ASPECTS OF THE LIFE HISTORY OF *Cichla temensis* (PERCIFORMES:

CICHLIDAE) AND ITS RELATIONSHIP TO THE AMAZON BASIN'S FLOOD

PULSE

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PAUL REISS

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ABSTRACT OF THE DISSERTATION

Aspects of the Life History of *Cichla temensis* (Perciformes: Cichlidae)

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Abstract – *Cichla temensis,* the largest of the Neotropical "peacock basses", is an economically important species for both sportfishing and human consumption in the central Amazon lowlands. As an apex predator, it is also an important component of its ecosystem. Due to taxonomic questions and a dearth of definitive information about the species' life history and relationship to its natural flood pulse habitat, there has been a lack of effective management tools and conservation policies to protect this economically and ecologically important species. The principle motivation for the work performed for, and described in, this dissertation is to clarify these topics and provide a foundation for the creation of effective future management tools.

In Chapter 1, a three-part study using morphometric, reproductive and molecular data showed that variants of *Cichla temensis* are members of a single species whose individuals undergo a yearly color and pattern variation correlated to their specific degree of seasonal sexual maturation.

Chapter 2 used geometric morphometric techniques to analyze shape changes in the variants and their relation to the deposition of fatty reserves necessary for prespawn

gametogenesis and postspawn maintenance during a protracted fast associated with brood guarding behavior. The study showed that the behavioral and physiological characters analyzed are related to each other and to their environment's yearly flood pulse.

Chapters 1 and 2, in conjunction with prior studies and personal observation, laid a foundation that served as the basis for Chapter 3. Habitat use and spawning behavior were analyzed in relation to flood pulse conditions and grade of color and pattern variation. The temporal and spatial linkages provided by these results enabled, for the first time, a synthesis of the species' life history with the Amazon basin's pulsative ecology.

This new body of information can help provide a foundation for future management tools and conservation policies to maximize the economic benefits provided by this species while simultaneously creating the sustainability to protect the unique ecosystem in which it plays an integral role.

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Dedication

I dedicate this dissertation to my future wife, Helen, whose generosity, graciousness and

strength allows me to share my heart with my other great love, the Amazon.

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Introduction

Cichla temensis is a large, Neotropical, predatory fish of considerable economic importance, both as a sportfish (Myatt *et al*., 2005; Holley *et al*., 2008; Thomé-Souza *et al*., 2014) and for human consumption (Smith, 1981; Goulding *et al*., 1996). Called 'tucunaré' in Brazil and 'pavon' in Spanish speaking countries, the term "peacock bass" is applied to all fifteen currently recognized species within the genus *Cichla*, a basal genus within the family Cichlidae (Stiassney, 1987; Farias *et al*., 1999; Kullander & Ferreira, 2006).

The family Cichlidae has received much attention due to its rapid speciation in African rift lakes (Barlow, 2000). African cichlid radiation has been attributed in part to the high degree of variability of cichlid pharyngeal jaw configurations (Konings, 1995) enabling the rapid exploitation of multiple ecological niches within a lacustrine system. Neotropical cichlid diversification, however, is less spectacular and more likely attributable to long-term genetic changes resulting from large-scale geological vicariance (Willis, 2007). Within the family, the South American genus *Cichla* has posed various taxonomic challenges. *Cichla* was long considered to be a sister genus to the elongate predators, *Crenicichla* (Stiassny, 1987). However, more recently, molecular analysis indicates that *Cichla* is more closely aligned within a clade that includes the genus *Astronotus* (oscars), (Farias *et al*. 1999, 2000). Until 2006, taxonomists had recognized only five species, although many researchers had suggested that several additional undescribed species existed. A revision of the genus based on morphological data

expanded the number of accepted species to fifteen (Kullander & Ferreira, 2006). Taxonomic uncertainty still remains, however. Karyological evidence suggests that hybridization may have occurred (Brinn *et al*. 2004) while molecular data suggests that groupings within the genus may differ from the morphologically based species descriptions (Willis, 2007). Within the species, *C. temensis* itself, there have also been ongoing taxonomic questions that are addressed by this dissertation.

Cichla temensis is found in lowland flood pulse rivers in the central Amazon basin and appears to be restricted to tannin stained, acidic, blackwater systems (Winemiller, 2001). It inhabits regions of the Amazon River basin, including the Rio Negro and Uatuma drainages as well as Venezuelan and Columbian tributaries of the Orinoco (Kullander, 2003). Additionally, an active sport fishery exists for *C. temensis* in blackwater tributaries of the Rio Madeira and Rio Branco (Kullander, 2006; Holley, *et al*., 2008; Reiss *et al*., 2012).

Within flood pulse systems, *C. temensis* are subject to large variations in available habitat. They occur in both lagoon (lentic) and river (lotic) habitats. Changing water levels and conditions associated with the flood pulse affect the selection of habitat, so that at varying times in the seasonal rainy/dry cycle, primary habitat selection can swing from lentic to lotic (Jepsen *et al*., 1997).

Cichla temensis can grow rapidly during its first year, although information from its natural flood pulse habitat is lacking. In aquaculture facilities, presumed *C. temensis* <50 mm were found to grow 1.0 mm per day, while fish 50-100 mm in length grew at 0.8 mm per day (Braga, 1953). A study of *C. temensis* reared in Texas ponds indicates that a cohort of 50 mm fish grew an additional 168 mm during a 5-month period, averaging 33

mm per month (Rutledge & Lyons, 1976). In their natural habitat, *C. temensis* larger than 350 mm all show some degree of gonad maturation during their peak regional spawning season (Jepsen *et al*., 1999). Based on otolith thin sections for age estimation and the relationships between length at presumed age, most fish reach sexual maturity at age I (Jepsen *et al*., 1999). Growth rates appear to slow significantly at larger sizes (Jepsen *et al*., 1999). *Cichla temensis* captured by sport and commercial fishermen can reach sizes exceeding 13 kg and 1 m in length (Myatt *et al*., 2005, 2012; personal observation).

      Cichla temensis is a substrate brooder whose pairs form monogamous bonds apparently for the duration of the spawning cycle (Jepsen, *et al*., 1997). *Cichla* in captivity or in reservoir environments can spawn several times per year (Chellapa *et al*., 2003a, 2003b), however, seasons in *C. temensis'* natural, pulsative habitat promote restricted reproductive seasonality, with a single yearly spawn per individual likely (Jepsen *et al*., 1999). After hatching and upon completion of the embryonic stage, the adults begin guarding the now free-swimming larva (Jepsen, *et al.*, 1997). They maintain guard over their brood for up to 2 months until the young begin to become piscivorous and the juveniles disperse at about 60 mm standard length. (Jepsen, *et al.*, 1997; personal observations). Spawning *Cichla* stop or drastically slow feeding activity during this period (Zaret, 1980; Correa, 1998; Jepsen *et al*., 1999).

      Little data regarding early life history in *C. temensis'* natural, pulsative habitat is available; however, *C. ocellaris* and *C. monoculus* have been thoroughly studied in reservoir environments. These species exhibit elaborate parental behaviors and are egg, embryo and larva "keepers" (Gomiero & Braga, 2004). *Cichla ocellaris* parents guard their young for approximately two months. Similar behavior has been observed with *C.*

temensis (personal observation). After leaving the parentally guarded school, these juveniles exist as solitary individuals living in dense cover. After about six months, these approximately 180 mm juveniles return to more open habitat and join schools of similarly sized juveniles (Zaret, 1977). The author has observed these same behaviors in juvenile *C. temensis* (personal observation).

Adult *Cichla temensis* is a piscivorous predator with a variable diet regime. Its large size relative to its trophic position in tropical food webs enables it to consume a wide variety of prey species and sizes and to exploit seasonal variations in prey availability (Layman *et al*., 2005). For example, during falling water periods in Venezuela, *Semaprochilodus kneri*, a relatively large, migratory detritus feeder makes up a majority of adult *C. temensis'* diet (Hoeinghaus *et al.*, 2006). In contrast, during low water periods, *C. temensis* will opportunistically feed on a wide variety of smaller species ranging from small water column predators to benthic armored catfishes (Jepsen *et al*., 1997; Layman *et al*., 2005). A study in a reservoir environment (Guri) demonstrated that *Cichla temensis'* introduction can have a dramatic impact on abundance of selected prey species (Williams *et al*., 1998).

Cichla temensis in turn is preyed upon by freshwater dolphins ('boto' - *Inia geoffrensis*), caiman ('jacare' – *Melanosuchus niger*) and large Amazon otters ('ariranha' – *Pteronura brasiliensis*) (Kircher, 1997). Piranha of several species are opportunistic fin and scale eaters of larger fishes (Goulding, 1980). Evidence of this predation can be seen in the commonly occurring torso scarring and irregularity of fin edges in *C. temensis* specimens (personal observation). Reported parasites of *Cichla sp*. include the trematode *Diplostomum compactum* (Santos *et al*., 2002), the tapeworm *Sciadocephalus*

megalodiscus, (Pertierra, 2003) and the coccidian, *Calyptospora tucunarensis,*
specifically infecting *C. temensis* (Santiago 2012)

 Ultimately, however, in spite of the presence of predators and parasites, it is most likely humans that are the most important source of mortality to *C. temensis*. *Cichla spp.* have long represented an important proportion of the commercial catch in much of central Amazonia (Smith, 1981; Goulding, 1996). Rapid human population growth in this region is motivating ever more aggressive and far reaching efforts at harvesting these species (Inhamuns *et al.*, 2009). Coupled with continuing loss of Amazon habitat, it is likely that *C. temensis'* naturally occurring lowlands population will diminish over time if not protected by effective management and conservation efforts.

 In spite of its economic importance, *Cichla temensis'* life history and ecology has not been extensively studied throughout much of its natural habitat. A series of important studies and a thorough ecological review of *C. temensis* that essentially laid the groundwork for this dissertation were performed in Venezuela (Jepsen *et al.*, 1999; Winemiller, 2001; Hoeinghaus *et al.*, 2003; Layman *et al.*, 2005; Arrington *et al.*, 2006). However, with the notable exception of Holley *et al.*, (2008) and Thomé-Souza *et al.*, (2014), who focused on the sport fishery and Willis (2005, 2015 - in preparation) who focused on population genetics, little work concentrating on *Cichla temensis'* has been carried out in the enormous central Amazon lowlands of Brazil. This region comprises the bulk of *C. temensis'* distribution and demonstrates the greatest effect of its flood pulse environment. It is in this region that the greatest need for management and conservation will occur.

