Caribbean Journal of Science, Vol. 37, No. 1-2, 81-87, 2001

Temperature and Hypoxia Tolerance of Selected Fishes from a Hyperthermal Rockpool in the Dry Tortugas, with Notes on Diversity and Behavior

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ABSTRACT.—**We documented the physical habitat characteristics and fish diversity of a hyperthermal rockpool on Loggerhead Key in the Dry Tortugas National Park during July 2000. Rockpool temperatures ranged from 30.5 °C to 35.8 °C and oxygen varied from 6.4 to 3.5 mg/L depending on depth and time of day. Seven fish species from five families inhabited the rockpool. Critical thermal maxima (CTMax) and critical oxygen minima (COM) were measured for three species. French grunt *Haemulon flavolineatum* was the most temperature tolerant fish (CTMax = 37.9 °C), followed by cocoa damselfish *Pomacentrus variabilis* (36.1 °C), and white mullet *Mugil curema* (35.0 °C), respectively. Cocoa damselfish were more tolerant of hypoxic conditions (COM = 0.8 mg/L) than either French grunt (1.2 mg/L) or white mullet (1.5 mg/L). French grunt and cocoa damselfish resorted to aquatic surface respiration at respective dissolved oxygen levels of 2.6 and 1.7 mg/L, whereas white mullet did not display this behavior at oxygen concentrations as low as 1.5 mg/L. High-temperature and low-oxygen responses of the three species were not exceptional, suggesting that behavior and not physiology is the major factor allowing Loggerhead Key fishes to exploit hyperthermal habitats.**

INTRODUCTION

Hyperthermal tidepools, desert springs, and mangrove ponds are relatively shallow, isolated water bodies that routinely experience hyperthermic, hypoxic or both extremes. Indeed, abiotic conditions in these habitats often approach the biokinetic limits for vertebrate life (Brock, 1967; Bennett and Beitinger, 1997). Fishes living in hyperthermal habitats are typically abiotic specialists relying heavily on exceptional physiological tolerance to survive hyperthermia (Lowe and Heath, 1969; Heath et al., 1993; Bennett and Beitinger, 1997) and hypoxia (Packard, 1905; Lowe et al., 1967; Hirshfield and Feldmeth, 1980). Hyperthermal pool fishes may further augment physiological adaptations with behavioral responses such as aquatic surface respiration (ASR) and diel movements among favorable microhabitats (Barlow, 1958; Kramer and McClure, 1982; Heath et al., 1993). Species richness in hyperthermal habitats is low because few fishes possess the necessary adaptations to persist in such rigorous environments. Most studies report species-poor assemblages dominated by livebearers (family Poeciliidae), pupfishes (family Cyprinodontidae), killifishes (family Fundulidae) or a mix of these. More than just evolutionary curiosities, hyperthermal and hypoxic tolerant fishes provide useful models for understanding temperature and oxygen adaptations and elucidate adaptive life-history strategies that are not obvious in fishes living in less rigorous habitats.

We studied shallow rockpools located on the northeast corner of Loggerhead Key within the Dry Tortugas National Park (Fig. 1) in June, 2000. Like other tropical and subtropical hyperthermal environments, the Loggerhead Key rockpools are shallow, relatively isolated, and experience intense insolation during the summer. The specific objectives of our research were to characterize the physical rockpool habitat, identify and observe fishes inhabiting the rockpools, quantify low-oxygen and high-temperature tolerance of selected rockpool fishes, and interpret the results relative to fish distribution and behavior. Our data are

