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Physiological tolerance to hyperthermia and hypoxia and effects on species richness and distribution of rockpool fishes of Loggerhead Key, Dry Tortugas National Park

J.L. Rummer *, N.A. Fangue, H.L. Jordan, B.N. Tiffany, K.J. Blansit, S. Galleher, A. Kirkpatrick,
A.A. Kizlauskas, C.M. Pomory, W.A. Bennett

University of West Florida, Department of Biology, 11000 University Pkwy., Pensacola, Florida 32514, USA

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ABSTRACT

Rockpools on Loggerhead Key in the Dry Tortugas National Park experience cyclic tidal changes in water quality and physical dimension resulting in ichthyofaunal assemblages that differ markedly from the adjacent coral reef. Within the beach rock formations and areas transitioning to the nearby reef, we observed 45 fish species; however, only four species – schoolmaster, *Lutjanus apodus*, French grunt, *Haemulon flavolineatum*, cocoa damsel, *Pomacentrus variabilis*, and frillfin goby, *Bathygobius soporator* – were found in all rockpools. All fishes were transient juveniles except for frillfin goby, which was a pool resident. High temperature tolerance, (Critical Thermal Maxima), and low oxygen tolerance, (Critical Oxygen Minima) for schoolmaster, French grunt, cocoa damsel, and frillfin goby were 40.9, 36.2, 37.6, 40.9 °C and 0.56, 0.77, 0.50, and 0.27 mg/L, respectively. All four species demonstrated thermal and hypoxia tolerance values similar to those published for species traditionally noted as abiotic specialists. Although fish distribution patterns in rockpools were likely influenced by structural complexity and spatial limitations, the relationship between pool morphology and species richness was weak, suggesting that physiological tolerance to high temperature and low oxygen among reef fishes may be more influential in determining which species inhabit the rockpools. Harsh thermal and oxic conditions that cannot be exploited by less tolerant species may be beneficial for some Loggerhead Key reef fishes in providing refuge from predators, foraging grounds, or potential nursery areas.

1. Introduction

Periodically isolated bodies of water associated with mangroves, sand flats, seagrass beds, and beach rock often experience rapid, large-scale, diel changes in morphology, temperature, oxygen, and salinity (Horn et al., 1999; Fangue et al., 2001; Nybakken, 2001). Another common feature is that they are found in close association with more stable open water systems such as coral reefs (Erftemeijer and Allen, 1993). Shallow pools (<0.3 m) off of the coasts of islands in the Great Barrier Reef (GBF) may experience diurnal temperature increases exceeding 14 °C (Orr, 1933; Potts and Swart, 1984), and other studies report similar temperature fluctuations in habitats bordering the Pacific and Indian Ocean atoll coral reefs (Cloud, 1952; Pugh and Rayner, 1981). Coral reef fishes, while commonly associated with warmer waters, are not specifically known for hypoxia tolerance (Wise et al., 1998; Renshaw et al., 2002). However, a few studies over the past decade have revealed that reef species may frequently encounter hypoxic conditions (Routley et al., 2002; Östlund-Nilsson and Nilsson, 2004; Val et al., 2006). For example, in the GBF, between the reef crest and Heron Island, the reef platform becomes isolated from the open ocean diurnally, causing large changes in habitat morphology as well as water oxygen levels falling below 20% saturation (Kinsey and Kinsey, 1966; Routley et al., 2002). Salinity has been noted to fluctuate dramatically in desert pools like the Salton Sea, Sonoran Desert, or Laguna Madre in North America or some of the well studied desert habitats in the Middle East and Africa (Barlow, 1958; Hurlbert, 2008), but has not yet been noted to fluctuate as markedly in habitats near coral reef ecosystems (Cloud, 1952). The term *marginal habitat* is a good descriptor for these environments because it conveys both the stark abiotic conditions as well as their proximity to the coral reef. While fish diversity is notably high on the reef, marginal habitats are typically occupied by species from relatively few fish families; for example, in North America, species of the Poeciliidae, Cyprinidontidae, and Fundulidae families dominate marginal habitats (reviewed in Beitinger et al., 2000; Fangue et al., 2001). Fish assemblages in marginal habitats may be influenced by several factors including space, cover, and habitat complexity as well as physiological tolerances to extreme temperature, hypoxia, salinity, and pH (Orr, 1933; Cloud, 1952; Barlow, 1958; Kinsey and Kinsey, 1966; Brett, 1970; Pugh and Rayner, 1981; Potts and Swart, 1984; Wise et al., 1998; Fangue et al., 2001; Fangue and Bennett, 2003; Renshaw et al.,

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* Corresponding author. Department of Zoology, University of British Columbia, 6270 University Blvd. Vancouver, B.C. Canada V6T 1Z4. Tel.: +1 604 822 3378; fax: +1 604 822 2416.
E-mail address: rummer@zoology.ubc.ca (J.L. Rummer).

