Reproductive tactics optimizing the survival of the offspring of
*Cichlasoma orientale* (Perciformes: Cichlidae)

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Abstract: High mortality rates have been observed in Teleost during early developmental stages, as well as great variations in reproductive tactics, which are related to adaptations towards environmental conditions and ecological niches for which different species have a specific response. The objective of this study was to describe reproductive tactics related to the survival of *Cichlasoma orientale* offspring, including aspects of body size, parental care, fecundity, oocyte size and spawning patterns. Samples were performed monthly from August 2011 to July 2013, in lentic and lotic environments at Curu river basin, Brazilian Northeastern semiarid region. Individual behavior (n=113) was observed underwater for over 50 hours by *ad libitum* sampling and focal-animal sampling. Collected individuals (males n=185, females n=95) were evaluated regarding the standard length, batch fecundity, oocyte size and spawning pattern. In females with mature ovaries, oocyte groups at different developmental stages were observed, these cells were counted and measured, and fecundity was estimated by the gravimetric method. Our results showed that the species displayed biparental care behavior and, on average, males were larger than females. Based on 46 ovaries, the average batch fecundity was 2 052±849 (range: 254-3 389). Standard length and batch fecundity were positively correlated, but no correlation was found between oocyte size and standard length. The maximum diameter observed in the most developed oocytes was 1.8mm. The observed distribution of oocyte size classes indicated synchronous oocyte development in three groups: previtellogenic, vitellogenic and mature, showing that *C. orientale* is a multiple spawner. Differences in the amount of oocytes among the three groups were observed, with the most developed group showing the smallest number of oocytes. The combination of low fecundity and large egg size is characteristic of demersal spawners due to a greater environmental stability. Multiple spawning increases chances of survival mainly because of increased fecundity per reproductive season, and the reduced competition among the offspring. We concluded that *C. orientale* makes a heavy investment in larval survival in detriment of the offspring number. Survival is favored by the large size of oocytes, large yolk reserve, biparental care and multiple spawning pattern. Rev. Biol. Trop. 62 (3): 1007-1018. Epub 2014 September 01.

Key words: fecundity, oocyte size, multiple spawning, biparental care, reproduction.

Teleosts make large investments in reproduction (Duarte, & Alcaraz, 1989) due to high mortality rates in early stages of life (McGurk, 1986). Offspring survival is safeguarded thanks to a range of reproductive tactics involving fecundity, egg size, spawning patterns and parental care, depending on environmental conditions and ecological niche (Winemiller, 1989; Murua, & Saborido-Rey, 2003).

Fecundity plays an important role in recruitment (Rickman, Dulvy, Jennings & Reynolds, 2000) and is regulated by environmental conditions, food availability, predation and genetic factors (Kjesbu, Hunter,
& Witthames, 2003; Ganias, 2013). Teleosts fecundity may be determinate or indeterminate (Hunter, Lo, & Leong, 1985). In the former case, oocyte recruitment is completed before spawning begins; thus, the number of mature oocytes in the ovary corresponds to the potential annual fecundity (Hunter, Macewicz, Lo, & Kimbrell, 1992). In the latter case, oocyte recruitment and spawning overlap; thus, potential fecundity is not determined prior to spawning (Hunter et al., 1992; Fernandes, Oliveira, Travassos & Hazin, 2012).

Recruitment is also dependent on egg size and spawning pattern. Thus, egg size is inversely proportional to fecundity (Reznick, 1985; Winemiller, 1995) and is a major determining factor of larval quality, with considerable impact on survival rates (McGurk, 1986). The spawning pattern is characterized by the number of batches per reproductive season and the duration of the season. These aspects have an influence on the number of eggs generated per season and on larval competition and predation (Nikolsky, 1963; McEvoy, & McEvoy, 1992).

Parental care increases substantially the quality of embryonic development and the chances of offspring survival (Korzelecka-Orkisz et al., 2012). Depending on the species, parental care may be provided by the female or the male, or both (Gross & Sargent, 1985). This behavior comprises a range of protective tactics, from aerating to guarding eggs and larvae against predators (Zworykin, Budaev & Mochek, 2000).

