Journal of Experimental Marine Biology and Ecology 497 (2017) 243–253

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Geographical variation in osmoregulatory abilities among populations of ten species of fiddler crabs from the Atlantic coast of Brazil: A macrophysiological analysis

Carl L. Thurman[a,*], Samuel C. Faria[b,1], John C. McNamara[b,c]

[a] Department of Biology, University of Northern Iowa, IA, USA
[b] Departamento de Biologia, FFCLRP, Universidade de São Paulo, Ribeirão Preto 14040-901, SP, Brazil
[c] Centro de Biologia Marinha, Universidade de São Paulo, São Sebastião 11600-000, SP, Brazil

ARTICLE INFO

Keywords:
Osmoregulation
Physiological ecology
Habitat diversity
Salinity acclimatization
Geographical distribution
Evolution
Fiddler crab

ABSTRACT

Geographical variation is often the earliest stage leading to divergence and speciation. Osmoregulatory ability was assessed in 64 populations of 10 species of semi-terrestrial fiddler crabs along the Atlantic coast of Brazil between Amapá and Santa Catarina. In the laboratory, crabs were exposed for 5 days to media ranging in osmolality from 15 to 3550 mOsm/kg H_2O. Hemolymph osmolality was measured in 10-μL aliquots using a Wescor 5520 osmometer. Survivorship, lower- (LLC_{50}) and upper (ULC_{50}) lethal osmolalities, and isosmotic concentrations [ISO] were estimated in populations for which habitat osmolality was also measured. All fiddler crab species were excellent hypo-/hyper-osmoregulators. Mean [ISO] was < 600 mOsm/kg H_2O in the lone oligosaline species, between 650 and 770 mOsm/kg H_2O in the seven mesosaline species, and > 800 mOsm/kg H_2O in the two eusaline species. Intraspecific variation in [ISO] was significant only in *Minuca rapax*, emphasizing the importance of this parameter as a physiological set-point. Although ULC_{50} varied intraspecifically in six species, habitat osmolality varied significantly for *M. rapax* and *M. victoriana* alone. Thus, intraspecific variation in ULC_{50} likely results from local osmotic acclimatization. Since genetic variation appears to be minor and unstructured across populations in most fiddler crab species, the consistency of [ISO] reflects its importance as a physiological property. In contrast, intraspecific differences in ULC_{50} among populations indicate that this character is ecophenotypic. Unquestionably, physiological studies on fiddler crab populations distributed over a wide geographical range can provide insight into the biological basis of adaptation and the evolution of species in this semi-terrestrial genus.

1. Introduction

The significance of biological patterns as expressed over large spatial and/or temporal scales is becoming more appreciated with time (Orovitz and Hoffman, 2007; Gaston et al., 2009). Many phenomena, once thought to be local, are now recognized to be the consequence of changes occurring on larger geographical and/or geological scales. For ecological and evolutionary studies, experimental observations conducted on a global scale have led to crucial discoveries, revolutionizing these disciplines (Levin, 1968; MacArthur and Wilson, 1973; Endler, 1977). However, in ecological physiology, *macrophysiology*, as it is now known, has received little attention. Provincial investigations focus on comparing physiological characteristics among related taxa: however, studies undertaken on a large spatial scale can reveal patterns of physiological variation that escape detection in locally focused investigations. Only when observations are made on an ample spatial scale can the full extent and significance of inter- and intra-specific physiological variation be assessed and ultimately appreciated.