To further challenge our understanding of *C. temensis*, there has also been a history of confusion about differing color and pattern variants within the species, itself. Disagreement existed as to whether these morphs actually comprised a single species or several species or subspecies. Some suggested the variants were evidence of sexual dimorphism. This uncertainty was demonstrated by a wide array of earlier scientific names (Kullander, 2003). Additionally, materials produced by governmental departments and associated offshoots (i.e., The Brazilian Agricultural Research Corporation (EMBRAPA) and the Brazilian Tourism Agency (BrasTUR) used a variety of common names and nonspecific scientific nomenclature, as did older publications (Smith 1981) and the sportfishing community (Myatt *et al*., 2005). Clearly, in order to effectively study a species and develop management criteria, it is critical that its taxonomy be definitive.

The overall objectives of this dissertation were to clarify *Cichla temensis'* taxonomy, to explain its color and pattern variation, to more definitively describe aspects of its life history and to improve our understanding of its relation to its flood pulse river environment, This dissertation is presented in three chapters, either previously published as stand-alone papers or written for future publication. Thus, these chapters include some similar introductory and supporting information.

The work performed in Chapter 1 of this dissertation (Reiss *et al*., 2012) has resolved the taxonomic question. Color and pattern variants of *Cichla temensis* were shown to be a single species whose individuals undergo a yearly cycle of color and pattern variation correlated to the specific degree of an individual's seasonal sexual maturation. In the process of completing this study, a semi-qualitative grading system of

color and pattern variation (CPV) was defined for this species. This made it possible to statistically analyze *C. temensis* life history data and to correlate it with other aspects of the species' ecology.

Future management will also be dependent on an understanding of C. *temensis'* specific physiological, morphological and behavioral characteristics. The questions addressed in Chapter 2 were posed by gaps in our prior knowledge, aspects of the species' pulsative environment and an apparent change in shape that relates to the individual's CPV grade (Reiss & Grothues, 2015). Although it is recognized that *C. temensis* is a capital breeder (Arrington 2006), the details of its reserve deposition and their utilization were not clear in relation to its spawning behavior and integration with its environment. This study confirmed temporal correlations between reserve deposition, spawning behavior and flood pulse characteristics. It further indicated that the deposition and consumption of liver reserves and corporal reserves are disjunct in this species.

Finally, Chapter 3 introduces new information enabling correlations between the life history aspects discerned in the first two chapters and the Flood Pulse Concept (FPC) (Junk *et al*., 1989; Junk & Wantzen, 2004). The FPC is the theoretical framework for limnological studies in river-floodplain systems, such as the Amazon. This has enabled, for the first time, the creation of a detailed synthesis of what is known about *C. temensis* in relation to its high amplitude flood pulse river environment. Hopefully, this will provide a foundation for the creation of effective management and conservation tools in this region as well as a fertile bed for future work to further clarify and expand our understanding of this unique and important animal.

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Chapter 1

Color pattern variation in *Cichla temensis* (Perciformes: Cichlidae):

Resolution based on morphological, molecular, and reproductive data

Abstract

Morphological variants of *Cichla temensis,* readily differentiated by their striking color pattern differences, are found in several Amazon basin flood pulse river systems. The adult variants have at times been thought to represent different species or sexual dimorphism. A three part study was performed in two regions in Brazil (rio Igapó Açú and rio Caures) to elucidate the nature of the variants. In part one; selected diagnostic morphometric characters were compared intraspecifically among the variants and interspecifically with *C. monoculus* and *C. orinocensis.* All of the *C. temensis* variants were found to differ significantly from their sympatric congeners while not differing among each other. In part two, mitochondrial DNA samples were compared intraspecifically among the variants and interspecifically with their sympatric congeners. There were no diagnostic molecular synapomorphies that would unambiguously distinguish the variants and all *C. temensis* variants were clearly diagnosable and divergent from their sympatric congeners. In part three, color pattern variation in both sexes was compared to a gonadosomatic index (GSI). A significant correlation between color pattern variation and gonadosomatic index was found. The results of this study

demonstrate that *Cichla temensis* variants are confirmed to be members of a single species and that the variation does not represent a sexual dimorphism. The color pattern variation is a cyclically occurring secondary sexual characteristic and is indicative of the specific degree of an individual's seasonal sexual maturation.

Variantes morfológicas de *Cichla temens*is, facilmente diferenciados por seus padrões de coloração marcantes, são encontrados em vários rios do sistema de inundação da várzea da bacia Amazônica. Variantes dos indivíduos adultos têm sido cogitados como pertencerem a uma diferente espécie ou apresentarem dimorfismo sexual. Um estudo em três etapas foi realizado em duas regiões no Brasil (rio Igapó Açú e rio Caures) para elucidar a natureza desta variação. Na primeira parte, caracteres morfométricos diagnósticos foram comparados intraespecificamente entre os variantes e interespecificamente com *C. monoculus* e *C. orinocensis*. Todos os variantes de *C. temensis* apresentaram diferenças significativas quando comparados com seus congêneres simpátricos enquanto não diferiram entre si. Na segunda parte, sequências de DNA mitocondrial foram comparadas intraespecificamente entre os variantes e interespeficamente com seus congêneres simpátricos. Não houve sinapomorfias moleculares diagnósticas para diferenciar inequivocamente os variantes e todos os variantes de *C. temensis* foram claramente divergentes de seus congêneres simpátricos. Na terceira parte, a variação no padrão de cor em ambos os sexos foi comparado ao índice gonadossomático (GSI). Foi encontrada uma correlação significativa entre a variação na coloração e o índice gonadossomático. Os resultados deste estudo demonstram que os variantes em *Cichla temensis* são membros de uma única espécie e que a variação não representa um dimorfismo sexual. A variação no padrão de cor é uma

característica sexual secundária que ocorre em ciclos, sendo um indicativo do grau de maturação sexual sazonal dos indivíduos.

Key words: Gonadosomatic index, Mitochondrial DNA, Neotropical fish, Secondary sexual characteristics.

Introduction

The genus *Cichla*, Cichlidae, comprises 15 valid species (Kullander & Ferreira, 2006) that are commonly known as peacock bass in English, tucunaré in Brazil and pavon in Spanish speaking countries (Kullander, 2003). They are large, diurnal predators and are primarily piscivorous (Jepsen *et al*., 1997; Winemiller *et al*., 1997). The genus *Cichla* has historically been a subject of mixed taxonomic opinions (Stiassny, 1987). Recently, molecular analysis has provided clarification regarding its place in the family Cichlidae (Farias *et al*., 1999, 2000; López-Fernández *et al*., 2010) and a revision of *Cichla* has done much to resolve taxonomy within the genus (Kullander & Ferreira, 2006). *Cichla temensis* has presented additional taxonomic questions at the species level, due to the existence of markedly differing color and pattern variants. Color and pattern changes have been described in many fishes within the family Cichlidae (Barlow, 2000) and specifically within the genus *Cichla* (Zaret, 1977; Winemiller, 2001; Kullander & Ferreira, 2006). There has not, however, been an empirical clarification of the marked adult color and pattern variation within the species.

Cichla temensis is the largest species of the genus and is of significant economic importance in the lowlands of Amazonia, both as a sportfish (Myatt *et al*., 2005; Holley *et al*., 2008) and for human consumption (Smith, 1981; Goulding, 1996). The species occurs

in blackwater flood pulse rivers with extremely variable seasonal environments (Val & Almeida-Val, 1995). It is found in Brazil, Venezuela and Colombia from the rio Negro and rio Orinoco drainages, in Brazil in the rio Branco basin and in the lower rio Madeira basin (Kullander, 2003). Populations are also recorded in several blackwater tributaries of the rio Solimões and rio Amazonas (Kullander & Ferreira, 2006).

In spite of its economic importance, study and management of *Cichla temensis* has been problematic in scientific, governmental and public domains. In comparison with many of its congeners who are readily accessible in less remote areas, the species has not been as extensively studied in its natural, flood pulse river habitat (Winemiller, 2001; Holley *et al*., 2008). The relative inaccessibility and complex physical and hydrological nature of the Amazon River basin have, until recently, made field collection and life history analysis of *C. temensis* difficult. Confusion caused by markedly different morphological variants has often led to those variants being identified as entirely different species by government agencies and the sportfishing community (Winemiller, 2001). Sound future management of this valuable species will benefit from a clear understanding of the nature of these variants.

Although the described variation in color pattern in this species occurs as a continuum (Winemiller, 2001), the common names used to describe these patterns are based on the two distinct extremes (Fig. 1a). The more brightly colored variant is known within the English-speaking sportfishing community as the three-barred peacock bass (Myatt *et al*., 2005) and in Brazil as tucunaré açu (Kullander, 2003). Its general body color is yellowish-gold, with brightly colored fins and a pattern of three sharply demarcated dark vertical bars on the trunk. (Fig. 1a, Grade 4). The other extreme, called

the speckled peacock bass or tucunaré paca, is dark and cryptically colored. Its mostly grey or brown body is dappled with a prominent series of light spots but the bright colors are absent. The three vertical bars are faint or almost invisible in relation to the spots and background color. (Fig. 1a, Grade 1).

Although much current scientific literature suggests a single species, it has long been thought, and continues to be suggested by some, that these two clearly discernable "types" or "variants" are two separate species or subspecies. As a result, this taxonomic issue is relevant to management of the species. It is evidenced by an array of earlier scientific names (Kullander, 2003), varying non-scientific nomenclature and regulations used by governmental agencies and non-governmental organizations (Embratur, Roraima Fish Chart, Myatt *et al*., 2005) as well as descriptions in earlier publications (Smith, 1981). In contrast, other publications have proposed that the variation results from sexual dimorphism, *i.e.*, that the brighter açu variants are sexually mature males and the darkly colored paca variants are immature forms or females (Braga, 1953; Kullander & Ferreira, 2006). These unresolved explanatory theories of separate species or sexual dimorphism are taken together to serve as the null hypotheses for this study.

