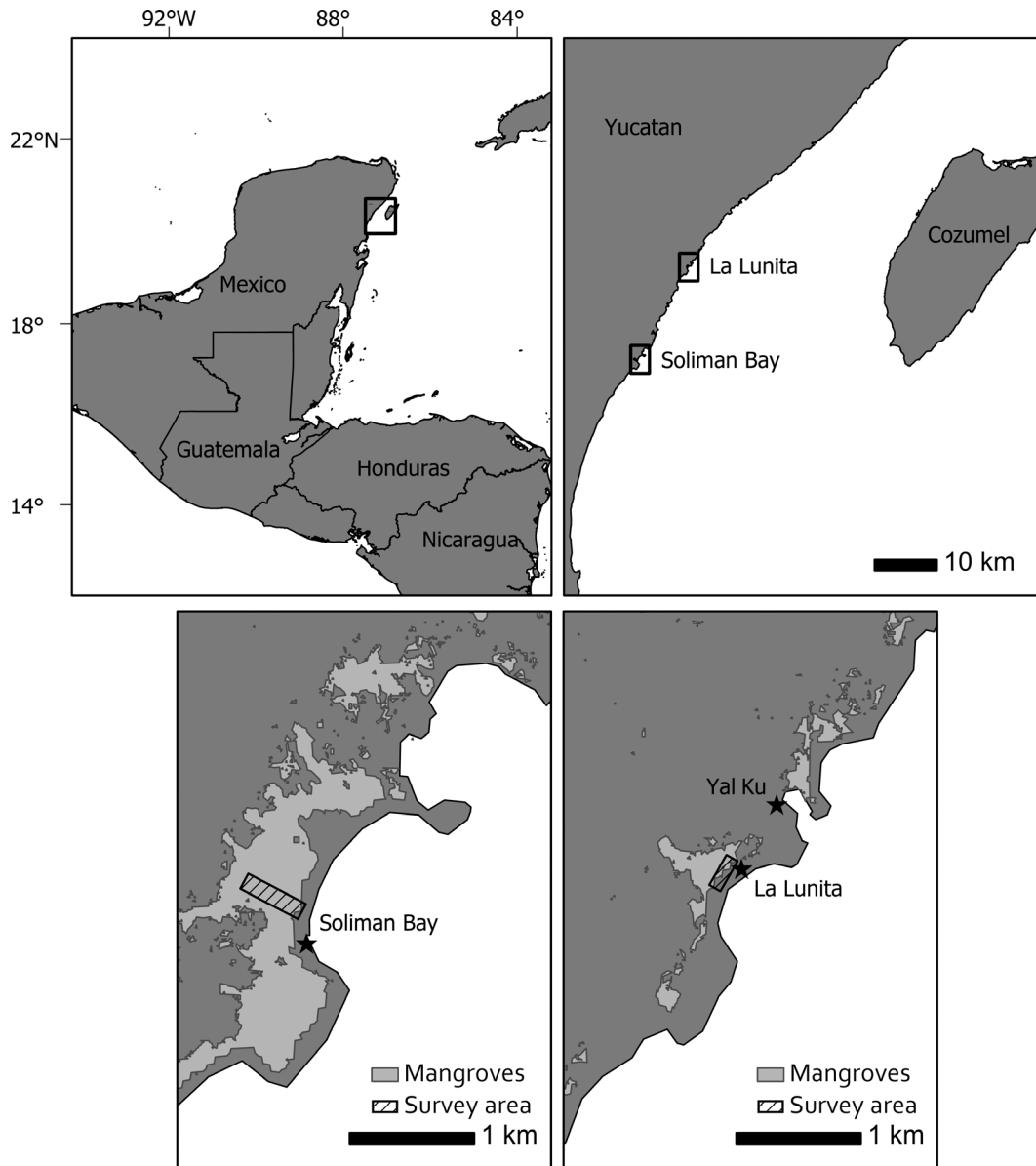
Fig. 1. Country, region and site-specific scale mangrove localities. The area surveyed within each mangrove forest is highlighted

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 and (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

2 mangrove systems, (2) characterise behaviour of mosquitofish and evaluate the significance of this behaviour for fish autecology, (3) assess food availability in both mangrove forests at morning and afternoon hours using plankton tows and (4) quantify mosquitofish size distributions across 2 mangrove forests.

2. MATERIALS AND METHODS

2.1. Sites used in the investigation

Observations of fish behaviour were made in 2 *Rhizophora mangle*-dominated mangrove forests in

Quintana Roo, Mexico. The Soliman Bay dwarf mangrove forest (Fig. 1, 380 ha, 20° 16′ 44.18″ N, 87° 22′ 55.32″ W) has a canopy height generally <1.5 m, but at the strandline, which was identified by the accumulation of buoyant plant detritus at the upper limit of tidal reach, has stands of *Conocarpus erectus*, *Laguncularia racemosa* and *Avicennia germinans*. Beginning at the strandline, mono-specific stands of dwarf *R. mangle* extend across the whole 380 ha of mangrove forest area. which is only connected to the sea via seepage through a sand bar and possibly via subterranean cenote connections. The other site is a higher canopied mangrove forest at La Lunita (Fig. 1, 2.9 ha, 20° 24′ 26.5″ N, 87° 18′ 27.5″ W), which has connections to the sea via a tidal creek that enters Yal Kul lagoon and via cenote-connected subterranean channels.

The lack of a channel connecting the Soliman Bay mangrove forest with the sea means that the fish population there is usually unable to move beyond the forest. The contained water there is micro-tidal (±0.5 m) (Teutli-Hernández & Herrera-Silveira 2018) with limited water flow and input via seawater permeating the sand bar and from rainwater, resulting in brackish water, ranging from 10 to 17 practical salinity units (psu). A channel runs through the forest with water depth ranging from 15 to 40 cm. Water temperature can reach up to 46°C and fluctuate by up to 20°C in a 24 h period. Dissolved oxygen (DO_2) is low.

During morning hours in the Soliman Bay mangrove, channels were populated by large numbers of mosquitofish *Gambusia affinis* Baird & Girard, 1853 (Fig. 2) which were observed foraging for food. During afternoon hours, almost all fish had vanished from those channels. We believe this sharp shift in behaviour is prompted by an extreme temperature change and that mosquitofish populations in the Soliman Bay mangrove forest seek the shade offered by *R. mangle* stands to avoid thermal stress.