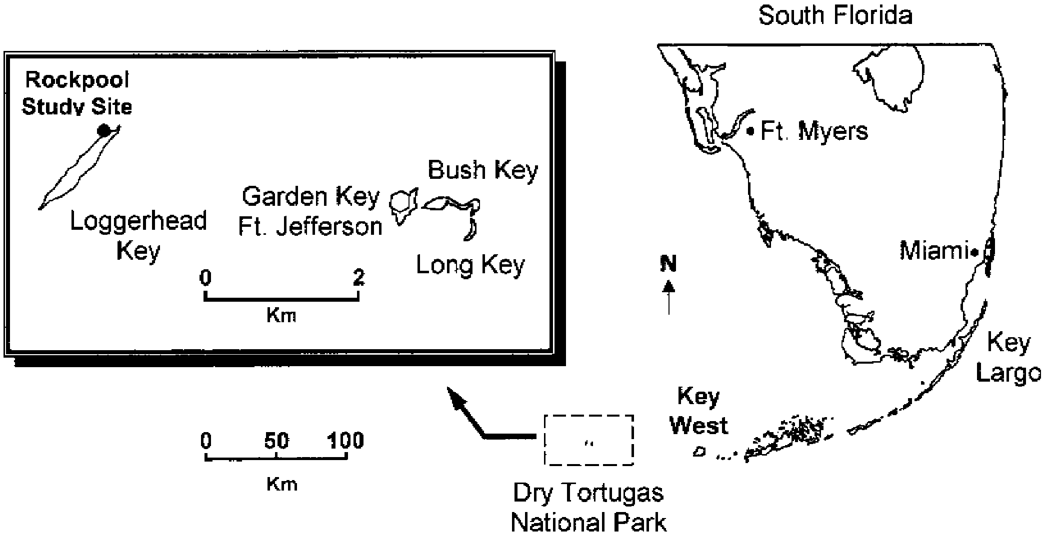

FIG. 1. Hyperthermal rockpool study site on Loggerhead Key, Dry Tortugas National Park, USA.

the first to document diversity, behavior, and physiology of fishes from the unique hyperthermal rockpool communities of the Dry Tortugas.

MATERIALS AND METHODS

The rockpools of Loggerhead Key occur along an expanse of old exposed limestone reef on the Key's northwest side. Our study site was the rockpool located immediately west of Loggerhead Key's northeastern tip (Fig. 1). The rockpool is roughly rectangular and measures 49.3 × 13.9 m. Depth varied from 0.1 to 0.8 m (mean= 0.4 m). Although mostly isolated from the sea, the rockpool received wave wash-over and tidal flow between fractures in the limestone formation. Plate-like limestone fragments 0.2 to 1.5 m long lie scattered over the rockpool bottom, with cover ranging from 20 to 30 % on the east end of the pool to near 80 % at the west end and rockpool margins. The bottom material was coarse coral sand. There was no macro-vegetation in the rockpool but the limestone fragments were markedly encrusted with algae and fouling organisms.

Characterizations of the physical habitat and water quality measures were done between 1100 and 1700 EST on 28 June 2000. We assessed the site's physical characteristics via maximum, minimum, and average depth (m), and described the bottom cover. Water quality variables included pH (±0.2 units), temperature (±0.1 °C), salinity (±0.1‰), and dissolved oxygen (±0.1 mg/L) measured at approximately hourly intervals. We determined pH at the center of the rockpool using a Fisherbrand® universal color indicator and a color comparison chart. We collected temperature, salinity, and dissolved oxygen from three evenly spaced sites along a transect through the middle of the rockpool, using a calibrated Yellow Springs Instrument Company (YSI) Model 30 Salinity-Conductivity-Temperature Meter and a YSI Model 95 Oxygen Meter. An Onset, Optic StowAway® temperature logger placed 0.25 m deep at the southeast corner of the rockpool collected water temperature (±0.1°C) every minute to quantify temperature change.

We carefully observed fish distribution and behavior in the undisturbed rockpool for one hour and continued to make observations periodically thereafter. We collected fishes using a 5-m bag seine, 1-m^2 lift net, 2-m cast net, aquarium dip nets or a combination of these. Captured fishes were held in a 170-L ice chest filled with clean seawater aerated by a Rule® submersible pump. Fish collection continued until approximately 1700 EST, at which time we transported the fishes to the *R/V Bellows* where they were counted and identified to species. Only juvenile French grunt *Haemulon flavolineatum* (Desmarest, 1823), cocoa damselfish *Pomacentrus variabilis* Castelnau, 1855 and juvenile white mullet *Mugil curema* Valenciennes, 1836 were captured in sufficient numbers to be used in temperature and oxygen tolerance experiments (Table 1). Fish were divided into two groups for use in temperature and oxygen tolerance trials.