The alternate hypothesis that was explored in this study was suggested by behavioral observation of wild specimens. Despite being an indirect measure, behavioral observations are satisfactory tools for the suggestion of hypotheses, which can then be experimentally tested (Rohwer & Rohwer, 1978). The principal difference observed between the color pattern variants centers around their reproductive behavior. Only fish of the brightly colored açu variant (Fig. 1a, Grade 4) have been observed engaged in either spawning or rearing activity. Although they build large nests and the attendant

spawning fish are clearly visible from the surface, no fish of the paca variant (Fig. 1a, Grade 1) have been noted on any of an estimated three hundred undisturbed nests viewed (various professional fishing guides and P. Reiss, pers. obs.). After nesting, and once fry become free swimming, fry-guarding parental pairs are particularly susceptible to capture by angling. Prior to and during specimen collection for this study, many fry-guarding adults were captured and released, all of which were observed only in the açu form (various fishing guides and P. Reiss, pers. obs.). Paca variants have not been observed in these reproductively related activities.

These observations suggest the alternate hypothesis that the *C. temensis* variants are a single species and that color pattern variation (CPV) in *Cichla temensis* occurs within individuals of both sexes in relation to the reproductive cycle. Some publications (Winemiller, 2001; Willis *et al*., 2007) have noted similar observations and reflect the hypothesis explored herein.

Morphometric characters, molecular data and the relationship between color pattern variation and reproductive status were considered to test these hypotheses. First, to test the hypothesis that the variation occurs in individuals of a single species, it was necessary to determine that the *C. temensis* variant specimens actually constitute a single species and are not members of other species. An array of morphometric measurements and meristic counts of characters were compared from specimens along the continuum of *C. temensis* color pattern variation and from specimens of *C. monoculus* (Fig. 1b) and *C. orinocensis* (Fig. 1c), two regionally sympatric congeners. Second, tissue samples from each group were collected and subjected to DNA analysis. All morphometric and molecular data were compared among the nominal *C. temensis* variants as well as

interspecifically with the sympatric congeners. Finally, to determine whether color pattern variation in *C. temensis* is related to the reproductive cycle, we compared the Gonadosomatic Index (GSI) among specimens representing grades along the continuum of *C. temensis* color pattern variability.

Material and Methods

Study sites. To ensure that results did not reflect unique local phenomena, two widely separated regions, with ostensibly separate breeding populations of *Cichla temensis* (Winemiller, 2001; Vasconcelos, *et al.*, 2005) were used for collection of specimens for the study (Fig. 2). Region 1, the Igapó-Açú basin, is a blackwater tributary complex of the lower rio Madeira, consisting of four cyclically flooding rivers interlinked with a large flooded forest lake situated south of the rio Amazonas main stem. Specimens of *Cichla temensis* and *Cichla monoculus* were collected here over a four week period during September and October, 2006. Region 2 encompassed the cyclically flooding rio Caures, a blackwater tributary of the rio Negro, situated north of the rio Amazonas main stem. Specimens of *Cichla temensis* and *Cichla orinocensis* were collected in Region 2 over three weeks during November and December, 2006. The collection periods in each region were selected to coincide with the dropping water period of each region's flood pulse, prior to the advent of the seasonal rains, and coinciding with a period of increased reproductive activity (Jepsen *et al*., 1997; Winemiller, 2001; Gomiero & Braga, 2004; Holley *et al*., 2008).

Collecting methods. Samples were collected by angling with artificial lures at a mobile sportfishing camp. Although, anglers changed weekly, the guides remained constant. The guides were trained in the study's specimen collection protocol and were furnished with the necessary materials to collect uniquely numbered, positively identified specimens of *C. temensis* with reviewable color pattern variation assessments. Simultaneously, locally available congeners (*C. monoculus* in the rio Madeira region and *C. orinocensis* in the rio Negro region) were also collected for morphometric and DNA comparison with the specimens of presumed *C. temensis*.

The fishing operation's policies prohibited sacrificing captured fish exceeding 650 mm SL and prohibited sacrificing any fry-guarding fish. This prohibition effectively excluded extremely large and post-spawning specimens from analysis. Adult *C. temensis* specimens greater than 300 mm standard length (SL), a size indicating sexual maturity (Jepsen *et al*., 1999), were collected in Region 1 (n = 101) and Region 2 (n = 79). *C. monoculus* (n = 7) and *C. orinocensis* (n = 35) are sexually mature at smaller sizes (Correa, 1998; Chellappa, 2003a), therefore specimens smaller than 300 mm SL but greater than 200 mm SL were included for those species. Tissue samples from *C. temensis* specimens in each color pattern variation grade and from the sympatric congeners were collected in the field and preserved in 95% ethanol for DNA analysis.

Definition of color pattern variation grades. Previous descriptions of *C. temensis* color and pattern have been based mainly on examination of preserved specimens. This analysis examined the wider range and subtlety of color and pattern variation (CPV) visible with live or fresh specimens. CPV in presumed *Cichla temensis* occurs as

continuous variation. In order to dependably categorize the degree of CPV of the *C. temensis* specimens, four readily differentiated CPV grades were defined along the continuum, providing a semi-quantitative metric for interpretation and application of statistical analysis (Fig.1). Because this study is intrinsically dependent on these characters, we have described in detail the characteristic color and pattern character suite associated with each of the defined CPV grades, following the format and terminology used in a recent revision of the genus (Kullander & Ferreira, 2006).

Grade 1 (paca) corresponds to the dark, cryptically patterned speckled peacock variant (Fig. 1a, Grade 1). It is distinguished by a dark grey or brown background color with a prominent series of light spots, typically arranged in four loosely delimited horizontal rows. There may be a total absence, ranging to a trace, of gold/brass coloration just beginning to appear on the lower edges of the interoperculum and suboperculum as well as the trunk just above the pelvic fins. The dorsal and upper caudal fins are dark grey or brown with white spots, recapitulating the body pattern. The pelvic, anal, and lower caudal fins are dark brown, ranging to dark red. Postorbital markings and dark vertical bars 1, 2, and 3 on the lateral body, although present, are rendered almost invisible by the dark background of grey/brown coloring.

Grade 2 (semi-paca) is distinguished by the appearance of significant gold/brass color radiating from the lower edges of the interoperculum and suboperculum and extending into the preoperculum and operculum (Fig. 1a, Grade 2). Noticeable gold/brass coloration extends into the body, encompassing the pelvic fins and the ventral lateral region anterior to vertical bar 2. The dorsal and posterior trunk remains a darker grey or brown. The upper fins remain dark while the lower fins become recognizably red. The

black vertical bars 1, 2, and 3 are visually equal in prominence to the background of lighter lateral spots. The postorbital markings on the operculum emerge from the dark background and their pattern is now distinguishable.

Grade 3 (semi-açu) specimens are defined by a trunk dominated by gold/brass color below and extending to the lateral line (Fig. 1a, Grade 3). The upper fins remain dark while the lower fins become a lighter, brighter red. The black vertical bars become visibly dominant on the trunk. The pattern of light lateral spots remains visible, although its contrast against the mostly gold trunk is diminished. The postorbital markings on the operculum are clearly visible.

Grade 4 (açu) corresponds to the brightly colored three-barred peacock variant (Fig. 1a, Grade 4). The entire lateral body is a bright yellowish-gold color except for the dorsal extremes. The dorsal fins and upper portions of the caudal fin show a blue cast, ranging up to a bright, electric blue. The pelvic, anal and lower portions of the caudal fins are bright red to reddish orange. The vertical black bars 1, 2, and 3 are sharply demarcated and dominate the lateral pattern. The light spots on the body are essentially absent, although traces may remain visible. The postorbital markings are sharp and distinct.

Treatment protocols. In order to standardize identification of the different grades, all live specimens were photographed by guides promptly after capture using compact digital cameras (Olympus Digital Zoom, 2.1 megapixels). Specimen color patterns were assessed against a waterproof CPV chart and the Grade 1-4 descriptions. An identifying waterproof numbered tag was placed in the specimen's buccal cavity for subsequent

identification. The specimen was then delivered to the field laboratory for treatment. In order to confirm the accuracy of initial grading and to ensure correct identification of each individual, specimens were re-photographed using a high-resolution digital camera (Nikon D200, 10.3 MP) and then re-graded by two other individuals at the time of dissection. Individual *C. temensis* possess an irregular postorbital stripe comprised of a variable series of black markings on the operculum (Kullander & Ferreira, 2006). These markings are effectively unique to individuals and useful for identification. All photographs were taken in a uniform fashion allowing the postorbital markings on the left operculum to serve as a second confirmation of positive sample identification between capture and field measurements.

Morphometric and meristic measurements. Following photographic recording and assessment of CPV grade, an array of morphometric measurements were taken for all specimens, including congeners of *C. temensis*. The characters recorded included; ray counts for first and second dorsal, caudal, anal, pelvic and pectoral fins; scale counts circumpeduncular, predorsal, on lateral line, above and below lateral line; gill raker counts; measurement of standard length, eye diameter, head height, maximum height, premaxilla to opercule and girth; weight of entire specimen, liver, stomach, and gonads. The measurements were made using American Fisheries Society techniques (Schreck & Moyle, 1990; Murphy & Willis, 1996) and were analyzed for the purpose of identifying the most useful, taxonomically diagnostic, species specific characters for this study and to determine if any significant differences existed among the *C. temensis* variants. Digital electronic calipers (Avenger Digital Caliper) were used for all measurements except

standard length, where an analog mechanical caliper was used (Aluminex). Certain morphometric and meristic characters have been shown to be diagnostically useful for identification purposes within *Cichla* (Lasso & Machado-Allison, 2000; Kullander & Ferreira, 2006), including lateral line scale count, height:standard length (SL) ratio and relative eye diameter. Since eye diameter can have an allometric component, we concentrated on the other two characters. Scale counts were made for all specimens of all species. The majority of *C. temensis* specimens possess continuous lateral lines (Kullander & Ferreira, 2006). In the case of specimens with discontinuous lateral lines, counts were made following the same pattern of scale rows as in complete lines. Total specimen weight was measured on a precision digital scale and recorded to the nearest gram.