Environment may influence tactics developed by a certain species to ensure its offspring survival, with each species presenting a specific response to environmental changes (Steams, 1976; Winemiller, 1989). This was evidenced in a study that evaluated how species presented changes in their reproductive characteristics in a seasonal environment (Winemiller, 1989). This author defined three strategies that indicate kinds of reactions to environmental variations. The first is an opportunistic strategy, in which species present a high fecundity, short reproductive season and low offspring survival. Concluding the triangular model of strategies, the equilibrium strategy is performed by density dependent species, that perform parental care, exhibit a long reproductive season and low fecundity with large eggs, and generally live in habitats with limited resources (Winemiller, 1989, 1995; Zeug, & Winemiller, 2007).

Among teleostean taxons, Cichlidae displays one of the greatest variations in reproductive strategies (Lowe-McConnell, 1969) with a highly variable fecundity (Araújo, Nascimento, Yamamoto, & Chellappa, 2012). This family invests great amount of energy in offspring survival, presenting parental care, which is performed by one or both genitors (Lowe-McConnell, 1969), and building nests that are the characteristics that allow eggs to experience a safe habitat during the initial development. This family is naturally distributed in Central and South America, Africa, Middle East, Iran, India and Sri Lanka (Kullander, 2003). The neotropical cichlid species *Cichlasoma orientale* Kullander 1983, object of the present study, is considered endemic to Brazilian semiarid regions (Kullander, 2003).

The objective of this study was to describe reproductive tactics related to offspring survival of *Cichlasoma orientale*, including aspects of body size, parental care, fecundity, egg size and spawning patterns, based on a population observed in the Curu river basin, Northeastern Brazil.

**MATERIALS AND METHODS**

Sampling was performed at the Curu river basin in three sites. The first site (3°47’03” S - 39°24’46” W) presents lentic characteristics due to a dam and the other two sites (3°49’08” S - 39°19’57” W and 3°57’08” S - 39°25’56” W) are lotic environment. In these lotic environments water volume varies greatly throughout the year, according to rain regimes, varying from approximately 6m wide during dry season to 50m wide during rainy season. The semiarid
regions of Northeastern Brazil are subject to irregular and sometimes extended drought periods (Krol, Jaegar, Brontert & Krywkow, 2001; Chiang & Coutavas, 2004). Rainfalls vary from 240 to 1500mm per year, with average temperatures in the range 24-26ºC (Nimer, 1972). The rainy season is characterized by intense showers, usually from March to July (Bouvy, Falção, Marinho, Pagano & Moura, 2000). Most rivers and streams in the region are intermittent, with little or no flow during the dry season (Maltchik, 1999). Due to precipitation variations in the region, the main basins have been modified with a great number of dams built in the main rivers stream beds, which ensures water to all human population in order to accomplish their activities during drought season (Andrade, Araújo, Rosa, Gomes, & Lobato, 2007; Chellappa, Bueno, Chellappa, Chellapa, & Val, 2009).

The description of parental behavior was based on 50 hours and 24 minutes of underwater observations covering a 100m stretch of Curu river from November 2011 to April and September 2012. Data were collected by ad libitum sampling (Lehner, 1996) and focal-animal sampling, observing a single individual for up to five minutes (sensu Sabino, 1999). Behaviors were classified into four categories (agonistic, offspring protection, nest patrolling, and pair formation) and quantified by a single stationary diver (LRM), far enough (approx. 2m) from observed individuals to not interfere with their behavior (Sabino, 1999).

Individuals sampled monthly from August 2011 to July 2013 were used to obtain information on standard length, batch fecundity, oocyte size and spawning pattern. Fishing gear consisted of seine nets with 0.5mm mesh size and gill nets with 40-100mm mesh size. Sampled individuals were anesthetized with eugenol (concentration = 100mg/L) and euthanized with ice, in accordance with Brazilian environmental legislation (sampling authorized by ICMBio under entry #28 385-2). Voucher specimens of *C. orientale* were deposited in the fish collection at the Federal University of Paraíba (UFPB 7 067; UFPB 7 081).