At the La Lunita mangrove forest strandline in the Yal Ku lagoon, *C. erectus*, *L. racemosa*, *A. germinans*, and *R. mangle* trees were found, but most of the forest consists of *R. mangle* reaching heights of up to 20 m (Fig. 3). The Yal Ku lagoon has a semi-decoupled water system with subterranean cenote connections (Gabriel et al. 2008). The tidal regime is also microtidal. Cenote water in La Lunita is deep, reaching approximately 4 to 5 m. Salinity was low,
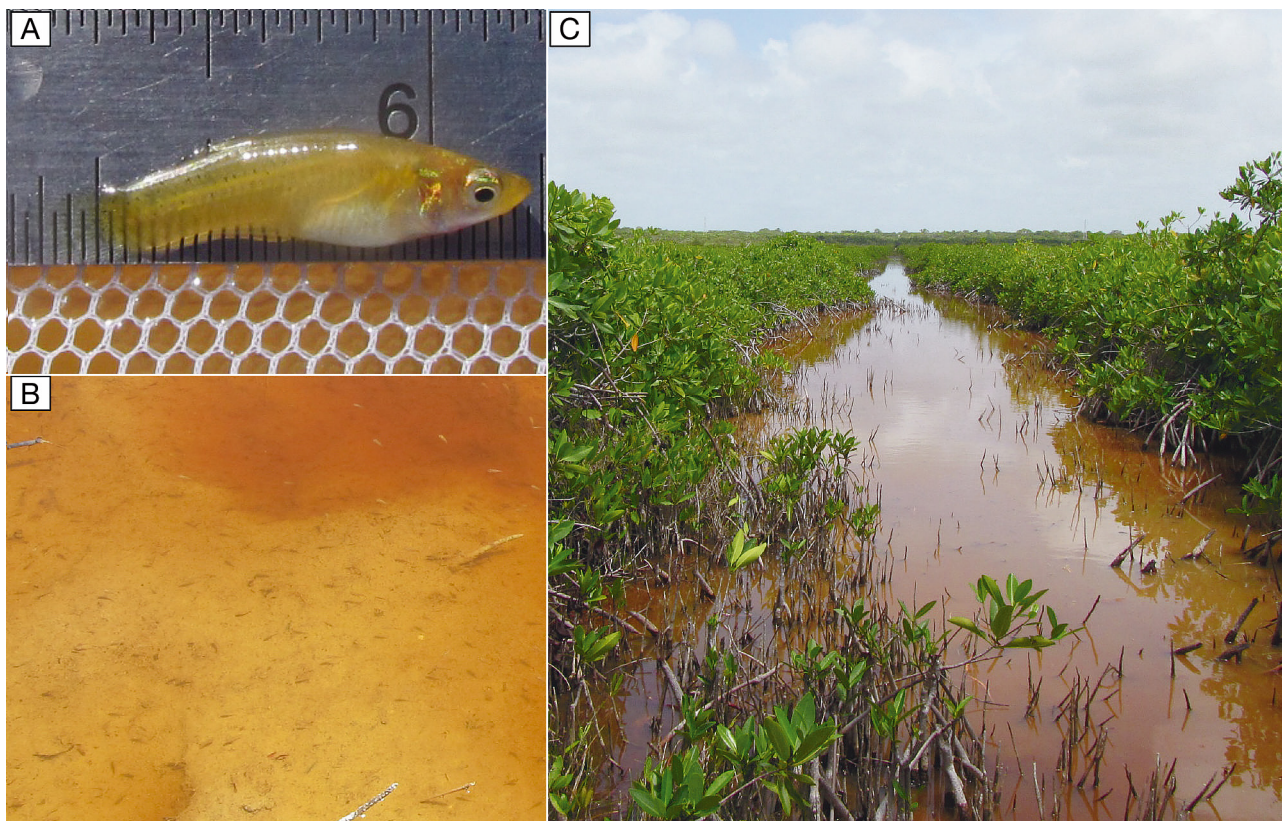

Fig. 2. Soliman Bay mangrove forest, Tulum, Mexico. (A) Adult female mosquitofish, (B) dense shoals of mosquitofish foraging for food in open channels, and (C) the main channel in the Soliman Bay mangrove forest where large numbers of mosquitofish forage for food in the morning
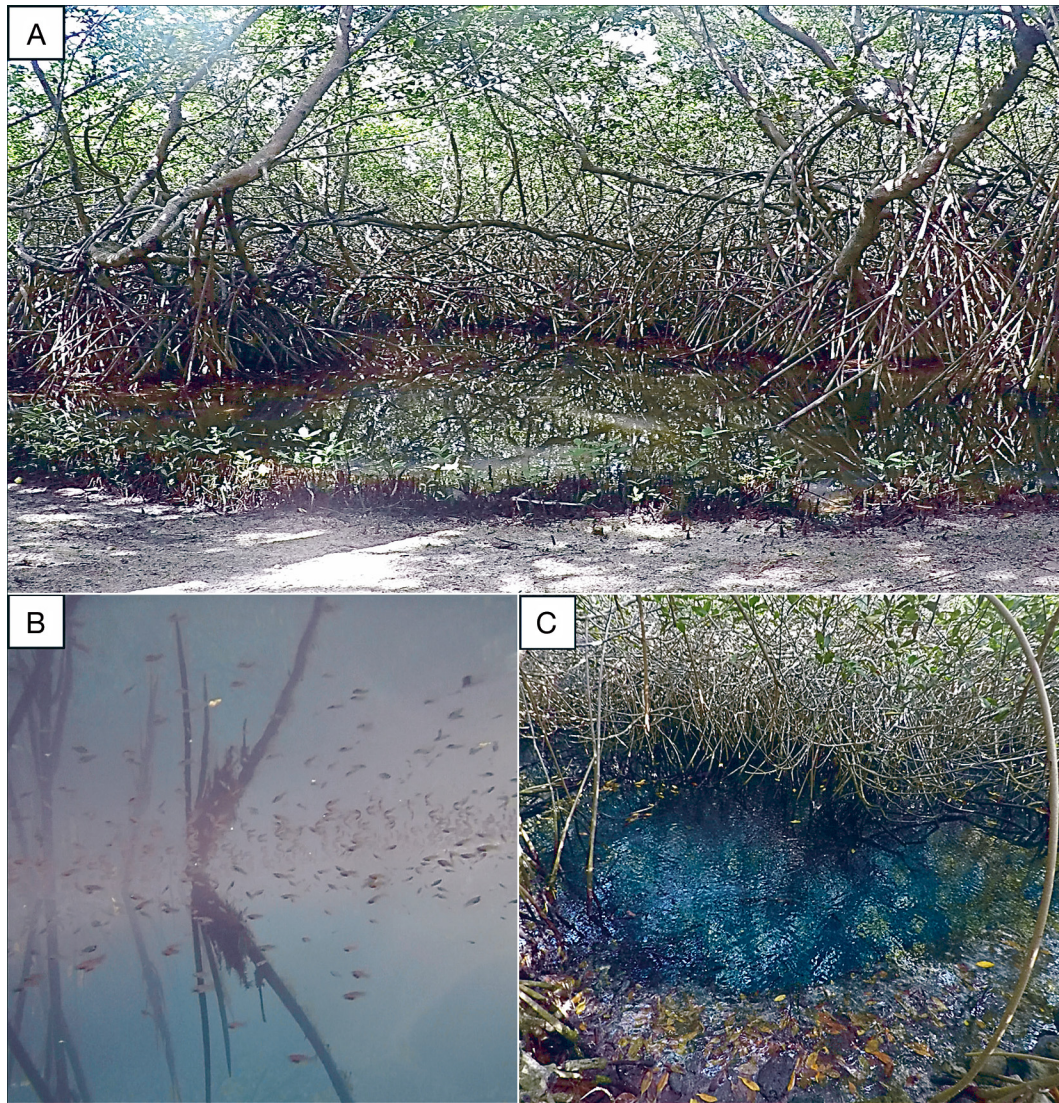
Fig. 3. La Lunita mangrove forest, Tulum, Mexico. (A) Strand line channel lined with large *Rhizophora mangle* trees, (B) dense shoals of large mosquitofish foraging for food in open channels and *R. mangle* roots, and (C) one of many cenote openings with deep flowing water