Gonadosomatic index. Upon completion of the above measures, all specimens were weighed (Sartorius model GE7101, precision 0.1 gram) then dissected to determine reproductive characteristics. Testes and ovaries were weighed on a digital scale to within 0.1 gram. Reproductive status was assessed by the Gonadosomatic Index [GSI = 100(wet wt gonads in g)/(total body wet wt in g – wet wt gonads in g)] (Le Cren, 1951). Gonads were examined microscopically to confirm sex determination. Egg size and maturity were examined (anecdotally, for the purpose of observing the relationship between GSI and gamete maturity) by microscopic examination of dissected ovaries (Baytronix x20 trinocular stereo microscope with eyepiece reticule). Photomicrographs of eggs, sperm, and gonadal tissues were made using a high-resolution digital camera body mounted on the trinocular head (Nikon D100 and D200, 10.3 megapixels).

Molecular methods. In addition to the data analyzed on-site in this study, mitochondrial DNA variation for specimens of each color variation grade and extremes of paca (n = 22) and açu (n = 28), as well as for samples of the other species, was examined at the Universidade Federal do Amazonas. DNA extraction was performed using the protocol described in Sambrook *et al*., (1989). We chose to analyze the mitochondrial control region because it is generally found to have the highest substitution rate in the mitochondrial genome (*e.g.* Donaldson *et al*., 1999), and mitochondrial DNA has a high rate of coalescence due to its (hypothetically) strictly-maternal inheritance in comparison to the nuclear genome (Hudson & Coyne, 2002). Therefore, the sharing of haplotypes and lack of reciprocal-monophyly at this locus would be highly indicative of ongoing gene exchange between the paca and açu phenotypes.

The mitochondrial control region was amplified via PCR using primers LProf (5'AACTCTCACCCCTAGCTCCCAAAG3') (Meyer *et al*., 1994) and primer 12SR5 (5'GGCGGATACTTGCATGT-3') (Farias & Hrbek, 2008). For the amplification of each sample we used 0.8 L of DNA (approximately 30ng), 2 L of each 2 M primer, 2.5 L of buffer (200 mM Tris-KCl, pH 8.5), 1.5 L 25 mM $MgCl_2$, 2.5 L of 10 mM dNTP, 0.2 L of Fermentas Taq polymerase enzyme (5U/ L) and 13.5 L of ddH_2O in a final reaction volume of 25 L. The PCR reactions were performed under the following cycling conditions: 35 cycles consisting of 1 minute at 92°C (denaturation), 35 seconds at 50°C (annealing of primers) and 1 minute at 72°C (primer extension) and final extension at 72°C for 5 minutes. The PCR products were subjected to purification by precipitation with sodium acetate (3 M, pH 4.8) and 95% ethanol, and resuspended in 25 L of ddH_2O.

The sequence reactions were performed in a final reaction volume of 10 L using the DYEnamic™ ET dye terminator kit (GE-Healthcare). Each reaction had the following composition: 4 L of amplified DNA, 2 L of 2 M primer, 2 L of ET-terminator and 2 mL of ddH$_2$O. The samples were then subjected to a 35 cycle thermocycling protocol with the following temperature profile: 20 seconds at 95°C (denaturation), 15 seconds at 55ºC (primer annealing) and 1 minute at 60ºC (primer extension). At the end of the PCR reaction, samples were purified via 3M NaOAc/ Ethanol precipitation, and resuspended in 10 mL MegaBACE loading solution (70% formamide/1 mM EDTA mix) according to manufacturer's instructions (GE-Healthcare). Subsequently, the samples were electro-injected and the sequences were resolved on an automatic sequencer MegaBACE 1000 DNA Analysis System (GE-Healthcare) following manufacturer's instructions. The sequences were manually edited and aligned by hand in the program BioEdit (Hall, 1999).

Data Analysis. Analyses of all morphological data were calculated using SPSS version 16.0 and Microsoft Excel. Analysis of variance of morphometric parameters between the variants was performed by ANOVA followed by post hoc t-test on this data. Differences in morphological parameters between *C. temensis* variants and other species were analyzed using two sample t-tests and ANOVA. Specimen height to standard length ratio (H/SL) is affected by the variable size of individual specimen's gonads. A girth to length ratio factor was calculated and applied to H/SL values as a specimen specific adjustment for varying reproductive state. The relationship between CPV and GSI was determined using the Spearman rank order correlation coefficient for non-parametric correlations.

We performed two types of molecular analysis on nominal *C. temensis* specimens. First we tested if the açu and paca phenotypes were monophyletic. Monophyly and diagnostic characters are a premise of the phylogenetic species concept (Mayden, 1997), and the majority of biological species are apparently monophyletic (Avise, 2000), although a review by Funk & Omland (2003) suggests that up to approximately 24% of the surveyed fish species may be non-monophyletic. We ran a constrained maximum-likelihood search under the HKY85 model of molecular evolution (Hasegawa *et al*., 1985), and tested if the most likely constrained phylogeny was a significantly worse phylogenetic hypothesis than an unconstrained phylogeny using the approximately unbiased test of Shimodaira (2002). Phylogenies were rooted with species of the clade A *Cichla* species (sensu Willis *et al*., 2010) available in GenBank (HQ230011 - HQ230016) of which *C. temensis* is a nested member, and thus represented potentially the best outgroup taxa. The outgroup species were *C. jariina*, *C. piquiti*, *C. melaniae*, *C. mirianae*, *C. pinima*, *C. thyrorus*, *C. vazzoleri*, and *C. kelberi*. Tests were implemented in the program Treefinder (Jobb *et al*., 2004) and the model of molecular evolution was selected with ModelTest (Posada & Crandall, 1998) using the Akaike Information Criterion. Recently diverged species may not be monophyletic, but because of lack of genetic exchange, should differ in haplotype frequencies (Avise, 2000). Therefore, we tested if the sequences observed in the açu and paca phenotypes were sampled from the same pool of haplotypes using an Analysis of Molecular Variance implemented in the program Arlequin 3.5.1.2 (Excoffier & Lischer, 2010). Inference of species-level differences and the number of molecular synapomorphies for the paca and açu phenotypes were inferred using the methodology of Davis & Nixon (1992).

Results

Comparisons between the *C. temensis* variants and their sympatrically occurring

congeners found morphometric differences (Fig. 3). ANOVA of lateral line scale counts

for *C. temensis*, *C. monoculus*, and *C. orinocensis* showed that the species were

significantly different. Post hoc t tests demonstrated that each of the species were

significantly different from each other; *C. temensis* and *C. monoculus,* t(6) = 11.00, p <

.0001; *C. temensis* and *C. orinocensis,* t(43) = 33.08, p < .0001; *C. monoculus* and *C.*

orinocensis, t(8) = 6.25 p = 0.0002. These results were similar to previously published

data and support their descriptions as different species (Lasso & Machado-Allison, 2000;

Kullander & Ferreira, 2006). No diagnostically useful, significant differences were found

in any of the other measured parameters. Genetic divergence between the *Cichla temensis*

variants and the other *Cichla* species was estimated to be 8.8-13.0% p-distance (Fig. 4),

and there were 46 molecular synapomorphies that unambiguously distinguished

C. temensis from all other *Cichla* species of the clade A (*sensu* Willis *et al*., 2010).

Cichla temensis is also distinguishable by these 46 and additional synapomorphies from

the sympatrically occurring *C. monoculus* and *C. orinocensis*, since *C. monoculus*

and *C. orinocensis* both occur in the clade B of *Cichla* species (*sensu* Willis *et al*., 2010).

With the exception of visible nuchal humps found in some male specimens of *C.*

temensis, as described in Gomiero & Braga (2004), no significant differences, including

differences in scale counts and relative body depth, were found among the color pattern

variants. Nor do any of the other measured morphometric characters differentiate any of

the variants from each other. The constrained maximum-likelihood phylogenetic

hypothesis assuming monophyly of the açu (Grade 4) and paca (Grade 1) phenotypes was

a significantly worse explanation of the data than the unconstrained most likely phylogeny (p = 0.0384), rejecting the hypothesis that the açu and paca phenotypes form monophyletic entities. Analysis of molecular variance also indicated the açu and paca sequences were sampled from the same gene pool (p = 0.0605±0.0025), and show no difference in haplotype frequencies between themselves. Furthermore, there were no molecular synapomorphies that would differentiate the açu and paca phenotypes. The molecular tests reflect extensive sharing of haplotypes between the full range of CPV grades. All intraspecific comparisons indicate that the variants are individuals of a single species.

Gonadosomatic index values increased in both males and females as color pattern variation grade progressed from the paca form (Grade 1) through the açu form (Grade 4), revealing a significant positive correlation between GSI and CPV in both sexes and in both study regions (Fig. 5). Females showed a higher correlation than males. Female specimens from the Igapo-Acu had a Spearman's rho = 0.833; P = < .0001 while those from the rio Caures had a Spearman's rho = 0.784; P = <

0.0001. Males from the Igapó-Açú had a Spearman's rho = 0.666; P = < 0.001 while those from the rio Caures also showed a significant correlation between GSI and CPV Grade, Spearman's rho = 0.589; P = < 0.0015.

The GSI values for both males and females in both regions rose most abruptly at grades 3 and 4, with Grade 4 having the highest mean value (Fig. 5). In addition, there was a consistently visible relationship between the grade assigned and the morphology and maturity of the gonads in both sexes. Although ovaries did not increase in length, they increased in relative thickness as they increased in weight. Ovary weights varied

from 0.5 grams to 42.9 grams. Grade 1 females had the lightest and thinnest ovaries.