We used the critical thermal methodology (CTM) as described by Heath et al. (1993) to estimate high-temperature tolerance. This method was especially well suited because it produces ecologically accurate and statistically precise thermal tolerance estimates using small numbers of fish (Becker and Genoway, 1979; Beitinger et al., 2000). We randomly placed seven to ten fish, one each, into 300-ml glass beakers with clean, well-aerated seawater. We then suspended the beakers in a CTM chamber containing 35 liters of freshwater and a 1250-W, Model E-10 Techne® re-circulating thremoregulator. Water temperature inside the beaker was increased at a rate of 0.4 °C/min when the thremoregulator was activated. Temperature increased until fish displayed final loss of equilibrium (LOE-inability of a fish to maintain dorso-ventral orientation for at least one minute). At this time we recorded the beaker water temperature (±0.1 °C), measured standard length (±0.1 cm), and returned the fish to pretest conditions to recover. We estimated the critical thermal maximum (CTMax) for each species as the arithmetic mean of the collective LOE temperatures.

We evaluated behavioral and physiological responses to low-oxygen by exposing fish to progressive hypoxia in a modified version of the counter-current oxygen depletion system described by Smale and Rabeni (1995). For these experiments, we placed up to ten fish, one each, into 1-L Plexiglas® test chambers filled with saturated seawater. A Supreme Mag Drive® model MD12 pump continuously recirculated water between the test chambers and a 180-cm × 5.1-cm OD counter-current exchange column made of polyvinyl chloride. Compressed nitrogen, forced through a fine-bubble diffuser stone at 1.5 L/min generated a bubble stream in the exchange column. Counter-current nitrogen/water flow in the exchange column reduced dissolved oxygen in the system by approximately one-half every 18 min. Oxygen depletion continued until fish displayed both ASR (except white mullet) – continuous ventilation of the surface film at the air-water interface – and LOE. We measured dissolved oxygen using the Model 95 Oxygen Meter at each endpoint. We removed each fish from its test chamber as it displayed LOE, measured standard length, and returned it to pretest conditions to recover. The ASR threshold and critical oxygen minimum (COM) were estimated as the arithmetic mean oxygen concentration at which ASR and LOE occurred.

We used parametric one-way analysis of variance to test for significant differences in mean CTMax or COM levels between species. To discriminate between individual mean CTMax and COM values, we used the Student-Newman-Keuls Multiple Range Test (SNK MRT). Statistical differences between ASR responses of French grunt and cocoa damselfish were tested using independent Students t-test; white mullet did not exhibit ASR. All statistical decisions were based on an α of 0.05.

RESULTS

Salinity and pH in the rockpool remained virtually unchanged at 34.4‰ and 8.2, respectively, while temperature and dissolved oxygen varied moderately. Average (±SD) water temperature along the center transect increased from 30.5 (±0.18) to 33.3 (±0.12) °C with no vertical stratification. The Onset logger data showed a rate of temperature increase of about 0.5°C/h until late afternoon (~1500 EST), when temperatures stabilized. As rockpool temperatures increased, dissolved oxygen fell from 6.4 (±0.42) to 5.2 (±0.72) mg/L. The most extreme temperature/oxygen conditions occurred in the shallow area at the rockpools west end. A spot check at 1400 EST found a temperature of 35.8 °C and corresponding oxygen concentration of 3.5 mg/L.

We observed or collected seven fish species (five families) at three general depth ranges (Table 1). Along the shallow margins at depths of 0.3 m or less, we found sergeant major *Abudefduf saxatilis* (Linnaeus, 1758), schoolmaster *Lutjanus apodus* (Walbaum, 1892), and French grunt juveniles congregating. Although relatively abundant, these fishes preferred areas of dense limestone cover and were difficult to capture. Away from the rockpool margins, at intermediate depths between 0.3 and 0.5 m, we observed cocoa damselfish aggressively defending territories among limestone fragments. Within this region we also captured two frillfin goby *Bathygobius soporator* (Valenciennes, 1837). In the relatively deep and open regions to the east side of the rockpool, we captured juvenile white mullet and observed one adult yellowtail snapper *Ocyurus chrysurus* (Bloch, 1791).