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2002; Routley et al., 2002; Östlund-Nilsson and Nilsson, 2004; Val et al., 2006; Hurlbert et al., 2007).

Loggerhead Key, located in the Dry Tortugas National Park (DTNP), USA, is the site of a series of rockpools formed by emergent beach rock formations on the Key's northwest edge immediately adjacent to the offshore coral reef. Longley and Hildebrand (1941) reported 446 total fish species inhabiting the Tortugas platform, and approximately 9% (41 species) were found inhabiting beach rock formations near Loggerhead Key. The number of fishes observed in the pools themselves was much lower; Fangue et al. (2001) reported only seven species occupied the shallowest pools at low tide. Following preliminary investigation, Fangue et al. (2001) suggested that space limitation at low tide, as well as high temperature and low oxygen could severely limit the total number and species of fish capable of consistently exploiting these habitats.

The purpose of our study was to quantify structural and water quality characteristics of all marginal rockpools formed during low tide on Loggerhead Key, identify the fish community inhabiting the rockpools, and elucidate physiological specializations allowing fish species to exploit these habitats. We also aimed to identify species richness within rockpools and transitional areas between the reef and the rockpools at low tide. High temperature and low oxygen tolerances were quantified for those species common to all rockpools at low tide. While previous studies have indicated the importance of life history patterns, feeding habits, and territoriality on fish distribution in more well-known marginal habitats (Orr, 1933; Cloud, 1952; Barlow, 1958; Kinsey and Kinsey, 1966; Pugh and Rayner, 1981; Potts and Swart, 1984; Wise et al., 1998; Renshaw et al., 2002; Routley et al., 2002; Östlund-Nilsson and Nilsson, 2004; Val et al., 2006; Verweij et al., 2006; Hurlbert et al., 2007), we hypothesize that fishes in the Loggerhead Key rockpools also rely on well-developed physiological tolerances to both high temperature and low oxygen to inhabit these rockpools. Our data contribute to the limited information on species composition of marginal habitats and provide a link to an ecosystem that is rarely the focus of studies, but nonetheless one of great importance to reef health and biodiversity.

2. Materials and methods

2.1. Site description

Loggerhead Key (24°38.08′N 82°55.24′W) is 846 m long and 110 m wide, and includes a prominent calcareous beach rock formation stretching for 523 m along the Key's northwest end (Fig. 1). Irregular layering of beach rock segments results in pools, undercuts, crevices, and channels that become inundated during high tide as water moves freely through the beach rock formation. While inundated at high tide, at extreme low tide the aquatic habitat is reduced to only 12 isolated rockpools containing various coralline and filamentous algae, invertebrates, and fishes (Fig. 2). The multi-layered beach rock shelf extends seaward approximately 12–15 m before ending abruptly at its northeastern edge.

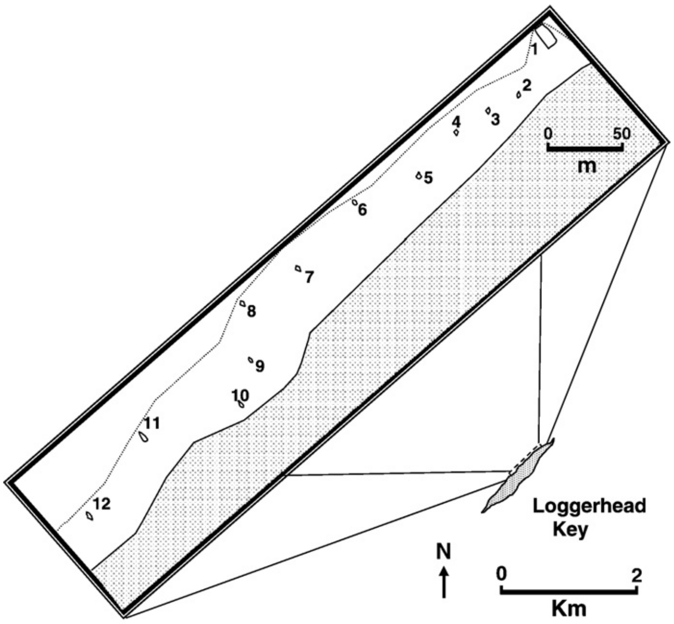

Fig. 2. The study area on Loggerhead Key and location of twelve rockpools. Shaded area denotes beach of Loggerhead Key, and rockpool study area is outlined with a dotted line. Area beyond dotted line represents transitional area as it leads to the adjacent coral reef.