Microscopic examination of ovaries from Grade 1 individuals, regardless of the

specimen's size, showed only immature oocytes, with no mature eggs. Ovaries from

Grade 2 individuals were also immature, although the mean relative ovary size was

greater. Grade 3 ovaries showed a marked increase in relative size, although variability

was greater than in any other grade. Vitellinization was apparent in oocytes from the

ovaries of Grade 3 individuals. Ovaries from Grade 4 individuals were largest. They were

thick and distended and contained large (approximately 3 mm) oval eggs. Testes ranged

from 0.1 to 6.0 grams. Although testes were far smaller in relation to ovaries at every

CPV grade, their relative weight increases were similarly proportional across the range of

grades (Fig. 5). Thickness of the testes increased in a similar, but reduced, fashion to that

of ovaries. Thus, overall coloration and pattern was not sexually dimorphic in the *Cichla*

temensis variants. Males and females had the same changes in color pattern as their

gonads developed, furnishing no visible, external means of distinguishing sex.

Discussion

The morphometric and genetic results of this study indicate that all of the variants of *C.*

temensis are clearly differentiated from sympatric *C. monoculus* and *C. orinocensis* and

that all of these species differ from each other. Moreover, these results confirm that the

variants of *C. temensis* are a single species. Further, the CPV and GSI results imply that

change in color pattern in *C. temensis* occurs in a cyclical fashion in individual fish. Since

both sexes follow precisely the same progression of color pattern variation, *C. temensis*

color pattern variation is neither a sexual dimorphism nor an external means of

differentiating sex. Rather, it is a secondary sexual characteristic indicating reproductive

status in both sexes. Similar results in both of the study regions confirm that the results are not regional phenomena.

Although *C. monoculus* and *C. orinocensis* were treated in the same manner as *C. temensis*, no comparable variation in color pattern occurred in either sympatric species. Although some specimens' body coloration appeared brighter than others, no significant relationship was found between this coloration and their GSI. In several cases, however, and as noted by Munoz *et al.,* (2006), *C. monoculus* displayed a bright orange coloration on its lower jaw, isthmus and operculum when GSI was high, while this coloration was absent or muted in specimens with low GSI.

Color and color pattern changes have been described in many other fishes, occurring in association with agonistic behavior, spawning behavior, maturation and sexual selection, including within the family Cichlidae (Barlow, 2000). Several authors have addressed color pattern changes in juvenile *Cichla* during maturational stages. Zaret (1977) described ontogenetic color and pattern changes and showed that certain color pattern development inhibits cannibalism in *C. ocellaris*. Kullander & Ferreira (2006) noted that a paca form occurs prior to first maturity in juveniles of several *Cichla* species other than *C. temensis*; however the observation in these species is that the paca pattern is lost altogether with increasing size. Winemiller (2001) described in detail the brightening coloration in breeding adults of several *Cichla* species, including *C. temensis*. This study, however, is the first to document that the conspicuous color and pattern changes occurring in both sexes in *C. temensis* are associated with seasonal maturity.

CPV Grade 1 and Grade 2 females consistently contained ovaries showing immature germ cells, inferred by Chellappa *et al*., (2003b) as "immature" stage. Ovaries

from Grade 3 individuals showed partially developed eggs with significant vitellinization, and ovaries from Grade 4 individuals were engorged with fully developed eggs apparently ready for deposition, stages denominated by Chellappa *et al*., (2003b) as "maturing" and "mature" stages, respectively. Since post spawn specimens were not sacrificed in this study, due to their fry-guarding status, no ovaries were examined showing vacant spaces or flaccid appearance, as in the "spent" stage inferred by Chellappa *et al*., (2003b). Gomiero & Braga (2004) described stages of development in their studies of *C. monoculus* and *C. ocellaris* using a different maturity scale but indicating a similar progression of maturation.

The data collected in this study form a series of snapshots of *C. temensis* color pattern variation taken during dry season conditions. They are not a complete cyclical set spanning the rainy season. Effective sampling by angling is almost entirely restricted to low water conditions in Amazon flood pulse river systems (P. Reiss, personal observation). Collection of *Cichla* by other means is also more difficult in high water conditions (Rabelo & Araujo-Lima, 2002). As a result, data of the type obtained by angling in this study is not as readily available from high water periods. Therefore, until a study is performed that observes individuals in the post-spawning condition, it cannot be known precisely how color pattern variation proceeds during the high water period and how individual açu (Grade 4) return to the paca (Grade 1) condition. Studies in artificial environments, however, may be inapplicable, since various species of *Cichla* have been shown to modify their behavior in reservoirs or other artificial environments (Zaret, 1980). Chellapa *et al*., (2003b) cite Brafield & Llewelyn (1982) in noting that different environmental conditions can yield different reproductive strategies in *Cichla*.

In spite of the lack of data collected in high water, several considerations make it seem likely that the color pattern cycle proceeds in a similar, albeit reversed fashion after spawning and rearing is completed. Jepsen *et al*. (1999) indicate that it is likely that individuals spawn only once per year in flood pulse environments. The majority of *C. temensis* individuals spawn toward the end of the dry season, immediately preceding the onset of the rainy season (Jepsen *et al*., 1997; Gomiero & Braga, 2004). Preliminary data from a study in progress suggests that a greater percentage of *C. temensis* are caught by angling in the açu pattern during the lowest water levels of the dry season and that paca appear to be more plentiful when water levels are higher (P. Reiss, unpublished data). Together with the routine capture by angling of paca in all size ranges above 300 mm (including the largest, approaching 900 mm), this information supports the likelihood of a reverse process returning açu variants to the paca form.

Many Amazon fishes have marked physiological plasticity enabling adaptation to highly variable environmental conditions (Val & Almeida-Val, 1995). Since the environment of *C. temensis* changes drastically from its low to high water extremes (Sioli, 1984), it is likely that their cyclical color and pattern variation may have adaptive value.

For example, the bright açu coloration is highly visible to observers both under and above water and could plausibly increase fitness in several ways. As the flood pulse cycles into the dry season and water levels decrease, more shallow areas with submerged wood, suitable for spawning, become available and greater percentages of the *C. temensis* population begin entering the spawning cycle. Since the positive correlation between CPV and GSI found in this study indicates that the açu variants are physiologically more

prepared to spawn than the paca variants, their bright coloration may provide a signal of

spawning readiness to potential mates prior to pairing and nest building, as has been

shown in other fishes and suggested for Cichlidae (Barlow, 2000). Following egg

deposition and during fry guarding, it is possible that the brightly colored and highly

visible açu color pattern of the parents acts as an aposematic signal to warn off egg or fry

predators (Schroder & Zaret, 1979; Rodrigues *et al*., 2009). Although *C. temensis* are

neither ovophilic nor larvophilic mouthbrooders, both fishing guides and local residents

have reported observing fry guarding parents gather their school of surface-feeding

offspring and depart an area at signs of danger. A mechanism to prepare and concentrate

fry would make such a tactic more effective and maximize survival of offspring,

improving reproductive success, and thereby increasing fitness of the parents. During the

period when fry are being guarded by one or both parents, the açu coloration and

individually unique postorbital markings may provide a recognizable bonding pattern or

signal of "safe haven" to the fry when warned by the parent. Warning signals to offspring

during parental guarding (Shennan *et al*., 1994) and even delayed mouthbrooding

(Goodwin *et al*., 1998) have been demonstrated in other Neotropical cichlids.

In contrast to the bright açu coloration, the dark background coloration of the paca

closely resembles the color of the tannin stained water and submerged wood in the

flooded forest (called "igapó" in Brazil), while the light spots on the body are reminiscent

of the dappling effect of light penetrating the flooded forest structure. In Amazon flood

pulse rivers, high water inundates the surrounding igapó and interfluvial marshes during

the rainy season (Goulding, 1988; Junk, 1997). Small forage fishes are able to feed in the

igapó (Forsberg, 2001) while finding shelter from predation (Marshall, 2007). A large

predator such as *C. temensis* entering the dense concentration of flooded trunks, stems and leaves to pursue prey species may not be able to rely solely on the speed and stamina that enable it to hunt in open, low water conditions. The paca color pattern may enhance crypsis of foraging *C. temensis* in the flooded igapó environment, thereby improving hunting effectiveness, survival and consequently fitness.

Despite a plethora of studies worldwide that have demonstrated the socio-economic value of sport fishing (Vander Zanden *et al*., 2007; Jensen, 2009) and its sustainable nature, *C. temensis* remains subjected to mostly uncontrolled commercial harvest (Holley *et al*., 2008). Regulatory efforts at balancing the sport fishing and commercial value of the species in its native habitat have often been equivocal or ineffective. Anecdotal conversations with commercial fishermen in several locales indicates that there have, at times, been regulations in place that protect the açu variant while the paca variant remained available for commercial harvest. These regulations can now be seen to have had no management value. The results of this study clarify the nature of *C. temensis* color pattern variation and serve to correct previously held assumptions that may have hindered the creation of successful management plans. Perhaps, by recognizing the variants as a single species and by designing management plans in concert with its life history and population dynamics, *Cichla temensis*, like other species with value in both sportfishing and commercial markets, can be effectively managed and protected.

Acknowledgements

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Figure Captions

Fig. 1. a) Four defined color pattern grades in *Cichla temensis* used to group live specimens in the field. Specimens within each grade occur along a continuum of color and pattern variation. Grades 1 and 4 represent the extremes of variation. The more brightly colored Grade 4 (specimen TL = 548mm) variant is known as the three-barred peacock or tucunaré açu in Brazil. At the other extreme, Grade 1 (specimen TL = 541mm) is known as the speckled peacock or tucunaré paca and is dark and comparatively non-descript. Grade 2 (specimen TL = 613mm) and Grade 3 (specimen TL = 585mm) represent transitional degrees of variation between the extremes of paca and açu. All variant grades were collected in both Region 1 and Region 2 (Fig. 2); b) *Cichla monoculus* was collected in Region 1 (specimen TL = 382mm) and displays a pattern of bars 1, 2 and 3 similar to, but shorter than those of *C. temensis* without postorbital markings but with an abdominal bar; c) *Cichla orinocensis* was collected in Region 2 (specimen TL = 412mm) and is differentiated by three ocellar marks in place of vertical bars 1, 2 and 3. Postorbital markings are absent 59

Fig. 2. Collecting regions in two cyclically flooding drainages in the rio Amazon basin. Region 1, the Igapó-Açu region, a blackwater tributary complex of the rio Madeira, provided specimens of *C. temensis* and *C. monoculus*. Region 2, the rio Caures, a blackwater tributary of the rio Negro, provided specimens of *C. temensis* and *C. orinocensis.*

Fig. 3. a) Mean (± SEM) lateral line scale counts for *C. temensis, C. monoculus*, and *C. orinocensis*. ANOVA showed no significant differences among the *C. temensis* variants but revealed significant differences interspecifically. Post hoc t tests (horizontal starred bar) revealed that all species were significantly different, $p < 0.0001*$. b) Mean (± SEM) body depth to Standard Length ratio (adjusted for gonad size differential) for *C. temensis*, *C. monoculus*, and *C. orinocensis.* ANOVA showed no significant differences among the *C. temensis* variants but revealed significant differences interspecifically. Post hoc t tests (horizontal starred bar) revealed that all *C. temensis* were significantly different from both sympatric species, $p < 0.0001*$.