In the laboratory, individuals were measured (standard length in cm) and dissected to determine sex and gonadal maturation stage. Gonads were classified into I – immature: very small gonads and translucent, characteristic of young individuals that have not reproduced yet. Histologically ovaries present only oocytes in chromatin nucleolar phase (stage I) and perinucleolar (stage II) and in testis primary and secondary spermatogonia are predominantly observed. II – in maturation: ovaries are orange and it is possible to observe some oocytes with the naked eye, while testis have a whitish color. Histologically it is observed the presence of oocytes in stages I, II and cortical alveoli (stage III) in ovaries and spermatogonia, spermatoocytes and spermatids in testis. Also, at the end of this stage some spermatozooids can be observed in testis lumen. III – mature: macroscopically ovaries present a color varying from yellow to light brown, and occupy most of the coelomic cavity and one can easily identify the oocytes with the naked eye, and testis are turgid and whitish. Histologically it can be observed the presence of oocytes in phases I, II, III and mature (stage IV) in ovaries, and a great number of spermatozooids in the testis lumen. IV – partially spawned: ovaries and testis are flaccid and it is possible to observe in some cases some hemorrhagic zones. Histologically, ovaries show empty spaces, oocytes in stages I to IV and atretic and post ovulatory follicles. In testis lumen there are some residual spermatozooids and spermatogonia.

Ovaries containing mature oocytes were preserved in Gilson’s fluid for oocyte dissociation (Bagenal, 1967), and after dissociation they were washed and preserved in ethanol 70% for subsequent measurement and count under a stereomicroscope (Hunter et al., 1985). Spawning was inferred based on histological observations of 76 ovaries from different stages (in maturation, mature, and partially spawned) considering oocyte size class.

For histology, ovary samples were immersed in Karnovsky’s fixative for 24 hours, dehydrated in a series of increasing alcohol concentrations, embedded in resin (Leica),
sectioned in 3µm sections and stained with hematoxylin-eosin (adapted from Junqueira, & Junqueira, 1983).

The batch fecundity was estimated gravimetrically based on the most developed oocytes (oocytes larger than 900µm), as proposed by Hunter et al. (1985) for species with indeterminate fecundity. The oocytes of three subsamples, corresponding to approximately 10% of the total gonad weight, of each ovary were counted. Based on the number of oocytes in each subsample, the total number of oocytes at each development stage was estimated, and average values were calculated for the ovary.

Sexes were compared with regard to standard length using the Mann-Whitney test for variables without normal distribution or homoscedasticity. The association between standard length, batch fecundity and oocyte size was evaluated with Pearson’s correlation test. Whenever correlations were significant ($p < 0.05$), the residues were tested for normality (Shapiro test) and homoscedasticity (Goldfeld-Quandt test). Once these assumptions were met, regressions were performed. The type of regression was defined by the highest $r^2$ values. The numbers of oocytes at different stages of development were compared using the paired $t$ test.

RESULTS

A total of 113 individuals of *C. orientale* were observed underwater. The fish remained mostly in still waters near the river bank, in groups of up to five individuals.

Four different categories of behavior were observed (Fig. 1). Agonistic behavior, the most frequently observed category (56.52%), was defined as persecuting any other fish, regardless of species. Pair formation, the second most frequently observed category (26.09%), was defined as swimming together as a pair for up to five minutes with some form of reproductive

**Fig. 1.** Categories of behavior observed to *Cichlasoma orientale* from Curu river, Pentecoste - CE (A=agonistic; B=pair formation; C=protecting offspring; D=patrolling nest).
interaction. Protecting the offspring (pair encircling group of juveniles) was observed three times (13.04%). Patrolling the nest (adult guarding leaf covered with eggs) was observed only once (4.35%).

Standard length analysis, batch fecundity, oocyte size and spawning pattern was based on 185 males and 95 females collected for the study. Median standard length was 11.91cm (range: 4.01-16.22) for males and 9.55cm (range: 4.07-13.87) for females. Thus, males were larger than females (U=4 518.000; p<0.0001) (Fig. 2).