2.2. Habitat characterization

At low tide, we measured the physical morphology of the twelve persistent rockpools on Loggerhead Key between 0900 and 1600 EST from 24 to 29 June 2001. Pools were numbered 1–12 from north to south, with the first being the most northern. Pool surface area (m^2) and volume (l) were calculated using the formulas of Kalff (2002). In addition, percent cover was estimated from densitometry data collected at a minimum of 50 points using 0.25 to 0.5 m grids set across each pool (Geographic Resource Solutions, Model GRS

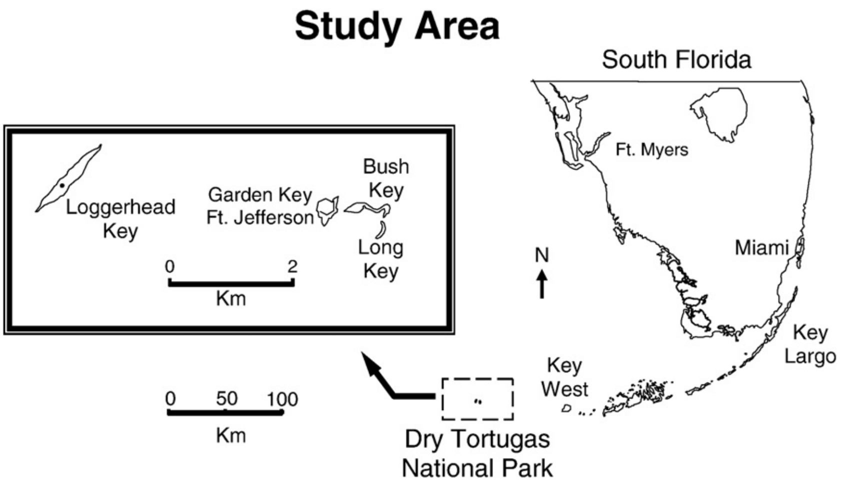

Fig. 1. Map depicting the Dry Tortugas National Park and location of Loggerhead Key with respect to the United States mainland, used with permission from Fangue et al. (2001).

Table 1
Physical measurements, bottom type, and calculated bottom cover complexity index (H') quantified in 12 rockpools on Loggerhead Key.

Rockpool #	Surface area (m^2)	Mean depth (cm)	Volume (l)	Bottom type (%)			Bottom cover complexity index (H')
				Sand	Coral rubble	Beach rock	
 #1 10m	45.99	17.01	79.23	24.62	3.08	72.31	0.35
 #2 1m	4.01	0.75	0.30	16.67	4.17	79.16	0.58
 #3 1m	4.69	2.46	1.15	16.67	4.17	59.84	0.64
 #4 1m	6.09	9.33	5.68	30.43	0.00	69.57	0.41
 #5 1m	8.33	19.23	16.01	17.65	2.94	79.40	0.42
 #6 1m	6.12	22.53	13.78	45.16	0.00	54.83	0.39
 #7 1m	20.18	18.52	37.38	50.85	3.39	45.76	0.42
 #8 1m	3.43	17.62	6.04	59.26	0.00	40.74	0.38
 #9 1m	3.44	17.30	5.96	34.21	5.26	60.52	0.48
 #10 1m	5.08	14.80	7.51	33.78	6.76	59.46	0.54
 #11 1m	11.56	16.90	16.53	24.62	3.08	72.31	0.35
 #12 1m	16.71	14.60	24.39	37.25	5.88	56.86	0.37

Densitometer). Bottom cover was categorized as small (<0.25 m long axis), medium (between 0.25 and 0.5 m long axis), large (>0.5 m long axis) beach rock plates, sand, coral rubble, or “other”. The number of rock layers was also noted when beach rock was observed. Densitometry data were incorporated into the Shannon–Weaver index (H') to estimate bottom cover complexity indices.

Water quality was measured in each pool 3–4 times daily encompassing all tidal stages. Temperature (±0.1 °C) and dissolved oxygen (±0.1 mg/l) were collected at the surface, middle, and bottom of each rock pool, using a calibrated Yellow Springs Instrument Company (YSI) Model 95 Oxygen Meter and corroborated by standard Winkler titration methods of selected water samples (Cox, 1974). Three Onset StowAway XTI temperature loggers were placed at similar depths (0.15–0.23 m at low tide), one each in a small, medium, and large rockpool to record temperature changes (±0.1 °C) every 15 min throughout the duration of the project. Salinity (±1.0‰) and pH (±0.1 unit) were determined using a light refractometer and a Fisherbrand® universal color indicator and color comparison chart, respectively. A one-way ANOVA was used to identify statistical relationships in water quality measures within rockpools (i.e. among three depths), and a Tukey–Kramer post-hoc test was used to identify differences between individual rockpools. An independent t-test was used to compare mean temperature, oxygen, and salinity values in each pool measured at low and high tide. All statistical decisions were based on $\alpha = 0.05$.