From a species perspective, geographical ranges vary greatly in dimension and complexity. Within a genus, some species may be limited in distribution to a few degrees of latitude or longitude while others cover a major portion of the planet. For each species, niche breadth may be related to phenotypic and genotypic variation (Levin, 1968; Endler, 1977). Should a species survive over a wide range of ecological conditions, variation in the expression of some traits such as carapace shape and feeding structures will be enhanced. In contrast, if niche preference is finely focused and the organism can survive only when specific habitat components or factors are present, the species is less

* Corresponding author at: Department of Biology, University of Northern Iowa, 144 McCollum Science Hall, Cedar Falls, IA 50614-0421, USA.
E-mail address: Thurman@uni.edu (C.L. Thurman).
[1] Present address: Instituto de Ciências Biológicas, Universidade Federal do Rio Grande, Rio Grande 96203-900 RS, Brazil.

http://dx.doi.org/10.1016/j.jembe.2017.07.007
Received 5 December 2016; Received in revised form 8 June 2017; Accepted 19 July 2017

likely to exhibit significant intraspecific variation across its range. Further, the magnitude of variation can be related to the extent of gene flow among populations across the range and to effective population size (Palumbi, 1994; Sotka, 2012). Consequently, a clearer understanding of the degree and significance of intraspecific physiological variation will arise if viewed from a broader geographical perspective (MacArthur and Wilson, 1973; Endler, 1977; Gaston et al., 2009).

Crustaceans first appeared in marine habitats about 500 million years ago during the Cambrian (Cisne, 1982; Lignot and Charmantier, 2015) and, as seen in other phyla, the early forms evolved to inhabit freshwater and terrestrial habitats (Brusca and Brusca, 1990). The terrestrial transition requires extensive modification of many characteristics, and radical changes in physiology, biochemistry, morphology, behavior and reproduction are required for life on land (Powers and Bliss, 1983; Wolcott, 1992). Crustaceans are most diverse in marine habitats. Today, there are few freshwater and semi-terrestrial crustacean taxa compared to other arthropod classes. Various brachyuran crabs have adapted independently to land using different ecological routes, resulting in unique combinations of physiological and behavioral features (Burggren and McMahon, 1988; Anger, 1995, 2003). However, all but a very few species are obligated to the marine environment through their planktonic larval stages. Adults from a single location release larvae that are transported to remote sites by the tidal and oceanic currents, widely broadcasting the genes of one generation to the next (Sotka, 2012).

Salinity plays a key role in the physiological ecology of decapod crustaceans, and osmoregulatory ability is a primary factor in the transition from the marine to freshwater and/or terrestrial habitats by brachyurans (Péqueux, 1995; Anger, 2003; Freire et al., 2008; McNamara and Faria, 2012; Faria et al., 2017a). Together with the elimination of ammonia-nitrogen metabolic products, maintaining water balance while confronting dehydration is a major challenge for semi-terrestrial crabs (Mantel and Farmer, 1983). Consequently, physiological mechanisms that attenuate internal variation against changing external osmotic conditions are particularly important for species inhabiting extreme niches (Schubart and Diesel, 1999; Tsang et al., 2014). In meeting osmotic demands, some brachyurans have become excellent hyper/hypo-osmoregulators, tolerating from freshwater/oligosaline to hypersaline conditions (Jones, 1941; Vernberg and Vernberg, 1972). Based on patterns of physiological tolerance and the value of internal osmotic set-points, different taxa have clearly adapted to habitats exhibiting different salinity and desiccation pressures (Faria et al., 2017a).

The fiddler crabs constitute a cohort of decapod crustaceans that exhibits numerous biochemical, physiological, morphological and behavioral adaptations to environmental conditions. Many species are distributed throughout tropical and temperate shores in mangroves, bays, lagoons and rivers around the Earth (Crane, 1975; see http://www.fiddlercrab.info/). Recently, Shih et al. (2016) organized the former single genus of *Uca* into nine genera based on a comparison of DNA sequences from several genes. In the southwestern Atlantic, we have studied the distribution of ten species along the coast of Brazil from just north of the Amazon River (Amapá, 2° 35′ 35.90″ N; 50° 50′ 55.8″ W) to the southern states below the Tropic of Capricorn (Santa Catarina, 28° 29′ 45.48″ S; 48° 45′ 49.85″ W) (Thurman et al., 2013). We examined intra- and interspecific variation in ≈7000 live fiddler crabs from 64 sites, recording the general habitat characteristics for each species, finding distinct patterns of habitat preference for salinity and substrate type that underpin the ecological distributions of each species.