ranging from 4 to 6 psu. Water temperature was consistent throughout the day, ranging from 27 to 28°C. A cut path that on the landward side of the strandline of the forest gave access to cenote openings above which mosquitofish were sampled to determine the population size distribution. This path was also used for observing fish behavior and measuring water temperature.

The semi-open system of La Lunita means that aquatic animals are less restricted in their ability to migrate out of the forest and the freer water circulation. Consequently, the environmental pressures found in Soliman Bay are not replicated in La Lunita. Large numbers of mosquitofish were also found here.

2.2. Behaviour and water variables measurements

At Soliman Bay, mosquitofish first occurred at 20 m from the strand line, and their abundance was estimated in open channels and in shaded areas of the *R. mangle* stands at stations between 20 and 200 m from the strandline. Surveys were conducted during morning (08:00 to 10:00 h) and afternoon (13:00 to 15:00 h) hours using 1 m^2 quadrats every 3 to 5 m along transect lines. After positioning quadrats, a 5 min period was left to pass prior to estimating fish numbers, allowing fish to acclimatise to the disturbance. Measurements were then made every 10 min.

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

R. mangle prop-root stands at 0 to 70 m from the strandline. Surveys were also conducted during morning (8:00 to 10:00 h) and afternoon (13:00 to 15:00 h) hours every 3 to 5 m along the transect lines. Due to practical constraints of water depth, estimates of mosquitofish abundance per m^2 were made by observing 1 m^2 areas of water surface.

Water temperatures at Soliman Bay were measured at 3 stations placed at 50, 100 and 150 m from the strandline. At each station, measurements were made in the open water and in the shade of the *R. mangle* canopy (±0.5°C, 30 min intervals). A total of 6 HOBO Pendant® Temperature/Light 64K Data Loggers were deployed for these measurements.

Water temperature recordings at La Lunita were also made in open water (exposed) and shaded areas, using LCD digital thermometers with submersible probes (www.tester.co.uk). Recordings were taken every 5 m from the strandline out to 70 m over a 6 d period in the morning (08:00 to 10:00 h) and afternoon (13:00 to 15:00 h) hours.

2.3. Fish behaviour in shaded and unshaded areas

Mosquitofish abundance was monitored at 3 stations in Soliman Bay starting at 10:30 h. Over a 6 d period, observations were made at 50, 100 and 150 m along the strandline. Each day before recordings took place, a 10 min interval was left to allow for any disturbed fish to acclimatise and for any disturbed sediment to settle. To establish mosquitofish distributions in the mangrove forests, water temperature (°C) in open channels was measured with an LCD digital thermometer with submersible probe, and mosquitofish abundance (per m^2) and in-channel water temperatures were concurrently recorded. Water temperature measurements and fish observations were recorded over a 4.5 h period or until channel water temperature failed to increase for ≥2 readings.

To establish whether mosquitofish at Soliman Bay were actively seeking cooler water provided by the *R. mangle* shade or simply seeking 'dark' cover, we positioned 3 parasols (3 m round parasol/umbrella, 6 spoke, Ikea Karlso) at 50, 100 and 150 m from the strandline in the open water channel during afternoon hours (12:00 to 14:30 h). Parasols were secured by driving their poles into channel sediment. Mosquitofish abundance was recorded every 10 min in the shade of the adjacent *R. mangle* prop-stands and in the cover of the parasol-shaded area. Concurrent water temperature measurements were also taken from within the open channel and in the cover of the parasol-shaded areas.

Salinity was measured in practical salinity units (psu) using a Bellingham and Stanley refractometer (https://refractometershop.com/) and dissolved oxygen was measured using a Tetra Oxygen Testing kit measuring in milligrams per litre (DO$_2$ mg l^{-1}). Concentrations of psu and DO$_2$ were measured at 10 locations in Soliman Bay situated every 20 m from the strandline and 10 locations in La Lunita every 7 m from the strandline.

2.4. Food availability

Mosquitofish diets contain large concentrations of zooplankton (Hurlbert & Mulla 1981). Crivelli & Boy (1987) found that some mosquitofish stomach contents consisted of >80% planktonic crustaceans. Thus, plankton tows were used to assess food availability within open water channels at both localities during morning and afternoon hours. The plankton net had a frame diameter of 300 mm, net length of 880 mm and mesh filter size of 250 µm. Tow-lines were pulled for 5 m in open water channels at 3 locations from the strandline (Soliman Bay: 50, 100 and 150 m and La Lunita: 0, 35 and 70 m). In each locality, ~1 m^3 of water was sampled for plankton diversity and abundance. A total of 6 samples were collected from each location and stored in 100 ml vials with water from the study site. Each sample was gently shaken, and 10 ml of sample water was extracted from each 100 ml vial using a Stempel pipette and analysed. Numbers were extrapolated to represent abundance of plankton per 1 m^3 of mangrove water between localities. Planktonic communities in the samples were examined at a magnification of × 40 using a stereo microscope.