2.3. Community characterization and collection methods

Teams of two observers made daily annotations of species richness at low tide within each rockpool as well as along the deep seaward beach rock margin leading away from the pool areas toward the open water patch reefs. Observations were made before entering the pools to collect water quality data or fish for experiments. Fishes used in temperature and oxygen trials were collected using a 5 m bag seine, 2 m cast nets, dip nets of various sizes, or 0.25 m^2 lift nets. Experimental fish were collected at least 24 h prior to experimentation and considered field-acclimatized to 28 ± 1.0 °C, as this was the mean collection temperature for all species tested. Fish were held in floating baskets near the collection site until transported to the *R/V Bellows* in 170 l ice coolers filled with clean sea water and aerated with a Rule® submersible pump.

2.4. High temperature tolerance

Critical Thermal Methodology (CTM) entails subjecting fish to progressive temperature increases at a set rate until a near-lethal but non-lethal endpoint is reached; this methodology was used to determine physiological thermal tolerance limits (Cowels and Bogert, 1944; Cox, 1974; Beitinger et al., 2000). Two 38-l insulated CTM chambers, heated with model E-10 1250 Watt, Techne re-circulating thermoregulators, produced water temperature increases (0.39 ± 0.03 °C per minute) during trials. Individual fish for each trial, following a 24-hour fasting period, were placed into 300 ml glass beakers containing clean seawater and suspended in glass aquaria. Beakers were moderately aerated during the trial to prevent thermal stratification. Heating continued until final loss of equilibrium (LOE) was observed, at which time water temperature (±0.1 °C), standard length (±0.1 cm) and wet mass (±0.01 g) of the fish were recorded, and the fish returned to pre-test water temperatures for recovery. Onset of muscle spasms and LOE are common CTM endpoints (Cox, 1974; Becker and Genoway, 1979; Beitinger et al., 2000); we chose LOE as our endpoint because not all fish demonstrated muscle spasms. The critical thermal maximum (CTMax) for each species was calculated as the arithmetic mean of the collective LOE endpoints (Cox, 1974). Because beakers were isolated from one another, each fish in a trial was considered to be an individual replicate.

2.5. Low oxygen tolerance

We used step-wise decreases in dissolved oxygen to quantify physiological and behavioral responses to hypoxia for various rockpool fishes. A modified version of the counter current oxygen depletion apparatus designed by Smale and Rabeni (1995) was used to determine the Critical Oxygen Minimum (COM) for each species. During low oxygen trials, following a 24-hour fasting period, individual fish were placed, one each, into 10 separate 500 ml Erlenmeyer flasks. Nitrogen gas was introduced to the system, and the oxygen was depleted from the flasks by approximately one-half every 12 min. Oxygen concentrations in each flask were monitored continuously using an oxygen meter (YSI model 55). Additionally, temperature was maintained at 28 ± 1.0 °C during oxygen depletion trials, as this was the mean collection temperature for all species tested and would facilitate COM comparisons between trials and species. Throughout oxygen depletion, fish were monitored for two

Table 2
Species observed within rockpool study site (rockpools 1–12) and transitional areas (transition) leading to the adjacent reef.

Species	Common name	Location observed												
		Transition	1	2	3	4	5	6	7	8	9	10	11	12
Class Elasmobranchiomorphi														
Order Lamniformes														
Family Rhincodontidae														
Ginglymostoma cirratum	Nurse shark	x												
Order Rajiformes														
Family Dasyatidae														
Dasyatis say	Bluntnose stingray	x												
Class Osteichthyes														
Order Elopiformes														
Family Elopidae														
Megalops atlanticus	Tarpon	x												
Order Clupeiformes														
Family Clupeidae														
Jenkinsia lamprotaenia	Dwarf herring	x												
Family Engraulidae														
Anchoa sp.	Anchovy			x										
Order Antheriniformes														
Family Antherinidae														
Menidia sp.	Silverside		x											
Order Scorpaeniformes														
Family Scorpaenidae														
Scorpaena plumieri	Spotted scorpionfish		x											
Order Perciformes														
Family Triglidae														
Prionotus ophryas	Bandtail searobin	x												
Family Echeneidae														
Remora remora	Remora	x												
Family Carangidae														
Caranx ruber	Barjack	x												
Family Lutjanidae														
Lutjanus apodus	Schoolmaster	x	x	x	x	x	x	x	x	x	x	x	x	x
L. campechanus	Red snapper	x												
L. griseus	Gray snapper	x												
L. synagris	Lane snapper	x												
Ocyurus chrysurus	Yellow tail snapper	x												
Family Gerreidae														
Eucinostomus argenteus	Spotfin mojarra	x												
E. gula	Silver jenny		x		x	x								
Gerres sp.	Unknown mojarra		x	x										
Family Haemulidae														
Haemulon sp.	Unknown grunt	x												
H. album	Margate	x												
H. carbonarium	Caesar grunt	x												
H. flavolineatum	French grunt	x	x	x	x	x	x	x	x	x	x	x	x	x
H. plumieri	White grunt	x												
Family Kyphosidae														
Kyphosus sectatrix	Bermuda chub		x											
Family Sciaenidae														
Equetus lanceolatus	Jackknife-fish	x												
Family Pomacanthidae														
Pomacanthus paru	French angel (juvenile)	x												
Family Pomacentridae														
Abudefduf saxatilis	Sergeant major	x	x	x	x	x	x	x						
A. taurus	Night sergeant		x											
Pomacentrus fuscus	Dusky damsel	x	x											
P. leucostictus	Beaugregory	x	x											
P. variabilis	Cocoa damsel	x	x	x	x	x	x	x	x	x	x	x	x	x
Family Mugilidae														
Mugil sp.	Unknown mullet	x												
M. curema	White mullet	x												
Family Sphyraenidae														
Sphyraena barracuda	Barracuda (juvenile)	x	x	x										
Family Labridae														
Bodianus rufus	Spanish hogfish	x												
Halichoeres bivittatus	Slippery dick	x	x											
Thalassoma bifasciatum	Bluehead wrasse	x												
Family Scaridae														
Scarus guacamaia	Rainbow parrotfish	x												
Sparisoma viride	Stoplight parrotfish	x												
Family Clinidae														
Malacoctenus triangulatus	Saddled blenny	x												
Family Gobiidae														
Bathygobius soporator	Frillfin goby	x	x	x	x	x	x	x	x	x	x	x	x	
Gobiosoma oceanops	Neon goby	x												