French grunt, cocoa damselfish, and white mullet showed significantly different physiological and behavioral responses to progressive hyperthermia and hypoxia. Critical thermal maxima differed significantly among these species (One-way ANOVA; $P < 0.0004$). Cocoa damselfish (mean = 36.1 °C) and white mullet (mean = 35.0 °C) showed no significant differences between CTMaxima, whereas French grunt (mean = 37.9 °C) were significantly more heat tolerant (SNK MRT; $\alpha = 0.05$). Likewise, physiological responses to hypoxia differed significantly between the fishes tested (One-way ANOVA; $P < 0.0001$), with COM values from French grunt and white mullet being statistically similar (1.2 and 1.5 mg/L respectively) but significantly higher than COM levels observed in cocoa damselfish (0.8 mg/L) (SNK MRT; $\alpha = 0.05$). Finally, French grunt used ASR at significantly higher mean dissolved oxygen levels than cocoa damselfish (2.6 vs. 1.7 mg/L) (Student t-test; $P = 0.0022$). White mullet did not display ASR behavior at dissolved oxygen levels as low as 1.5 mg/L.

TABLE 1. Species of fishes listed by family, collected from a hyperthermal rockpool at Loggerhead Key, Dry Tortugas National Park. Mean ± 1 standard deviation (SD) are given for standard length (SL), critical thermal maximum (CTMax), aquatic surface respiration (ASR) and critical oxygen minimum (COM) of selected-species.

Species	Common name	Collection depth (m)	n	SL ± SD (cm)	CTMax ± SD (°C)	ASR ± SD (mg/L)	COM ± SD (mg/L)
Family Lutjanidae							
Lutjanus apodus[1]	Schoolmaster	0.1-0.3	4				
Ocyurus chrysurus[1]	yellowtail snapper	0.8	1 observed				
Family Haemulidae							
Haemulon flavolineatum	French grunt	0.1-0.3	14	2.6 ± 0.39	37.9 ± 1.64	2.6 ± 0.31	1.2 ± 0.57
Family Pomacentridae							
Abudefduf saxatilis[1]	sergeant major	0.1-0.3	3				
Pomacentrus variabilis	cocoa damselfish	0.3-0.5	20	5.8 ± 0.76	36.1 ± 0.80	1.7 ± 0.57	0.8 ± 0.21
Family Mugilidae							
Mugil curema	white mullet	0.5-0.8	19	5.3 ± 0.64	35.0 ± 0.95	Not observed	1.5 ± 0.13
Family gobiidae							
Bathygobius soporator[1]	frillfin goby	0.3-0.5	2				

[1] Number of captured individuals was insufficient for either high-temperature or low oxygen tolerance determinations.

DISCUSSION

The Loggerhead Key rockpools exhibit all the key physical hallmarks of hyperthermal environments. They are shallow, isolated, and exposed to intense direct sunlight. As a result, rockpool water temperatures increased rapidly 3 to 5 °C during the day, ultimately approaching 36 °C in some shallow reaches. Meanwhile, open sea temperatures remained nearly stable at about 30 °C. Heating rates and maximum temperatures measured are similar to those of hyperthermal subtropical tidepools (Bennett and Judd, 1992), but are less extreme than in desert pools and mangrove ponds (Feldmeth, 1981; Heath et al., 1993). However, we certainly did not measure the highest temperatures of the year; for example, Pearse (1934) measured August water temperatures of 42.9 °C in brackish pools on Long Key in the Dry Tortugas. Most rockpool locations remained normoxic throughout the day; although we measured oxygen concentrations as low as 3.5 mg/L at one site. High surface area, periodic mixing, and photosynthetic activity in the system probably make daytime hypoxic events rare. However, since Congleton (1980) and Schmidt-Nielsen (1996) suggest that nocturnal hypoxia is common in shallow tidepool environments with moderate biological oxygen demand, night sampling may have uncovered a different environment.