2.5. Size distribution

The seine-net haul technique was used in random locations from the strandline and further out at both localities to determine mosquitofish size distributions (±0.1 mm; standard length from the anterior tip to the caudal peduncle). The seine-nets used had a mesh size smaller than the diameters of juvenile mosquitofish. Four seine-net hauls were undertaken to capture mosquitofish at Soliman Bay, and 5 seine-net hauls were undertaken to capture mosquitofish at La Lunita. Photographs of each of the hauls were taken using a Nikon Coolpix A100. Images were analysed using the digital analysis package ImageJ.

Pooled sample sizes of 391 fish from each study site (391 fish were caught at 1 site and 391 were selected at random from the other site) were used to distinguish mosquitofish size distributions (https://imagej.nih.gov/ij/).

2.6. Statistical analyses

Temporal and spatial differences of fish distributions, and temperature (°C) differences from the quadrat data were examined using a general linear model (GLM) with time of day and habitat (between roots or out in open channel) used as factors. Differences of temperature and fish abundances in Soliman Bay were examined using a GLM, with distance from the strandline and time as factors between sites and parasol cover compared to open channels. Paired t-tests were used to examine differences of temperatures measured in the open channels with temperatures measured within the shade of the *R. mangle* trees; mean morning and afternoon temperatures and mean morning and afternoon fish abundance in both study locations; and dissolved O_2 and salinity measurements on the dimensionless practical salinity scale from both study locations. Two-way analysis of variance (ANOVA) was used to analyse variations in food availability in both study locations during morning and afternoon hours with site and time of day (morning and afternoon) as factors. A 1-way ANOVA was also used to examine relationships before and after temperatures reach their critical thermal limit for the mosquitofish in the Soliman Bay mangrove open-water channels and shaded areas. All count data were square root transformed, and all measurement data were log transformed, with the suitability of the transformations scrutinised by examining residuals. Post-hoc, descriptive statistics and Tukey's pairwise comparison tests were used to separate values into statistically distinct subsets in all ANOVAs. All statistical analyses were performed using MINITAB (v.13.20).

3. RESULTS

3.1. Fish abundance

During morning hours in Soliman Bay, the greatest abundance of mosquitofish was found in the open water channel. In the afternoon, mosquitofish abundances were significantly greater in the shade amongst the roots of the *Rhizophora mangle* trees (Fig. 4A: GLM, area [channel and roots] and time [am and pm] vs. fish abundance: area vs. fish abundance: $F_{1,87} = 8.5$, p ≤ 0.01; time vs. fish abundance: $F_{1,87} = 39.1$, p ≤ 0.001; and area × time vs. fish abundance:
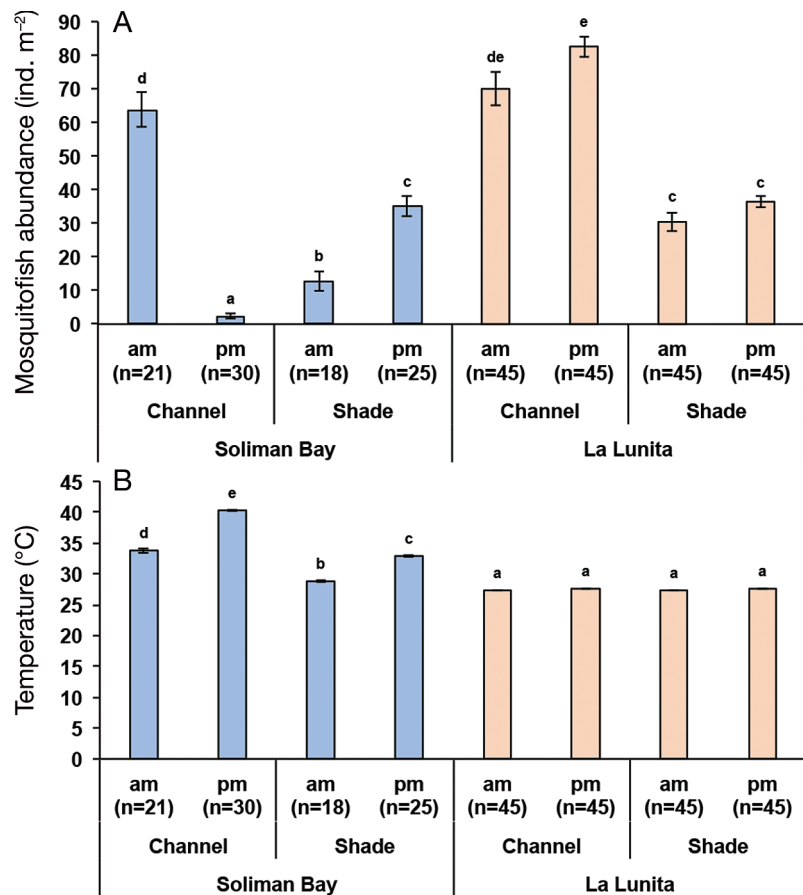

Fig. 4. Temporal and spatial variations of mosquitofish abundance and water temperature in open channels and prop root-shaded areas in the Soliman Bay and La Lunita localities: (A) mosquitofish abundances (ind. m^{-2}, mean ± SE) during morning (am) and afternoon (pm) hours in open water channels and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

$F_{1,87} = 177.2$, p ≤ 0.001). Water temperature (°C) in the open channel was significantly warmer during the afternoon (12:00 to 18:00 h) compared to water temperatures recorded from within the adjacent *R. mangle* tree shaded areas at the same time.

Water temperatures in Soliman Bay during afternoon hours were on average > 6°C cooler in the *R. mangle* tree stands than in open-water channel temperatures. Afternoon water temperature measurements within *R. mangle* shaded areas were only 1.7°C warmer than temperatures measured in the adjacent channels during morning hours (06:00 to 12:00 h) (Fig. 4B: GLM, area vs. temperature: $F_{1,87} = 799.2$, p ≤ 0.001; time vs. temperature: $F_{1,87} = 606.2$, p ≤ 0.001; and area × time vs. temperature: $F_{1,87} = 28.5$, p ≤ 0.001).