Almost half the females (46/95) carried mature oocytes. These females were present in all months of the year (Fig. 3). The mean standard length of females carrying mature oocytes was 10.38cm (range: 4.34-12.80). Batch fecundity was 2 052±849 on the average (range: 254-3 389). The two parameters (batch fecundity and standard length) were directly related when analyzed by power function regression (r=0.87; t=11.53; gl=44; p<0.0001) (Fig. 4).

The oocytes of *C. orientale* are elliptical (measurements refer to the greatest diameter).

Three stages of oocyte development were identified (Fig. 5):

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**Fig. 2.** Distribution of relative frequency according to standard length class interval for males and females of *Cichlasoma orientale* based on 280 specimens from the Curu river basin (Ceará, Northeastern Brazil).

**Fig. 3.** Relative monthly frequency of developmental gonadal stages in females and males of *Cichlasoma orientale* from Curu river basin, Ceará, Northeastern Brazil (years grouped).

**Fig. 4.** Relation between batch fecundity and standard length of *Cichlasoma orientale* based on 46 individuals from the Curu river basin (Ceará, Northeastern Brazil).
• **Previtellogenic oocytes.** Size <150µm. Under a stereomicroscope, these oocytes appear whitish. Under light microscopy, the cytoplasm is characterized by intense, homogenous basophilia. Average number of previtellogenic oocytes per ovary: 10 570±4 813.

• **Vitellogenic oocytes.** Average size 400µm (range: 250-500). These oocytes are orange-colored when observed macroscopically. Under light microscopy, yolk granules are visible, especially in the peripheral cytoplasm. Average number of vitellogenic oocytes per ovary: 2 550±1 087.

• **Mature oocytes.** Size ≤1.8mm. Clearly visible to the naked eye, these oocytes vary in color from orange to light brown. Under light microscopy, the cytoplasm appears filled with yolk granules. The nucleus is difficult to locate due to its small size in relation to the oocyte. The number of fully mature oocytes is on the average 20% smaller than the number of vitellogenic oocytes (t _par_ =180; gl =44; p=0.044). The oocytes in this group are released in the following spawn.

The oocytes at the same maturation stage varied very little in size within each ovary, but variation was somewhat greater between different individuals, especially with regard to mature oocytes. The latter measured as little as 900µm in less developed ovaries, and up to 1 800µm in more developed ovaries. No correlation was found between oocyte size and standard length (r=0.22; t=1.19, p=0.20).

In addition to the three stages described above, 82% of the analyzed ovaries contained whitish and brittle cells identified as atretic oocytes. These varied up to three times in size within the same ovary.

**DISCUSSION**

Compared to most Teleosts, *C. orientale* produces few and large oocytes. This pattern has been observed for other species in this genus (Townshend & Wootton, 1984; Martinez-Palacios, & Ross, 1992; Chavez-Lopes, Peterson, Brown-Peterson, Morales-Gómez, & Franco-López, 2005) and family (Coward & Bromage, 1999; Campos-Mendoza, McAndrew, Coward,
Teleosts fecundity vary greatly regardless of size (Mazzoni & Caramaschi, 1997; Serezli, Guzel, & Kocabas, 2010; Casimiro, Garcia, Almeida, & Orsi, 2011; Juchno & Boroń, 2012; Peressi, Gonçalvez, & Braga, 2012; Fernandes et al., 2012; Trujillo-Jiménez, Sedeño-Díaz, Camargo, & López-López, 2013), indicating the existence of an array of reproductive tactics, concentrating resources on the production of either many and small or few and large eggs (Wooton, 1984, Casimiro et al., 2011). The relation between size and number is among other things associated with the habitat in which spawning takes place (Duarte & Alcaraz, 1989). According to these authors, larval mortality among pelagic spawners is mainly regulated by environmental variability. In the pelagic zone, greater fecundity means more eggs hatching in a favorable habitat, thus greater reproductive success. On the other hand, demersal spawners have more control over the circumstances in which their eggs will hatch, thereby reducing the variability of growth conditions. Thus, because demersal fishes invest more heavily in larval survival, oocytes tend to be larger (Duarte & Alcaraz, 1989).