Table 2 (*continued*)

Species	Common name	Location observed												
		Transition	1	2	3	4	5	6	7	8	9	10	11	12
Class Osteichthyes														
Family Acanthuridae														
Acanthurus sp.	Unknown tang	x												
A. coeruleus	Blue tang	x												
Order Tetraodontiformes														
Family Balistidae														
Monacanthus ciliatus	Fringed filefish	x												

endpoints with corresponding oxygen concentrations recorded for both. We defined the behavioral endpoint, Aquatic Surface Respiration (ASR), as the point at which a fish ventilated persistently at the air–water interface for at least 1 min (Kramer and McClure, 1982). This was followed by the physiological endpoint, loss of equilibrium (LOE), and was equivalent to the fish's critical oxygen minimum (COM). Both ASR and COM were calculated for each fish species as the arithmetic mean of the collective oxic endpoints. A modified Winkler titration (Cox, 1990) and colorimetric analysis (Oulman and Baumann, 1956) were used to verify oxygen concentrations (mg/l) at ASR and LOE. Wet mass, standard length, ASR, and COM were recorded for all fish.

2.6. Statistical analyses

To investigate the relationship between rockpool physical morphology and species richness, a stepwise forward multiple regression analysis was performed, and an equation fitting the physical morphology data to species richness data from the 12 rockpools was calculated. This method is preferable to classical multiple regression when correlation between factors is suspected. For this reason, we also investigated correlative relationships between physical characteristics with the Pearson Product Moment Correlation. The final regression model included only parameters not found correlated with one another. One-way ANOVA was used to test for statistical differences between species' CTMax, ASR, and COM values, and statistical relationships between multiple means were determined using a Tukey–Kramer post-hoc test. Fish that did not survive 24 h post-trial were not included in analyses. By definition, CTMax and COM endpoints must be non-lethal (Coutant, 1969) and if 24-hour survival is not observed it is assumed that the measured endpoint overestimated the CTMax or COM. SigmaStat 3.0 (Systat software, San Jose, California) statistical software was used to perform analyses, and all statistical decisions were based on $\alpha = 0.05$.

3. Results

3.1. Habitat characterization

Each of the twelve rock pools chosen for observation varied in shape, size, bottom type, mixing between tides, and tidal influx (Table 1). Pools ranged from 34–460 m^2 surface area and comprised bottom types of sand, coral rubble, beach rock of one, two, and three layers, and occasional debris. Rockpool #1 was the largest of all observed pools and marked the beginning of the transition between the adjacent patch reefs and the shallow water habitats. Sand was the dominant bottom type in the pools near the beginning of the gradient, and coral rubble was observed throughout the study area except for in the pools closest to the shore. Pools closer to shore were dominated with beach rock comprised of at least two and sometimes three layers and therefore exhibited higher complexity than those close to the open water. Consistent with our observations, beach rock and sand bottom types were negatively correlated with one another ($r = -0.890$, $P < 0.001$), and pool surface area and total volume were positively correlated ($r = 0.988$, $P < 0.0001$). Complexity index was negatively correlated with mean depth ($r = -0.746$, $P < 0.05$), indicating that shallower pools tended to exhibit greater habitat complexity. Ultimately, the physical characteristics showed a transition between the sandy-bottom and coral rubble patch reef habitat and the protected areas nearshore, creating a distinct physical gradient to study.