Mosquitofish abundance in La Lunita remained high in open water channels throughout both morning and afternoon hours. Abundance of mosquitofish increased in the open water channels and among the *R. mangle* prop-root stands from morning hours (GLM, area vs. fish abundance: $F_{1,176} = 170.3$, p ≤ 0.001; time vs. fish abundance: $F_{1,176} = 7.9$, p ≤ 0.01; and area × time vs. fish abundance: $F_{1,176} = 1$, p ≥ 0.05). Water temperatures in La Lunita remained the same in the open channels and between *R. mangle* prop-roots. Morning and afternoon water temperatures remained constant, only changing by an increase of 0.3 ± 0.9°C (mean ± SE) from morning to afternoon hours (GLM, area vs. temperature: $F_{1,176} = 0$, p ≥ 0.05; time vs. temperature: $F_{1,176} = 79.7$, p ≤ 0.001; and area × time vs. temperature: $F_{1,176} = 0$, p ≥ 0.05).

3.2. Mosquitofish behaviour

The open-water mosquitofish abundance in Soliman Bay significantly dropped across all 3 monitoring stations on 6 different sampling days. Areas further away from the strandline in Soliman Bay became warmer later in the day compared to shallower waters closer to the strandline. Mosquitofish avoided the extreme water temperatures by swimming to the cooler areas of the open channels further out. This resulted in areas closer to the strandline having fewer mosquitofish compared to areas further out from the strandline as time passed.

In the open channels of Soliman Bay, mosquitofish abundance dropped by >90% above an average (±1 SE) water temperature of 38.8 ± 0.1°C (Fig. 5A). Thus, when channel water temperatures exceeded 38°C during afternoon hours, a large shift in mosquitofish abundance was recorded, with 44.1 ± 2.8 ind. m^{-2} (mean ± SE) before water temperatures reached critical limits reducing to 10.4 ± 1.1 ind. m^{-2} after critical water temperatures were reached. Declines above the critical water temperature in open channels occurred at all stations on all days (ANOVA, temperature before and after critical thermal temperature vs. mosquitofish abundance in open water channels: $F_{1,327} = 107.1$, p ≤ 0.001).

After 14:00 h, mean water temperatures in the open water channel were almost 10°C hotter compared to morning water temperatures. Water temperatures were highest at the 50 m station (GLM, time vs. temperature: $F_{2,135} = 170.7$, p ≤ 0.001; distance from the strandline vs. temperature: $F_{2,135} = 45.2$,
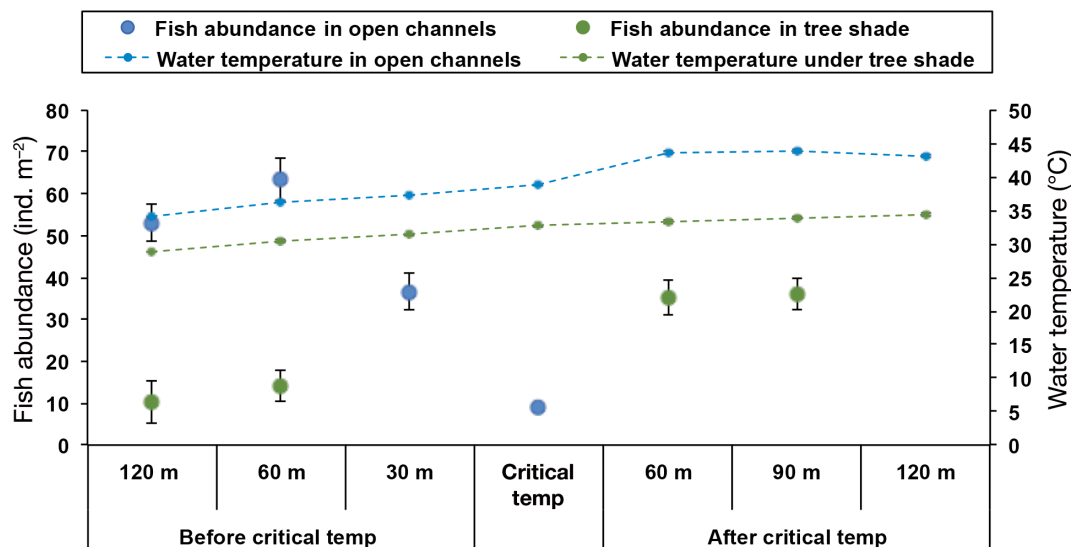

Fig. 5. Abundance of mosquitofish (ind. m^{-2}) and water temperatures estimated in open channels and among tree shade areas in Soliman Bay (mean ± SE), recorded 120 min before and after critical temperatures were reached

$p \leq 0.001$; and time × distance from the strandline vs. temperature: $F_{4,135} = 1.02$, $p \geq 0.05$). All open-water Soliman Bay mangrove forest areas showed similar temperature increases between days. Mosquitofish abundance (mean ± SE) in the shade of the *Rhizophora* trees (35.1 ± 2.6 in m^{-2}) was significantly greater after open water reached critical temperatures (Fig. 5B) compared with mosquitofish abundance in the shade of the trees before open water temperatures reached critical temperatures (13.3 ± 2.5 fish m^{-2}; ANOVA, temperature before and after critical thermal temperature vs. mosquitofish abundance in shaded areas: $F_{1,52} = 28$, $p \leq 0.001$). Burying behaviour and an increase of air-gulping behaviour by the mosquitofish in Soliman Bay was observed when water temperatures reached > 40°C.