The demersal spawning pattern observed for *C. orientale* (few and large eggs) indicated that the species invests heavily in larval survival. Indeed, *C. orientale* also engages in biparental care, as observed for many other cichlids (Winemiller, 1995; Teresa & Gonçalves-de-Freitas, 2011; Korzelecka-Orkisz et al., 2012). These characteristics are associated with the equilibrium strategy guild proposed by Winemiller (1989), which to a large extent coincides with the K strategy for adaptation to life in resource-poor or density-dependent environments (MacArthur & Wilson, 1967; Pianka, 1970). The species in this guild make great efforts to ensure the survival of their offspring by providing them with significant amounts of yolk and/or by way of direct parental care during the early stages of life (Winemiller, 1995; Korzelecka-Orkisz et al., 2012). The individuals observed underwater in the Curu river basin engaged in biparental care, thereby increasing the chances of larval survival. Parental care includes any form of direct protection of the offspring after fertilization (Gross & Sargent, 1985), whether it be the eggs (e.g. nest patrolling) or free-swimming larvae and juveniles (e.g. accompanying juveniles) (Baylis, 1981). Larger eggs are associated with increased survival potential as larger larvae are subject to lower mortality rates (McGurk, 1986): they feed on larger food items, swim faster and carry larger amounts of yolk with which to resist periods of food scarcity while searching for a favorable habitat (Duarte & Alcaraz, 1989). Larger larvae are also easier to defend from predators (Souza, Fragoso-Moura, Freneich-Verani, Rocha & Verani, 2008). The elliptical shape of the oocytes of *C. orientale* makes it possible to deposit eggs in smaller clusters, facilitating patrolling and aeration (Korzelecka-Orkisz et al., 2012). Several species of cichlids have elliptical eggs, with similar benefits (Coward & Bromage, 1999; Korzelecka-Orkisz et al., 2012).

*C. orientale* differs from the majority of teleosts by the larger size of the male. These differences in sexual ratio have been explained by different hypothesis, such as physiological differences between sexes, mechanisms that makes males more inclined to be captured or less inclined to natural mortality (Boussou et al., 2010), growth rates (Kume & Joseph, 1966) and longevity (Wu, Su, & Kawasaki, 2001). Usually, a larger body volume would allow the female to allocate more eggs and therefore might be considered an evolutionary advantage (Moraes et al., 2013). In Cichlidae, however, males tend to be larger than females (Garcia-Lizárraga et al., 2011, Araújo et al., 2012), possibly because larger males protect the offspring more efficiently, thereby ensuring greater reproductive success (Keenleyside, 1991). Likewise, while both parents of the cichlid species *Laetacara araguaiae* patrol the nest, the male displays more developed parental behaviors, especially in the premating stage and when guarding eggs and larvae (Teresa & Gonçalves-de-Freitas, 2011). Size also seems to make a significant difference in courtship.
Experiments with the cichlid species *Pterophyllum scalare* have shown that larger and more aggressive males have more mating opportunities than smaller males (Cacho, Yamamoto, & Chellappa, 2007).

The positive correlation between batch fecundity and standard length among females of *C. orientale* has been observed for other fish species (Mazzoni & Iglesias-Rios, 2002; Ortega-Salas, Cortés, & Reyes-Bustamante, 2009; Rodrigues & Macchi, 2010; Serezli et al., 2010; Araújo et al., 2012; Peressin et al., 2012; Reid & Chaput, 2012). The correlation may be explained by the fact that larger individuals have more energy available for the production of oocytes (Patimar & Mohammadzadeh, 2011) and larger females have more space in the abdominal cavity, providing greater fecundity.

In many teleosts, when analyzed intra-specifically, egg size and standard length are positively correlated: older and larger females tend to produce larger oocytes, increasing chances of larval survival (Rana, 1988; Kjesbu, 1989; Reid & Chaput, 2012). However, no such correlation was observed in the present study, matching reports for other species of cichlids (Rana, 1988; Coward & Bromage, 1999) and for some species of teleosts (Rodrigues & Macchi, 2010; Peressin et al., 2012). Within a given population, egg size may be influenced by detrimental habitat changes (Hainfellner, Souza, Moreira, Makaghi, & Batlouni, 2012) or by the number of previous spawns in the reproductive cycle (oocytes tend to be larger in the first spawns) (Kjesbu, 1989).