Fig. 4. Maximum-likelihood phylogeny of 50 sequences sampled from the paca and açu variants of *Cichla temensis* (Genbank accession numbers HQ230011 - HQ230016) The phylogeny was rooted a posteriori with *Cichla* species of the clade A (*sensu* Willis *et al.,* 2010) (GU295691- GU295704). The scale represents an HKY85 genetic distance

Fig. 5. Mean Gonadosomatic Index for *C. temensis* variants grouped by CPV grade. **a**) Females from the Igapó Açú (Region 1). **b**) Females from the rio Caures (Region 2). **c**) Males from the Igapó Açú region. **d**) Males from the rio Caures. A significant correlation between GSI and CPV Grade was found for males and females in both collecting regions, $p < .01$ for a, b, d, and d.
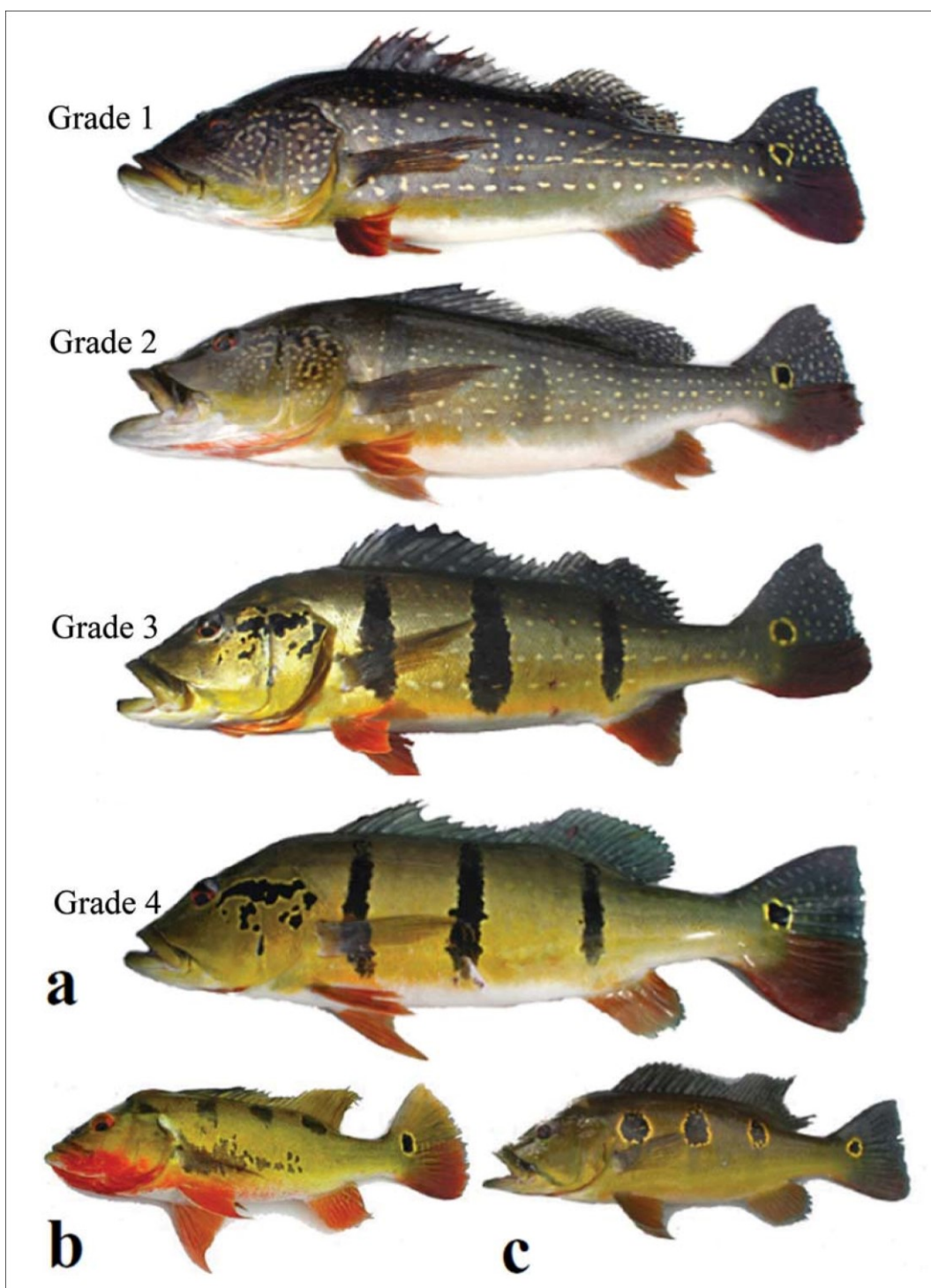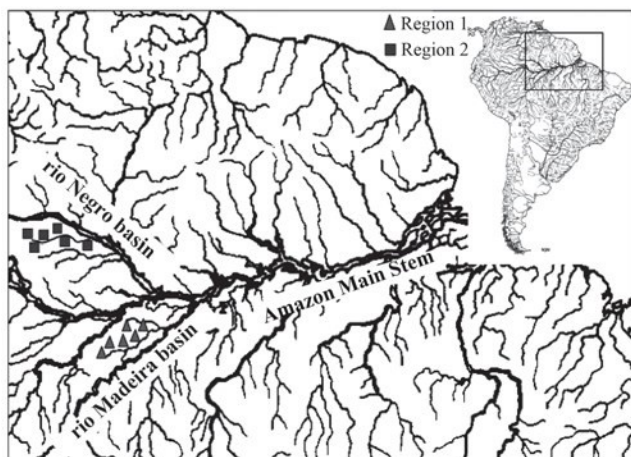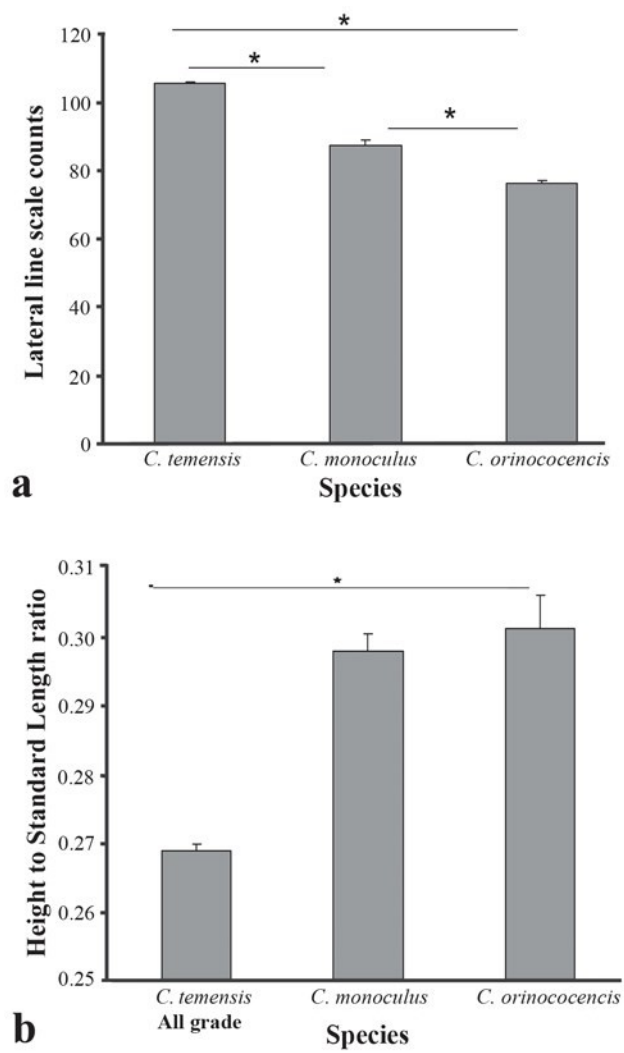
Figure 1.

Figure 2.

Figure 3.