Given the habitat preference of each species, here we describe the osmoregulatory capability of each, aiming to answer several questions from two distinct perspectives: ecological physiology and macrophysiology. Firstly, do all fiddler crab species from Brazil exhibit the same osmoregulatory abilities regardless of habitat preference? If not, is habitat difference among species reflected by variation in osmoregulatory physiology? Secondly, does intraspecific osmoregulatory pattern vary over each species' range in Brazil? If so, is the magnitude of variation similar among species? Thirdly, for those species that are more widely dispersed between North and South America, does osmoregulatory capability vary between populations in the two hemispheres? Although focused primarily on fiddler crab osmoregulatory physiology, this study contributes to an integrated understanding of ecological adaptation, diversification, and the evolution of intertidal organisms challenged by habitat heterogeneity.

2. Materials and methods

Field collections, authorized by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA, permits #2009/18559-1 and #2010/23976-1) were made at 64 localities along the Atlantic coast of Brazil between the states of Amapá (AP) and Santa Catarina (SC) (Fig. 1, Supplementary Table S1). From June to November 2009, 49 locations were sampled from Pernambuco (PE) [Itapissuma, Ilha de Itamaracá (northern point)] to SC [Palhoça, Barra da Passagem, and Rio Massiambu]. Fifteen habitats were sampled from AP [Calçoene, Rio Cacoal] to Ceará (CE) [Fortaleza, Rio Cocó] between June and August 2010. These sites were not randomly chosen but represent convenient accesses to coastal habitats by road, track, or boat. However, collections were made randomly at each locality to obtain every species.

Crab collections were taken from a variety of habitats together with recordings of substrate type and surrounding vegetation. A general description of the terrain, including the nature of the substratum, was also recorded for each collecting site (Supplementary Table S1; Thurman et al., 2013). Surface sediments were classified according to Shepard's (1954) nomenclature. Tides along the coast of Brazil are semi-diurnal with amplitudes varying from 0.7 to 3.3 m (Doty, 1957). At each collecting site, a 25-mL sample of habitat water was collected in a 25-mL polyethylene bottle from the nearest significant surface source (bay, river, stream or tidal pool) and habitat osmolality (in milliOsmoles/kg H_2O, mOsm/kg H_2O) was measured later. Habitats ranged from oligosaline rivers ($0 \leq 360$ mOsm/kg H_2O; $0 \leq 12$‰ S [parts per thousand salinity]) through upper to lower intertidal zones to hypersaline salt flats (≥ 1050 mOsm/kg H_2O; ≥ 36‰ S) (Hedgpeth, 1957). Microhabitat temperatures were measured between 1000 and 1600 h on the sediment or substrate surfaces with an infrared thermometer (Icel TD-965, Manaus, Brazil, 0.1 °C precision) and ranged from about 22 to 36 °C among the populations of fiddler crab studied (Faria et al., 2017b).

The ten species of fiddler crabs were identified using traditional morphological characters. Five of these species also occur in North America, and details of their morphologies are described elsewhere (Barnwell and Thurman, 1984). A dichotomous key (Melo, 1996) was used to identify the Brazilian fiddler crabs, together with supplementary descriptions of the cryptic species (Crane, 1943; Holthius, 1967; Chase and Hobbs, 1969; Coelho, 1972; von Hagen, 1987; Tavares and de Mendonça, 2003; Bedé et al., 2007). The species collected during this study were classified among three genera, *Minuca*, *Leptuca*, and *Uca* as proposed by Shih et al. (2016). None of the ten species are either endangered or protected.

2.1. Laboratory maintenance of fiddler crabs

After capture at low tide, crabs were transported to the Centro de Biologia Marinha (CEBIMar), Universidade de São Paulo, on the São Sebastião Channel near São Sebastião, SP, in plastic boxes containing water from the collection site (CEBIMaR Project #2009/06). Within 24 h, crabs and water samples were prepared for analysis at 23 °C. The crabs were divided into two groups, the first consisting of five crabs of each species held with free access to their natural habitat water, and on which measurements were made immediately after arrival at the