Even in mature ovaries, oocytes continue to grow due to constant vitellogenesis. The ovaries examined to estimate batch fecundity were considered mature, but oocytes in the third stage of maturation displayed different stages of development according to the proximity of spawning. Further investigations determining oocyte size at the moment of spawning are required to confirm the absence of a correlation between oocyte size and standard length in *C. orientale*.

Group-synchronous oocyte development is discernible in the distribution of absolute frequency of oocyte size in *C. orientale*, indicating the species is a multiple spawner. Teleosts with this spawning pattern usually produce few eggs, several times over several months (Growns, 2004). A common tactic in tropical and subtropical species, multiple spawning increases the survival chances of a species (Nikolsky, 1963; Hunter et al., 1985) and offers several advantages: i) it increases the number of eggs spawned throughout the reproductive period (Nikolsky, 1963), ii) the spawn is exposed to a variety of environmental conditions so that at least some of the offspring may develop under ideal conditions (Growns, 2004), and iii) it reduces competition between larvae/juveniles and predation of eggs and larvae (McEvoy & McEvoy, 1992). Many ciclids are multiple spawners (Winemiller, 1989; Coward & Bromage, 1999; Paugy, 2002; Campos-Mendonza et al., 2004; Araújo et al., 2012; Korzelecka-Orkisz et al., 2012); in such species, fecundity is high (considering the length of the reproductive period) and survival is favored as larval competition is minimized.

The fact that the number of fully mature oocytes was approximately 20% smaller than the number of vitellogenic oocytes may be explained by atresia. Atretic oocytes have been described for many teleosts species (Byskov, 1978). Cichlids present a complex reproductive behavior which can be influenced by biotic parameters and environmental dynamics (Keenleyside, 1991b). Environmental characteristics such as diminished amount of food or high predation levels can be associated with a greater amount of females with atretic oocytes. Thus, in females with limited energy reserves, oocyte development may be interrupted, thereby regulating the number of eggs produced (McEvoy & McEvoy, 1992), generating just the ideal number to the conditions the environment presents at that moment. The seasonal environment of the semiarid region may yet be of great influence in the great amount of females found carrying atretic oocytes due to drastic variation in water level and consequent availability of mesohabitats to build nests.
El manejo de táticas reprodutivas observado para *Cichlasoma orientale* en el curu río muestra que la especie hace un gran esfuerzo por el desarrollo larvario en hembras. Con base en 46 ovarios, el tratamiento de cuidado biparental y, en promedio, los machos presentan un patrón de desove. Las especies muestran un comportamiento de cuidado biparental y una gran variación en las tácticas reproductivas, que están relacionadas con adaptaciones a las condiciones ambientales y nichos ecológicos con cada especie y que presentan una respuesta específica. El objetivo de este estudio fue describir las tácticas reproductivas relacionadas con la supervivencia de la descendencia de *Cichlasoma orientale*, incluyendo aspectos tales como el tamaño corporal, el cuidado parental, la fecundidad, el tamaño de los ovocitos y los patrones de desove. Las muestras se recolectaron mensualmente desde agosto de 2011 hasta julio de 2013, en ambientes lénticos y lóticos en la cuenca del río Curu, región semiárida del noreste de Brasil. El comportamiento individual se observó de desove múltiple. Se observaron diferencias en la cantidad de ovocitos entre los tres grupos, con el grupo más desarrollado que muestra el menor número de ovocitos. La combinación de la baja fecundidad y gran tamaño del huevo es una característica de las reproductoras demersales, debido a una mayor estabilidad del medio ambiente. Múltiples desovos aumenta las posibilidades de supervivencia, principalmente debido al aumento de la fecundidad por temporada reproductiva y reducción de la competencia entre la descendencia. Llegamos a la conclusión de que *C. orientale* hace una fuerte inversión en la supervivencia de las larvas en detrimento del número de descendientes. La supervivencia se ve favorecida por el gran tamaño de los ovocitos, gran reserva de yema de huevo, cuidado biparental y patrón de desove múltiple.

**Palabras clave:** fecundidad, tamaño de los ovocitos, desovos múltiples, cuidado biparental, reproducción.

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