a

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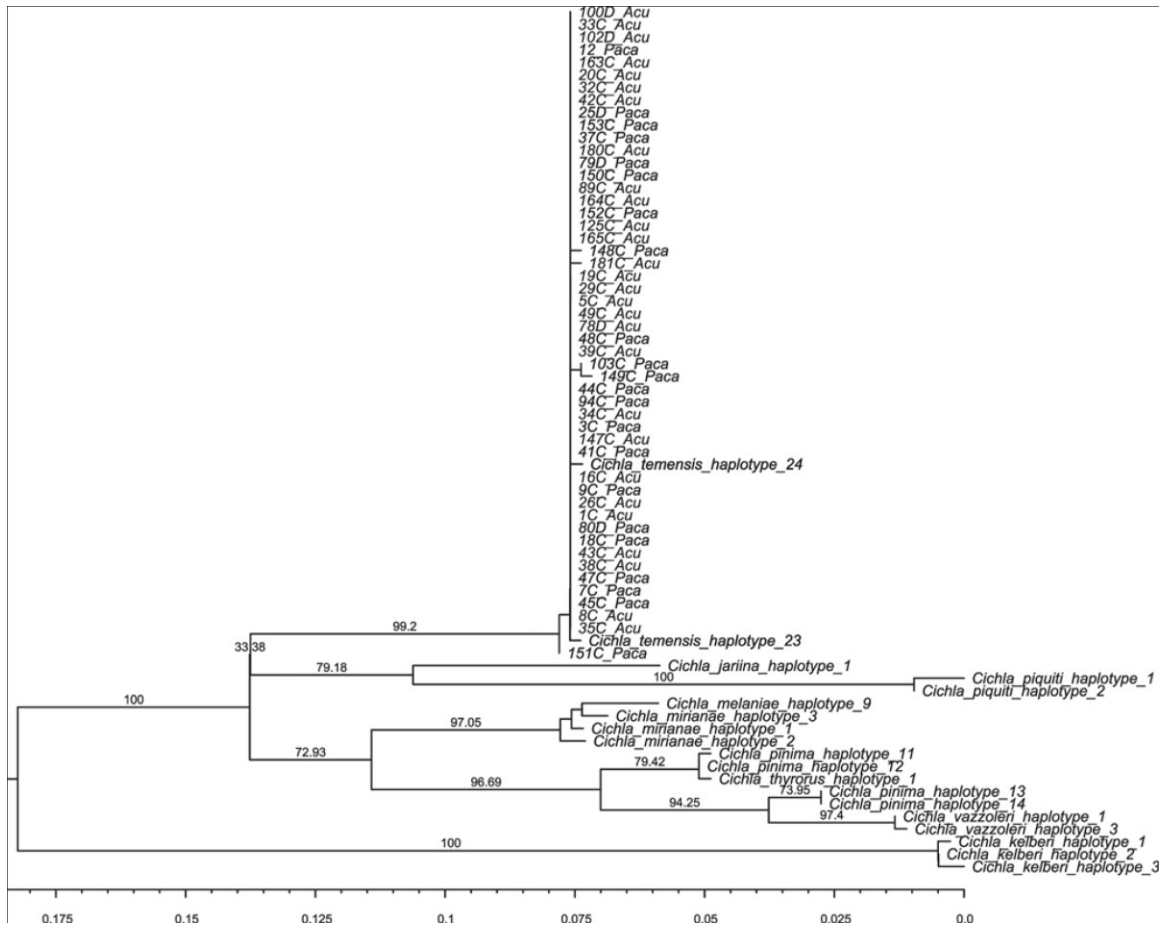
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Figure 5.
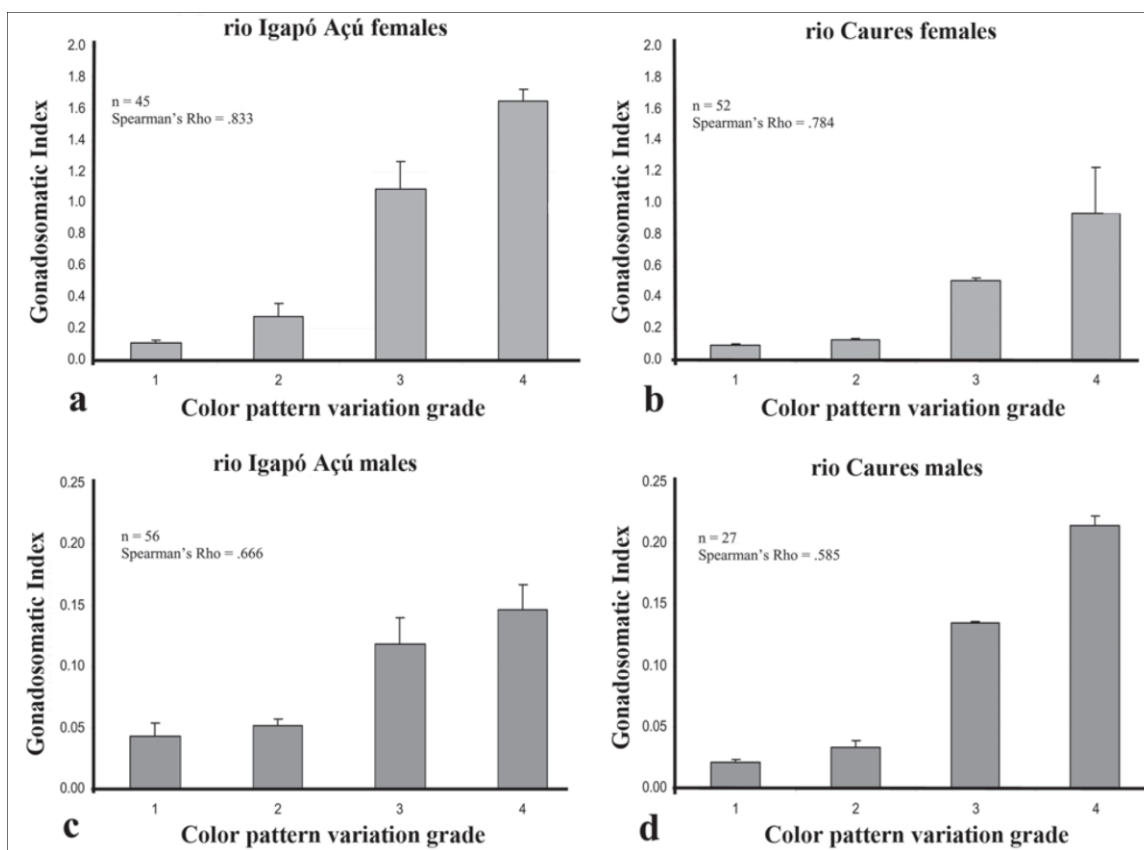
Chapter 2

Geometric morphometric analysis of cyclical body shape changes in color pattern variants of *Cichla temensis* Humboldt, 1821 (Perciformes: Cichlidae) demonstrates reproductive energy allocation

Abstract

Previously recognized color and pattern variants of adult *Cichla temensis* in Amazon flood pulse river environments reflect the cycling of individuals through seasonal sexual maturity and spawning. Individuals also vary in shape from blocky to fusiform. To determine if shape differences are related to patterns of fat reserve deposition and utilization, and to quantify the relationship of shape with color and pattern variation and life history status, specimens in each of four previously defined grades of color and pattern variation were compared using geometric morphometric techniques. Progressive shape changes occurred between grades independent of sex and correlated to gonosomatic index (GSI). Thin plate spline deformation visualizations indicate that the observed shape differences are related to fat deposition patterns. The seasonal timing of shape change and its link to color pattern variation, sexual maturity and local water level conditions suggests a relationship between the physiological and behavioral characteristics of *C. temensis* and the cyclical flood pulse pattern of its habitat.

As variantes de cor e padrão previamente reconhecidos dos adultos de *Cichla temensis* em ambientes alagáveis amazônicos refletem o ciclo de indivíduos durante o período de maturidade sexual e desova. Indivíduos também variam na forma de oblonga a fusiforme. Para determinar se as diferenças de forma estão relacionadas aos padrões de deposição e utilização das reservas de gordura e quantificar a relação da forma com cor, variação de padrão e status da história de vida, espécimes de cada quatro níveis de cor e variação de padrão previamente definidos foram comparados usando técnicas de morfometria geométrica. Mudanças progressivas ocorreram na forma entre os níveis independentes do sexo e correlacionadas ao índice gonadossomático (IGS). Visualizações de finas deformações na placa da espinha indicam que as diferentes formas observadas estão relacionadas aos padrões de deposição de gordura. O período de mudança de forma e sua ligação com a variação do padrão de cor, maturidade sexual e as condições do nível da água local sugerem uma relação entre as características fisiológicas e comportamentais de *Cichla temensis* e o padrão de inundação cíclico do seu habitat.

Keywords: Fat reserves, Functional morphology, Gonadosomatic index, Hepatosomatic index, Physiology.

Introduction

Cichla is a genus that comprises 15 Neotropical species. All are large, diurnal and primarily piscivorous predators. *Cichla temensis* is the largest species with a natural range that consists primarily of blackwater flood pulse rivers with extremely variable

seasonal environments (Val & AlmeidaVal, 1995) in Brazil, Venezuela and Colombia. The species is economically important throughout its distribution, both in recreational (Myatt *et al.*, 2005; Holley *et al.*, 2008) and artisanal fisheries (Smith, 1981; Goulding, 1996).

Markedly different color and pattern variants of *Cichla temensis* coexist in their natural environments. The morphological variation within *C. temensis* was previously attributed to assumptions that the variants were different species, subspecies or an effect of sexual dimorphism (Braga, 1953; Myatt *et al.*, 2005; Kullander & Ferreira, 2006). A recent study has shown that the variants are all members of the same species and the differences in color and pattern among the variants are due to individual fish cycling through a series of changes correlated to seasonal gonad maturity (Reiss *et al.*, 2012).

Although color pattern variation occurs along a continuum, the common names used to describe the variants are based on the two distinct extremes (Winemiller, 2001; Reiss *et al.*, 2012). The more brightly colored variant is known within the English-speaking sportfishing community as the Three-barred Peacock Bass (Myatt *et al.*, 2005) and in Brazil as tucunaré açu (açu) (Kullander, 2003). Its general body color is yellowish-gold, with brightly colored fins and a pattern of three sharply demarcated dark vertical bars on the torso. The other extreme is called the Speckled Peacock Bass or tucunaré paca (paca). Dark and cryptically colored, its mostly grey or brown body color is dappled with a prominent series of light spots. Bright colors are absent. The three vertical bars are faint or almost invisible in relation to the spots and background color. Earlier work by Reiss *et al.* (2012) developed a color and pattern variation (CPV) scale,

dividing the continuum of variation into four readily definable CPV grades (fig. 1 in Reiss *et al*. (2012)).

In addition to the previously analyzed color and pattern variations, the variants also differ in form. The paca extreme of variation (CPV Grade 1, fig. 1, Reiss *et al*. (2012)) appears fusiform relative to the açu extreme of variation (CPV Grade 4, fig. 1, Reiss et al. (2012)). The açu extreme presents what is best described as a block-like shape. It appears proportionately deeper bodied due to an apparent increased ratio of height to standard length (SL). The abdominal region appears to be extended beyond the skeletally constrained morphological features. Enlarged fleshy areas at the base of the anal fin and second dorsal fin further exacerbate the torso's squared-off appearance. Specimens in the defined grades between paca and açu (CPV grades 2 and 3) show intermediate levels of this shape change.