No large shifts of mosquitofish abundance in La Lunita were found in the open water channels throughout morning and afternoon hours. Mosquitofish abundance increased by ~20% from morning to afternoon hours within the open water channels (GLM, time vs. fish abundance: $F_{1,84} = 4.8$, $p \leq 0.05$; distance from the strandline vs. fish abundance: $F_{2,8} = 1.6$, $p \geq 0.05$; and time × distance from the strandline vs. fish abundance: $F_{2,84} = 1.5$, $p \geq 0.05$). No difference of fish abundance was found between the 3 stations.
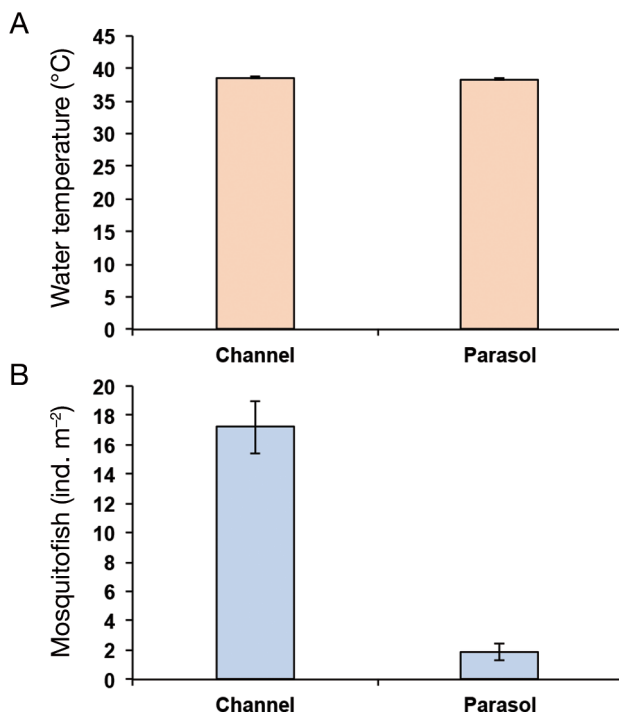

Fig. 6. (A) Water temperatures and (B) distributions of mosquitofish (paired *t*-test: $p \geq 0.05$ and $p \leq 0.001$, respectively, mean ± SE) in open water and in shaded parasol areas in Soliman Bay observed during afternoon hours

3.3. Parasol shaded area

Mosquitofish abundance (mean ± SE) was significantly higher in the open channels at Soliman Bay (17.2 fish ± 1.8 fish m^{-2}) compared to mosquitofish abundance under shading provided by parasols (1.9 fish ± 0.6 fish m^{-2}) during afternoon hours (Fig. 6A,B, paired *t*-test: $p \leq 0.001$, n = 30. Water temperatures in the parasol-shaded areas (38.4 ± 0.1°C) matched those of the ambient open water channel (38.6 ± 0.1°C; n = 30).

Salinity (mean ± SE) concentrations at Soliman Bay were 11.5 ± 0.8 psu and were higher than those in La Lunita of 5.8 ± 0.2 psu (paired *t*-test: $p \leq 0.001$, n = 10). Dissolved O$_2$ readings were significantly lower in the Soliman Bay water channels, 5.4 ± 0.7 mg l^{-1}, compared with La Lunita water channels, 11 ± 0 mg l^{-1} (paired *t*-test: $p \leq 0.001$, n = 10).

3.4. Food availability

Zooplankton abundance (mean ± SE) in the open water channels was similar between both localities during the morning and afternoon hours (Fig. 7, 2-way ANOVA, site × time vs. zooplankton abundance: $F_{1,8} = 0.3$, $p \geq 0.05$). During the morning in Soliman Bay, there were 301 ± 33.9 zooplankton individuals per 1 m^3, and in the afternoon, there were 415 ± 65.9 ind. m^{-3}. In the La Lunita mangrove forest, there were 301 ± 98.5 ind. m^{-3} in the morning compared with 320.5 ± 80.6 ind. m^{-3} in the afternoon.

3.5. Size distribution

Mosquitofish from Soliman Bay were on average >10% smaller (Fig. 8A) compared to mosquitofish from La Lunita, with mean (± SE) sizes of 18.7 ± 0.2 mm and 21 ± 0.2 mm, respectively (paired *t*-test, $p \leq 0.001$). A greater abundance of mature mosquitofish was found in La Lunita (Fig. 8B). The largest mosquitofish measured in Soliman Bay was 36 mm, and the largest mosquitofish measured in La Lunita was 47 mm.

4. DISCUSSION

Sites were not replicated during this study, so it was not possible to draw firm conclusions about site-specific environmental factors. However, our data reveal that when channel water was warmest in Soliman Bay, the mean water temperature shaded by the
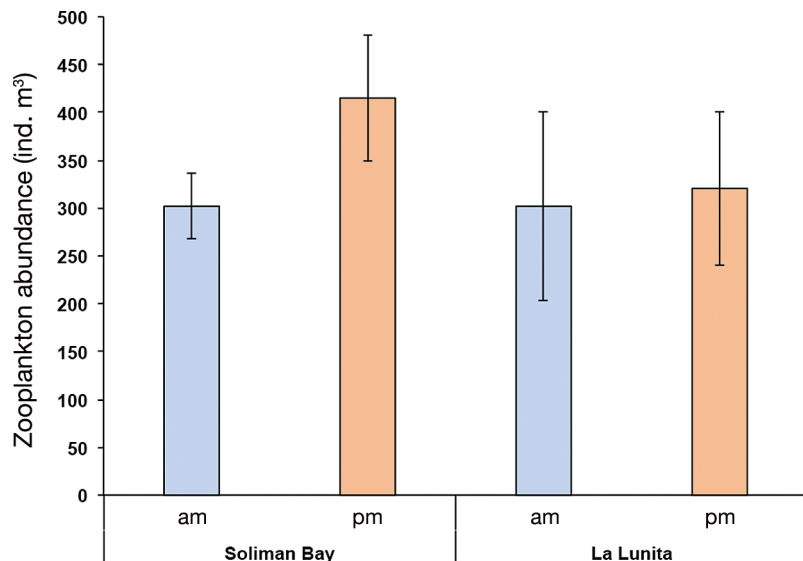
Fig. 7. Zooplankton abundance estimated from 6 plankton tows in the Soliman Bay and La Lunita mangrove forests for morning hours (am) and afternoon hours (pm) (mean ± SE, 2-way ANOVA, p ≥ 0.05)

Rhizophora mangle trees was >6°C cooler, a significant temperature difference for mosquitofish, especially if reduced dissolved oxygen in the open water impacts feeding rates (Chipps & Wahl 2004), reduces growth rates (Wurtsbaugh & Cech 1983) and increases mortalities (Otto 1973). Water temperatures at the La Lunita mangrove forest were driven by daily flushing from inshore coastal water, and the temperature remained constant throughout the day with little change and with a maximum water temperature reaching 28°C. At times, water temperature in Soliman Bay was ~18°C hotter. Such an extreme water temperature will place very high physiological demands on the flora (Alongi 2018) and fauna.