While the physical profile of the Loggerhead Key rockpool was characteristic of a hyperthermal habitat, the number and type of fishes inhabiting the site were not. Previous investigations have found that the demanding nature of hyperthermal waters results in low species richness. For example, Heath et al. (1993) found three hyperthermal species in mangrove ponds in Belize, Pearse (1934) found two species in Dry Tortugas brackish ponds, and Barlow (1958), Gunter (1967), and Lowe and Heath (1969) found a single species each in south Texas tidepools, the Salton Sea in California, and a Sonoran desert spring in Arizona, respectively. In all cases (except Pearse, 1934), the fishes present were pupfishes, livebearers or both. On the other hand, the Loggerhead Key pool contained seven fish species, six of which have not been reported in association with hyperthermal environments (Pearse, 1934 reported white mullet from two hyperthermal brackish ponds on Long Key). Furthermore, the familiar hyperthermal residents (pupfishes, killifishes, and livebearers) were absent. We propose that the absence of these abiotic specialists has allowed some of the more tolerant reef species to establish themselves in the rockpools, thereby producing the unusual faunal characteristics.

Perhaps more than any other group, fishes from hyperthermal environments are notorious for their ability to withstand temperature and oxygen extremes. Fishes in our experiments demonstrated no remarkable tolerance abilities. Beitinger et al. (2000) observed that all pupfishes, killifishes, and livebearers tested exhibit CT-Maxima above 40 °C when acclimated at 30 °C or higher. No fish in our experiments attained a CTMax above 40°C, despite being captured at temperatures well above 30 °C. French grunt came closest, with a CT-Max of 37.9 °C, but cocoa damselfish (36.1°C) and white mullet (35.0°C) were captured within a few meters of potentially lethal temperatures. Likewise, hypoxia tolerance in our experiments (0.8 to 1.5 mg/L) was unremarkable when compared to fishes from other adverse habitats. Several studies have documented the ability of hyperthermal pool fishes to tolerate dissolved oxygen levels below 0.5 mg/L and, in some cases, even complete anoxia for an extended time (Packard, 1905; Moore, 1942; Lowe et al., 1967). This somewhat paradoxical finding-sensitive fishes in an unforgiving environment-suggests that physiological tolerance adaptations at Loggerhead Key rockpools are not adequate or necessary for survival. The question then becomes one of how a relatively diverse group of fishes handicapped by limited physiological tolerance limits survives potentially lethal conditions during the warmest parts of the year.

Behavioral adaptation, rather than superior physiological tolerance, is likely the dominant factor sustaining fishes in the rockpools of Loggerhead Key. The site's physical layout insures that wave action and tidal flux keeps some areas of the pools within acceptable tolerance limits. Even without observing exceptionally harsh conditions, we saw that fish enhanced their physiological adaptations by exploiting different microhabitats within the pool environment. French grunt, sergeant major, and schoolmaster juveniles exploited pool margins where extreme conditions and dense cover offered maximum protection. A moderately high CTMax and well-developed ASR response were no doubt helpful to French grunt in these areas as summer progressed. It would be interesting to determine if sergeant major and schoolmaster juveniles use similar tactics. Cocoa damselfish and frillfin goby generally occupied the middle depths. Cooler temperatures in these areas were probably attractive to the heat-sensitive damselfish, but good oxygen tolerance and concomitant reduction in ASR dependence may have been useful as well, since this area was more accessible to predators. White mullet, being intolerant of high temperature and low-oxygen, were probably confined to the relatively deep and cool open-rockpool areas. A yellowtail snapper observed in this region used a different tactic by leaving the pool prior to low tide. With exception of the yellowtail snapper, no large predators entered the rock pool; however, predatory fishes were observed patrolling rockpool openings, which may have dissuaded small fish from entering or leaving.

Loggerhead Key fishes clearly benefit from their association with rockpools, including access to resources, reduced competition, and refuge from predators; however, the ecological benefits to the nearby reef community, if any, are unclear. These habitats did not support fishes other than typical reef or open water species. Moreover, rockpool fish populations and the pools themselves are probably too small to play an important role as a nursery-recruitment area or genetic reservoir. Although the rockpools probably have little impact on reef population structure and ecology, their novel character and simple organization provide opportunities to understand ecological and physiological relationships of some fishes in ways not possible in the complex reef environment.

Acknowledgments.—We thank Tom Schmidt, Robert Brock, and Sonny Bass from the National Park Service, Everglades/Dry Tortugas National Parks for their suggestions and help in developing this project. We also thank Dr. Richard Snyder, Dr. Jim Van Tassell, and the crew of the *R/V Bellows* for their various contributions. Funding was provided by the Florida Institute of Oceanography and the study was conducted under National Park Service Permit #2000053.

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