Thorough abiotic characterization also demonstrated that temperature fluctuated both on a diel basis and over the course of the study. High tide thermal changes ranged from 27.2–32.1 °C and low tide thermal changes ranged from 28.1–32.2 °C during the daylight sampling hours. Temperatures at high tide and temperatures at low tide were significantly different ($T = 1.71$, $P = 0.0900$, and $n = 108$). However, results from the temperature loggers broadened the scope of thermal variability and reflected temperature changes at night when we were unable to sample. Results from the logger secured in the largest pool demonstrated that, over a 24-h period, temperatures could range between 27.2 and 31.6 °C with a mean of 29.1 ± 1.06 °C. Logger #2, launched in a medium sized pool at a location more protected from the tide, resulted in data ranging between 26.2 and 33.2 °C with a mean of 28.9 ± 1.10 °C. Finally, logger #3, which was placed in the smallest pool and the one most distant from the open water, displayed temperatures between 25.9 and 33.2 °C with a mean of 28.8 ± 1.52 °C. Fluctuations in pH were minor, ranging from 8.0 to 8.4, while salinity ranged between 35 and 41‰ (mean 35.9 ± 0.92‰) at high tide and between 35 and 40‰ (mean 37.2 ± 1.79‰) at low tide. Salinity at high tide was significantly lower than that at low tide ($T = 4.20$, $P = 0.0001$, and $n = 80$). Oxygen tensions ranged between 3.06 and 7.42 mg/l (mean 6.2 ± 1.91 mg/l) and between 4.60 and 7.20 mg/l (mean 6.4 ± 0.59 mg/l) at high and low tide, respectively, but we found no significant differences between high tide and low tide oxygen readings ($T = 0.23$, $P = 0.8181$, and $n = 20$) and cannot speak to the changes we may have observed if nighttime sampling was possible.

3.2. Species richness

Fish species observed in the entire study area represented 2 taxonomic classes, 8 orders, 25 families, 32 genera, and 45 species (Table 2). Species observed within the transitional area outside of the 12 rockpools were more numerous and larger in size. Qualitatively, fish numbers and size decreased with pool size, and fewer species were observed within pools closest to the shoreline. A total of 26 species were observed only within the transition area, while 10 species were able to exploit at least one of the rockpools. Only 4 species – juvenile schoolmaster, *Lutjanus apodus*, juvenile French grunt, *Haemulon flavolineatum*, cocoa damsel, *Pomacentrus variabilis*, and frillfin goby, *Bathygobius soporator* – were observed consistently within all 12 rockpools.

Conclusions from a forward stepwise regression model indicated that, relative to the morphological characteristics measured for each pool, species richness could only be predicted from a linear combination of mean depth and pool volume; $r = 0.894$, $P < 0.001$, equation: species richness $= 5.936 - (0.211 * \text{mean depth}) + (0.170 * \text{volume})$. Pool surface area significantly influenced species richness but was removed by the model due to significant positive correlation with pool volume ($r = 0.988$, $P < 0.001$). Unquestionably, only a few small fish can

Table 3
Aquatic surface respiration (ASR), critical oxygen minimum (COM), as well as critical thermal maxima (CTMax) are displayed for all species.

Species (common name)	*n*	ASR (mg/l)	*n*	COM (mg/l)	Standard length (cm)	Wet weight (g)	*n*	CTMax (°C)	Standard length (cm)	Wet weight (g)
Schoolmaster	11	1.41 ± 0.46^{b}	11	0.58 ± 0.11^{c}	2.02 ± 0.12	1.40 ± 0.13	8	40.9 ± 0.73^{a}	3.30 ± 1.17	1.10 ± 0.87
French grunt	5	1.54 ± 0.51^{b}	10	0.80 ± 0.38^{c}	2.39 ± 0.39	2.14 ± 0.40	15	36.17 ± 2.20^{b}	3.20 ± 0.59	1.40 ± 0.72
Cocoa damsel	8	1.15 ± 0.29^{a}	10	0.52 ± 0.13^{b}	5.75 ± 0.14	7.68 ± 0.15	12	37.58 ± 1.59^{b}	4.80 ± 1.35	5.30 ± 4.27
Frillfin goby	1	1.25 ± 0.00^{a}	6	0.26 ± 0.04^{a}	2.92 ± 0.05	0.97 ± 0.06	5	40.92 ± 0.78^{a}	3.20 ± 2.36	2.10 ± 2.32

Values represent mean ± SD, and significant differences, as per Tukey–Kramer post-hoc analyses, are noted by letters. Mean ± SD for standard length and wet weight are also reported. Not all individuals that were tested for COM also displayed ASR, hence the different sample sizes.

physically occupy a small pool, but contrary to our original hypotheses, it does not seem that structure and complexity significantly influenced the number of species occupying an individual pool. In three of the pools, where temperature was logged over several days to include at least one full 24-hour cycle, we observed a relationship between the number of species and the scope of temperature change. The pool that exhibited the smallest change in temperature over a 24-hour period also had the greatest number of species present. Conversely, the two pools where the range of temperatures observed was greater and achieved absolute temperatures higher than in the largest pool accommodated only 4 or 5 species. This correlative relationship, however, could not be statistically supported due to low sample size and low power.