The high abundance of mosquitofish foraging within open water channels at both localities during daytime hours are consistent with fish behaviours observed from other studies (Ling & Willis 2005, Pyke 2005). However, dramatic declines in abundance of mosquitofish within the open channels at Soliman Bay during afternoon hours did not mirror the distribution patterns of mosquitofish observed in La Lunita during the same time. The high abundance of mosquitofish in Soliman Bay

shifted from the open channels during morning hours to cooler *R. mangle* prop-root shaded areas in the afternoon. During the same time, mosquitofish abundance in La Lunita remained the same in open water channels from morning to afternoon hours, and no significant change in mosquitofish abundance was observed. The sharp rise in water temperature observed in Soliman Bay exceeded the critical thermal tolerance for the mosquitofish, which triggered their rapid migrations to seek environmental buffering.

Benoit et al. (2000) report that mosquitofish prefer habitats with lots of cover to avoid predation, but Casterlin & Reynolds (1977) report that mosquitofish do not favour such habitats. Our study highlights that mosquitofish behaviour is driven by water temperature and availability of a mangrove root refuge from predation. Our data are similar to Winkler (1979), who found that mosquitofish prefer water temperatures of 31 to 35°C, avoiding a critical water temperature limit of 39°C (Cherry et al. 1976) and an upper lethal limit of 38°C and above (Otto 1973). Despite the availability of many vegetated areas and complex habitats in Soliman Bay, mosquitofish only appeared to show
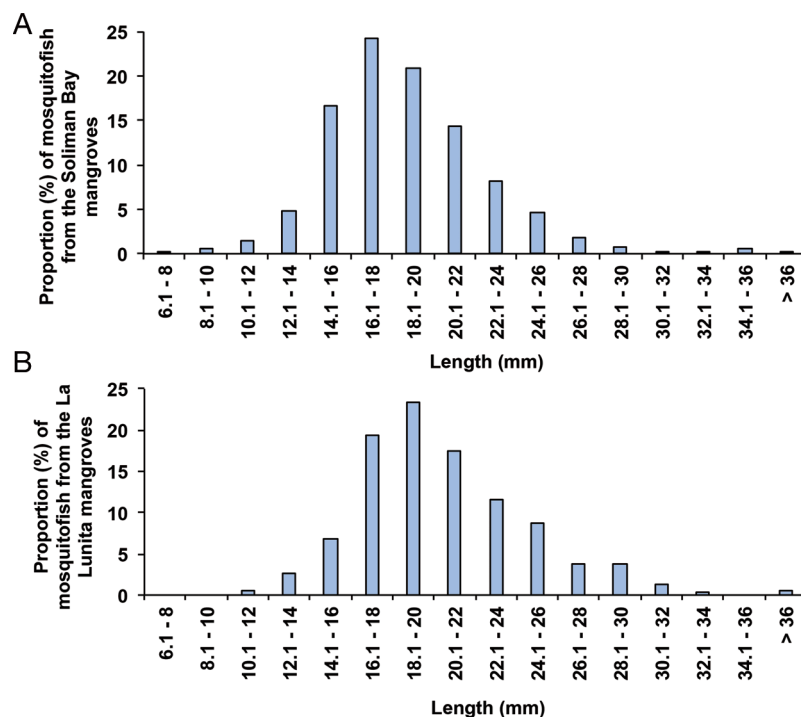

Fig. 8. Estimated lengths (mm) of mosquitofish caught from (A) 4 nettings within Soliman Bay (n = 391) and (B) 5 nettings from La Lunita (n = 391) mangrove forests

preference for these habitats when water temperatures reached ≥38°C during afternoon hours. We found that mosquitofish in Soliman Bay preferred channel water temperature ranges of 35 to 37°C with a maximum thermal tolerance of 38 to 39°C, which agrees with previous studies of mosquitofish (Otto 1973, Cherry et al. 1976, Winkler 1979). Our results demonstrate that mosquitofish are actively seeking areas provided by *R. mangle* prop-roots when open channel water temperatures reach lethal limits of ≥38°C to avoid thermal stress. Prop roots also provide greater in-water complexity at the same time as the canopy provides shade. Antipredator responses could also play a role as predatory birds were often observed. In Soliman Bay, large numbers of egrets fed on thermally stressed mosquitofish showing a loss of equilibrium. During this time, water levels were low, and water temperatures were >43°C (Fig. 9A–C).

Mosquitofish are opportunistic omnivores, consuming algae and zooplankton (Crivelli & Boy 1987). They are known to have extremely high feeding rates (up to 83% of their total body weight per day) when exposed to water temperatures of 10 to 35°C (Wurtsbaugh & Cech 1983, Chipps & Wahl 2004), very similar to the water temperatures at Soliman Bay from 10:00 to 12:30 h. Mosquitofish metabolic rates increase with temperature until DO_2 availability becomes too low (Cech et al. 1985). This may explain the high abundance of mosquitofish in the open channels at the Soliman Bay because the perfect feeding environment for mosquitofish would be between these times. Their appetites were corroborated by the fact that mosquitofish were observed in high abundance in open areas throughout the day continuously foraging for food, particularly on the water surface and occasionally on the surface of the sediment. The optimum feeding temperatures may also explain why mosquitofish abundance in the open water channels at La Lunita increased by almost 20% during afternoon hours when water temperatures reached the ideal conditions for feeding. In addition, mosquitofish in Soliman Bay were not as numerous in the shade of the parasols compared to the open channels and shade of the mangrove trees. Unlike the mangrove prop roots, the parasols provided no additional underwater structure and did not provide any water temperature buffering.