3.3. High temperature tolerance

We collected a total of 40 fish representing 4 species for use in CTMax trials (Table 3). Of the four species tested, the frillfin goby (3.20 ± 2.36 mm and 2.10 ± 2.32 g standard length and wet weight) and juvenile schoolmaster (3.30 ± 1.17 mm and 1.10 ± 0.87 g) displayed the greatest tolerance to high temperatures with CTMaxima of 40.9 ± 0.78 and 40.9 ± 0.73 °C, respectively. No significant differences were detected in CTMaxima between the two species ($P = 0.05671$). Values were significantly higher than recorded for juvenile French grunt (3.20 ± 0.59 mm standard length and 1.40 ± 0.72 g wet weight) and cocoa damsel (4.80 ± 1.35 mm and 5.30 ± 4.27 g) displaying CTMaxima of 36.17 ± 2.20 and 37.58 ± 1.59 °C, respectively.

3.4. Low oxygen tolerance

We collected a total of 34 fish representing 4 species for use in low oxygen tolerance trials (Table 3). ASR for Frillfin goby (1.25 ± 0.00 mg/l) and cocoa damsel (1.15 ± 0.29 mg/l) differed significantly from all other species, and ASR for juvenile schoolmaster (1.41 ± 0.46 mg/l) and juvenile French grunt (1.54 ± 0.51 mg/l) differed significantly from all other species but not each other. Although, it should be noted, not all individuals displayed ASR. Frillfin goby displayed LOE at a significantly low oxygen tension, resulting in a COM of 0.26 ± 0.04 mg/l. Standard length and wet weight were 2.92 ± 0.05 mm and 0.97 ± 0.06 g. COM values for the cocoa damsel were 0.56 ± 0.12 mg/l and significantly different ($P<0.001$) from the frillfin goby. Standard length and wet weight were 3.01 ± 0.13 mm and 1.02 ± 0.14 g, respectively. COM values for schoolmaster 0.58 ± 0.11 mg/l and French grunt 0.80 ± 0.38 mg/l were not significantly different from each other but were significantly different from the other two species ($P<0.001$). Standard lengths and wet weights were 2.02 ± 0.12 and 2.39 ± 0.39 mm and 1.40 ± 0.13 and 2.14 ± 0.40 g, respectively.

4. Discussion

Tidal fluctuations and intense insolation dramatically alter available space and water quality, both of which may ultimately dictate fish distribution in rockpool habitats on Loggerhead Key. Low volumes, coupled with relatively high structural complexity, make rockpools an attractive refuge to smaller fishes. However, several small damselfish, grunt, and wrasse species present in adjacent areas did not enter the rockpools, suggesting that factors other than habitat complexity may dictate species richness (Table 2). Because of the weak relationship between most of the morphological characteristics measured and calculated for individual pools and species richness, we suspect that water quality parameters such as temperature, oxygen, salinity and pH may be influencing species distribution more heavily than structural complexity. We logged temperature increases from 25.9 to 33.2 °C over the course of this study in some pools, and while a wider range than measured in similar studies, it is unlikely that we saw the most extreme thermal conditions experienced by fishes living in these habitats. Temperatures ranging 30.5–35.8 °C were reported in 2000 (Fangue et al., 2001), and Pearse (1934) measured August temperatures in pools near Long & Garden Keys as high as 42.8 °C. Furthermore, increasing water temperature often results in decreasing oxygen tension, evaporation, and therefore increases in salinity. While rockpool oxygen tensions remained relatively high during our sampling period, they may have been less hospitable at night as biological oxygen demand increases in the absence of photosynthesis. Salinity increased by 4–5‰ over the course of any given sampling day, and pH remained stable. Our data contribute to the existing body of evidence suggesting that conditions in rockpool habitats may be comparable to those documented in other extreme habitats worldwide, like desert pools or shallow saltwater basins, where the most temperature and hypoxia tolerant fish species have been well studied.