Plankton tows from this study demonstrated that food availability remained constant throughout morning and afternoon hours at both localities and consisted of gastropods and crustaceans. High concentrations of zooplankton were found in all samples, providing further evidence that mosquitofish shifting abundance from open channels to cooler *R. mangle*
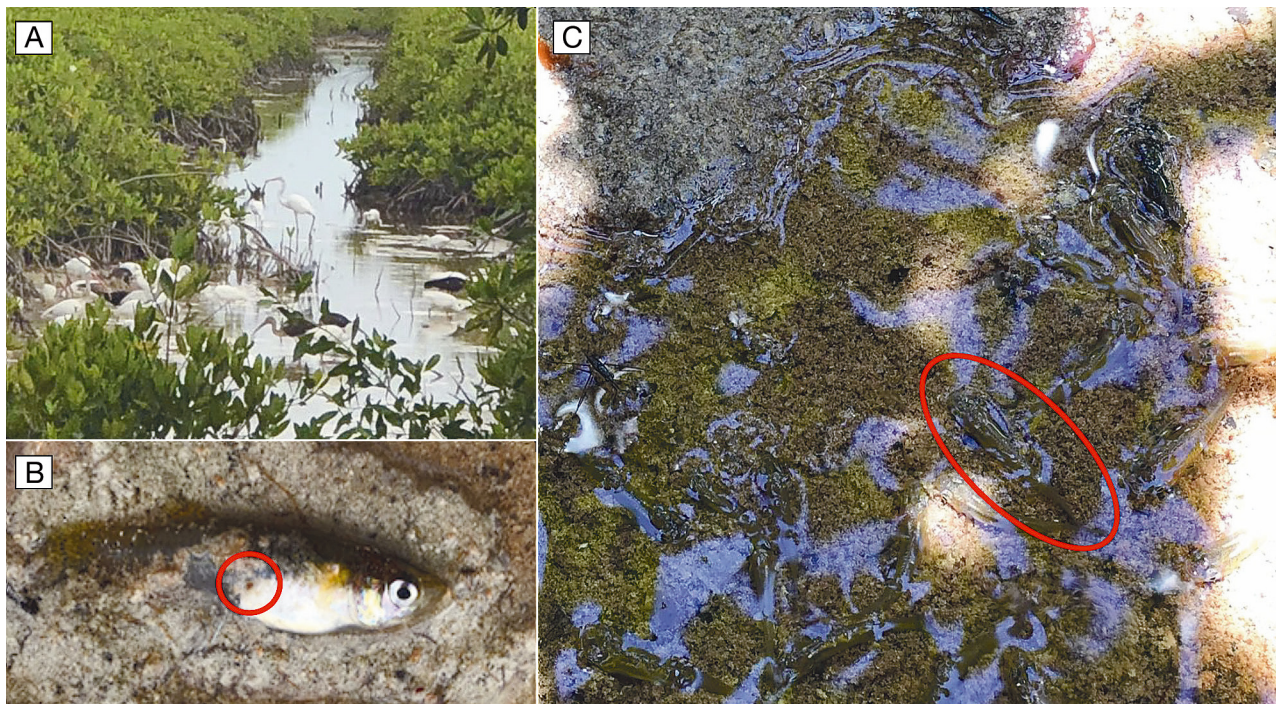

Fig. 9. (A) Egrets feeding on thermally stressed mosquitofish. (B) Dead mosquitofish, attacked area highlighted in red. (C) Mosquitofish (highlighted in red) burying into sediment when channel water temperatures reached >43°C during the afternoon in the Soliman Bay mangrove forest

shaded areas during afternoon hours at Soliman Bay were not driven by food availability but by avoiding extreme thermal stress. The lack of thermal stress and extreme water temperatures in La Lunita may explain why mosquitofish remained foraging in open channels throughout the day, as food was plentiful.

At birth, mosquitofish are between 6 to 8 mm in length and grow at a rate of ~1 to 2 mm wk^{-1} (Stearns 1983). This means that the greatest proportion of mosquitofish caught from both localities were aged between 3.5 and 6.5 wk old. However, mosquitofish populations caught in La Lunita possessed higher ratios of fish aged ≥6.5 wk. Male mosquitofish mature after 8 wk and females mature after 10 wk (Pyke 2005). This means that sexually mature individuals are more abundant in La Lunita. The size of mosquitofish caught in Soliman Bay were on average >10% smaller than fish measured from La Lunita. Different predators and predation pressures coupled with extreme water temperatures in Soliman Bay may have caused changes with the *Gambusia* fish communities (Phenix et al. 2019). Stressful temperatures may have created developmental consequences for juvenile mosquitofish. Studies found that when tropical shallow-water fish are exposed to extreme water temperatures, they exhibit energetic trade-offs to survive the heat stress, such as smaller body sizes, reduced energy reserves and increased rate of protein and lipid depletion (Madeira et al. 2017). Under stressful environments, juvenile and vulnerable individuals divert more energy into dealing with stress instead of gonad and somatic development (Shahjahan et al. 2017, Wang et al. 2017). Such stress can also lead to higher mortalities (Madeira et al. 2017). Without cooler water provided by the *R. mangle* shade, populations of mosquitofish in Soliman Bay would suffer serious thermal stresses combined with increases of mortalities.

Improved fitness levels in mosquitofish are reported to occur at water temperatures of 30°C, with female mosquitofish investing higher proportions of energy into reproduction at average water temperatures of 25°C (Wurtsbaugh & Cech 1983). A similar water temperature environment was found in La Lunita and may explain why the abundance and size distribution of mosquitofish were greater at this locality. Mosquitofish growth can be reduced by lower food availability and by water temperatures above 35°C (Wurtsbaugh & Cech 1983). Due to the extreme water temperatures at Soliman Bay, mosquitofish sacrifice feeding in open water channels in favour of reduced environmental stressors provided by the *R. mangle* stands.

Air-gulping behaviour expressed by mosquitofish was observed in Soliman Bay. This behaviour was previously documented with fish known to survive in DO_2 concentrations as low 0.28 mg l^{-1}, providing they can breathe atmospheric air from surface waters (Pyke 2005). As dissolved oxygen in the general water column approaches zero, mosquitofish survival depends on their ability to gain access to the surface water (Homski et al. 1994). They either take in water that is relatively oxygen-rich at the atmosphere-water interface (Lewis 1970) or gulp air from the atmosphere. Their dorsally oriented mouth and dorso-ventrally flattened head is the ideal morphology for breathing at the surface-water interface (Lewis 1970).

The mosquitofish burying behaviour observed in this study has not previously been reported. Further studies would be needed to confirm this novel behaviour, as our study indicates that mosquitofish are also utilising cooler temperatures provided within sediments shaded by *R. mangle* trees.

5. CONCLUSION

Ecosystem services derived from mangrove roots in the form of the nursery function have been well-documented (Beck et al. 2001, Nagelkerken 2009, Alongi 2014). However, the role of environmental buffering is less understood. This study gives an example of how *R. mangle* prop-roots and canopy may reduce thermal stress and 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 eco-

systems, 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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