Abiotic specialists were traditionally thought to come from only a few fish families, namely Cyprinidontdae, Fundulidae, and Poecilidae, but dominant ichthyofaunal assemblages expected to occupy marginal habitats may also include Apogonid, Blenniid, Cichclid, Gobiid, Haemulid, and Pomacentrid species (reviewed in Beitinger et al., 2000; Fangue et al., 2001; Östlund-Nilsson and Nilsson, 2004; Hurlbert et al., 2007). Only cocoa damselfish, sergeant major, frillfin goby, and schoolmaster were found consistently in all Loggerhead Key rockpools and under all tidal conditions, indicating that they possessed physiological mechanisms that allow them to survive the harsh, abiotic conditions observed in the pools. The schoolmaster and frillfin goby displayed CTMaxima above 40 °C, which is among the highest CTMaxima reported to date (reviewed in Beitinger et al., 2000; Fangue and Bennett, 2003). CTMaxima for the French grunt and cocoa damsel ranged between 36.2 and 37.6 °C, significantly lower than for the schoolmaster and goby but nonetheless within the upper range of thermal tolerance reported for other species collected from or acclimated to temperatures near 30 °C, the approximate temperature from which these species were collected (reviewed in Beitinger et al., 2000). Patterns of low oxygen tolerance followed a similar trend, with the frillfin goby as the most hypoxia tolerant, displaying LOE at the lowest oxygen tension, while the other species tolerated levels from 0.80 to as low as 0.52 mg/l. Although not anoxia tolerant like the well-studied goldfish and carp, hypoxia tolerance in these four species is comparable to some of the most well-known hypoxia tolerant species such as the Cichlids, Cyprinodondtids, and Gobiids (Muusze et al., 1998). Other studies report reef species like cardinalfish, other damselfish and goby species, blennies, and even a shark species (*Hemiscyllium ocellatum*), also tested at 30 °C, as surprisingly hypoxia tolerant, maintaining righting reflex, ventilation, and rhythmic swimming at oxygen levels below 1.0 mg/l (Muusze et al., 1998; Wise et al., 1998; Renshaw et al., 2002; Östlund-Nilsson and Nilsson, 2004). Indeed, it may be that hypoxia tolerance in tropical and subtropical species is more widespread than previously thought, and may be particularly advantageous after dark when resident flora deplete oxygen and even more so if low tide occurs at night. Physiological capabilities clearly play a role in species distribution patterns and habitat utilization strategies for reef fishes utilizing marginal habitats.

Varying degrees of physiological tolerance may also play a role in how fishes utilize shallow rockpools both spatially and temporally. Many researchers have concluded that fishes physiologically capable of inhabiting harsh habitats are taking advantage of untapped resources, namely refuge from aquatic predators (Laegdsgaard and Johnson, 2001), foraging grounds (Vega and Arreguin, 2001; Laegdsgaard and Johnson, 2001), and nursery areas (Bennett, 1987, Chong et al., 1990; Nagelkerken et al., 2000; Vidy, 2000; Laegdsgaard and Johnson, 1995, 2001; Huijbers et al., 2008). Species were classified as either transient or resident to the rockpools based on several aspects of distribution and physiology (Mahon and Mahon, 1994). The cocoa damsel, French grunt, sergeant major, and schoolmaster demonstrated notable physiological tolerance to high temperature and low oxygen but did not spend all of their time in the shallow pools. Species that did not use the marginal habitats as both juveniles and adults may only possess the ability to do so during one life stage or another. For example, we observed juvenile schoolmaster and French grunt transiently utilizing the pools, and we know that both of these species can be observed on the reef as adults (Huijbers et al., 2008). Some species may not have the maneuverability to, if necessary, easily escape rockpools via the narrow channels that occasionally connect to the reef, and therefore may only be utilizing marginal habitats on a diel basis (Verweij et al., 2006) to avoid large visual predators on the reefs. Fangue and colleagues (2001) observed juvenile white mullet (*Mugil curema*) occupying 30 °C waters adjacent to the rockpools, but when the CTMax was quantified as 35 °C for this species, it was concluded that the isolated rockpools might be too warm for white mullet to fully exploit (Fangue et al., 2001). Lowe-McConell (1987) suggested that many transients move back to the reefs after dusk, at which time predation may not be a substantial threat. Species may also exploit the marginal habitat opportunistically. During our study, we observed a small nurse shark briefly entering a rockpool at high tide, but whether this was a feeding strategy or one to avoid predation is unknown. According to our findings, marginal habitat transients probably distribute themselves according to thermal and oxic gradients that can be heavily influenced by tidal stage and time of day.

5. Conclusions

We conclude that the frillfin goby was the only permanent resident of the rockpools of Loggerhead Key due to its physiological tolerance to the unstable abiotic profile of the area and because this fish was observed occupying all rockpools as both juveniles and adults. In addition to exceptional high temperature tolerance, the frillfin goby, when compared to the other fishes, displayed loss of equilibrium at the lowest oxygen concentration. This is not entirely surprising as past studies report that rockpool and tidepool residents are typically of small body size and most commonly gobies and blennies (Mahon and Mahon, 1994; Lam et al., 2006). However, the frillfin goby's life history strategies also agree with the resident profile, as they invest a lot of energy in caring for their eggs (Lowe-McConell, 1987) and would clearly benefit from residing in harsh, secluded areas due to an increased likelihood of offspring survival (Gross and MacMillan, 1981). Additionally, it may be that this species cannot compete elsewhere and use their physiological tolerance to reside in a habitat unattractive to predators. Regardless of provisional or residential utilization strategies, fish species that use marginal habitats must have superb tolerance to hyperthermia and hypoxia, which ultimately influence species richness and distribution.

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