Cichla temensis form breeding pairs that guard their nest, eggs and fry for extended periods (Winemiller, 2001). They fast for up to two months during their reproductive period (Jepsen *et al.*, 1999). Although some species of *Cichla* may spawn multiple times per year in lake and reservoir environments (Gomiero & Braga, 2004), *C. temensis* likely spawn only once per year in their natural, flood pulse river habitat (Jepsen *et al.*, 1999), probably due to a combination of fluctuating availability of prey, spawning sites and potential forage for their fry. In consideration of these factors, Arrington *et al.*, (2006) described *Cichla temensis* as a "capital" breeder (*sensu* Bonnet *et al.*, 1998), requiring the deposition of energy stores in the form of lipid reserves prior to the initiation of its reproductive activity. There is seasonal variation in dorsal muscle lipid content in *C. temensis* from the Cinaruco River in Venezuela (Arrington *et al.*,

2006). Inhamuns (2008) described the deposition of fatty tissue in dorsal muscle of

Cichla during dry season conditions in Lago Janauaca, Amazonas, Brazil, also a flood

pulse habitat of *C. temensis*. These studies suggest that in flood pulse environments, *C.*

temensis begins to deposit reserves during falling water periods following the end of the

rainy season, when prey become concentrated and most readily available. This seasonal

variation in both the total body lipid content and the deposition of fat in specific areas in

this species (Arrington *et al.*, 2006; Inhamuns *et al.*, 2009) further suggests that reserve

deposition may be responsible for observed shape changes and may be related to cyclical

color and pattern variation and the reproductive behavior of *C. temensis* in its natural

habitat.

Changes in body shape are quantifiable when isolated from size and allometric

effects. Shape, following the definition by Kendall (1977), is all the geometric

information that remains when location, scale, and rotational effects are filtered out from

an object. A recently developed method, geometric morphometry, provides more

insightful analysis of shape than traditional multivariate analysis of a suite of linear

measures ('trusses') (Parsons *et al.*, 2003). By analyzing geometry among the locations

of all points of consideration simultaneously, rather than serially pairwise, geometric

morphometric techniques analyze shape variation that retains information on spatial

covariation by using selected morphological "landmarks" (Rohlf & Marcus, 1993). This

is particularly relevant to the study described herein. In other Neotropical cichlids,

geometric morphometrics applied to a common data set of similar but morphologically

variable interspecific specimens, *i.e. Amphilophus citrinellus* and *A. zaliosus*, (Parsons *et*

al., 2003) yielded strong evidence of morphological differences. In this application,

where the differences to be examined are intraspecific, this fine degree of resolution is essential.

 In this analysis, we apply geometric morphometric techniques to quantify the shape variation observed in *C. temensis* and we examine the hypothesis that there is a relationship between this shape change with CPV grade and cyclical seasonal environmental fluctuations.

Materials and Methods

Specimen Selection Criteria. The analyzed materials consist of uniformly posed photographs of live specimens. All were captured by angling with artificial lures by anglers traveling with a sport-fishing operation in the Amazon during falling and low water periods from 2009 through 2012. Fish were placed on a horizontal boat platform and a lateral image of the left side of each individual was captured using a digital camera (Nikon D200) held parallel to the platform, after which fish were returned to the water alive. *A priori* specifications of the image characteristics of this lens-camera system were examined to ensure that it would not produce visual distortions of shape (*e.g.* fish-eye effects or excessive parallax). Images were captured at a resolution of 10 megapixels. All photos were treated identically with Photoshop CS2 software to enhance clarity. An overall sample pool consisting of 824 specimen photographs in each of the CPV grades (see Fig. 1 in Reiss *et al.*, 2012) was assembled from 7 widely separate habitat regions (rios Igapó Açu, Matupiri, Caures, Unini, Xeriuini, Urubaxi, Uneuixi) within the species' recognized range in Brazil in order to sample separate populations and eliminate the possibility of observing a local effect. The overall pool of specimen photographs was reviewed to eliminate those with improperly angled photos, flexed torsos, flattened fins,

excessive glare and other mechanical deficiencies. Samples were then selected from the remaining pool, leaving selection of source location as a random effect.

Sizes of angler-captured fish were widely distributed (ranging from <200 mm to >920 mm standard length (SL); therefore, we defined specimen selection criteria to keep the analysis of body morphology size-independent. Juvenile *C. temensis* grow rapidly and undergo significant morphological change in their early life history (Kullander & Ferreira, 2006). Fishes at larger size extremes display slower growth but often exhibit ontogenetic exaggeration of certain morphological features (Zelditch *et al.*, 2004; von Bertalanffy, 1938). Because confounding allometric effects can occur in both of these size ranges (Richter *et al.*, 2000) the specimen photographs used were restricted to images of individuals between 350 mm and 740 mm. This range effectively also excluded specimens not capable of sexual maturity (Jepsen *et al.*, 1999) and approximated the middle of the size range of the species (Myatt *et al.*, 2005). Each of the four CPV grades (Reiss *et al.*, 2012) was represented by 20 photographs with a relatively uniform distribution of specimen sizes, within the size range limitation and independent of sex. Grade 1 specimens averaged 507.6 mm with a Standard Deviation (SD) of 56.02. Grade 2 specimens averaged 518.7 mm with an SD of 83.8. Grade 3 specimens averaged 513.2 mm with an SD of 67.1. Grade 4 specimens averaged 510.4 mm with an SD of 96.3. ANOVA showed size distributions between CPV grades to be random and unordered ($p = 0.97$) further ensuring that no size trends confounded the shape analysis. The 80 selected specimens were randomly ordered for analysis.

Shape Analysis. Analysis was conducted using programs (referenced individually below) within the software suite tpsDig2, available from the Rohlf website at SUNY,

Stony Brook, NY (http://life.bio.sunysb.edu/morph) (Rohlf, 2000). Shape variation was characterized using tpsDig2 v 2.05, a landmark-based geometric morphometric program (Adams *et al.*, 2004; Zelditch *et al.*, 2004). The eighty uniformly posed photographic specimens provided the raw shape data necessary to compare individuals among the four CPV grades. To summarize, beginning at CPV Grade 1 (paca extreme), they proceed stepwise through Grades 2 and 3 until reaching Grade 4 (açu extreme). Grade 2 specimens are those that are beginning to move from the paca form to the açu form and are distinguished by the appearance of distinct gold/brass color radiating from the lower edges of the interoperculum and suboperculum and extending into the preoperculum and operculum. Gold/ brass coloration extends into the body, encompassing the pelvic fins and the ventral lateral region. The dorsal and posterior trunk remains a darker grey or brown. Grade 3 specimens are approaching the açu form and are defined by a trunk dominated by gold/brass color below and extending to the lateral line. The dorsal fins and upper half of the tail remains dark while the anal and pelvic fins along with the ventral portion of the tail become a lighter, brighter red.

For ease of description, particularly when discussing shape extremes and spawning behavior throughout this work, the local term "paca" is used to refer to the most fusiform and non-sexually mature extreme of variation (CPV Grade 1), while "açu" refers to the most blocklike, brightly colored and sexually mature extreme (CPV Grade 4). The intermediate levels of variation do not have common local names and are instead referred to here by their CPV grades (2 and 3). The CPV grades as described in Reiss *et al.* (2012) are used when specifying numerical and statistical results and when referring to the continuum of CPV and shape variation (Table 1).

An initial analysis of the specimen's overall body form assessed variation in the overall shape characteristics of each CPV grade. Twelve two-dimensional landmarks on the left side of each fish were defined (Fig. 1). Locations were selected to encompass the central torso, including; (1) the anterior point of the skull; (2) the anterior edge of the base of the first dorsal spine; (3) the center of the base of the 10th dorsal spine; (4) the base of the first long ray of the second dorsal fin; (5) the base of the 9th ray of the second dorsal fin; (6) the posterior point of termination of the second dorsal fin; (7) the posterior base of the anal fin; (8) the base of the 7th anal fin ray; (9) the anterior insertion of the anal fin; (10) the cloaca; (11) the ventral termination of the scale row emanating from the insertion of the 10th dorsal spine; (12) the ventral termination of the scale row emanating from the anterior point of the insertion of the pelvic fin. To avoid confounding data points relative to the overall shape change, several anatomical structures were excluded. Points on the caudal peduncle and caudal fin were deemed unnecessary for this analysis and were excluded to avoid variation due to caudal fin damage or position of the tail during photography. The nuchal hump, a protrusion on the forehead of many reproductively active fishes, particularly Cichlids, is highly variable in form and occurs primarily in CPV Grade 4 males in *C. temensis*. It is outside of the area of overall shape change we studied and was excluded from this analysis by avoiding selection of any points defining it or occurring within it.

We rotated, translated and scaled landmark coordinates into alignment via generalized least squares superimposition, and described the variation in relative landmark positions using thin-plate spline visualization with tpsRegr following Bookstein (1991). Superimposed landmark configurations were then used to calculate

partial warps (also with tpsRegr) for each of the CPV grades. Partial warps are analogous to bending energies necessary to deform a shape from a standard and thus describe morphological variation as variables in geometric morphometric analyses (Bookstein, 1991; Rohlf & Marcus, 1993; Marcus *et al.*, 1996).

Secondary Analyses. Based on the resulting thin-plate spline visualizations of overall shape and on potential fat deposition locations (Arrington, 2006; Inhamuns, 2008), a series of secondary analyses used landmarks and semilandmarks (software-generated secondary points dependent on the defined landmarks) to more closely assess areas other than the abdomen that appeared to generate large contributions to shape change. This ensured a second point of view that avoided the potential confounding effects of enlarged gonads, engorged stomachs, or liver size variation. This focused on two areas defined in the initial analysis; the shape of the fleshy bases of the second (soft) dorsal and the anal fin. Fixed landmarks defined the limits of the areas to be analyzed and sliding, equally spaced, semi-landmarks defined the outlines of the areas to be analyzed. The second (soft) dorsal fin was delimited by a landmark defined at the base of the first spine of the second dorsal fin and a second landmark at the point of termination of the second dorsal fin, identical to the corresponding landmark in the initial overall shape analysis. The tpsUtil curve creation function was then used to define a curve with 11 points and to convert these to semi-landmarks. Finally, the semi-landmarks were reviewed to confirm adherence to the structure's outline, adjusted as necessary to optimize position and then resampled to ensure even spacing. The same procedure was repeated with the anal fin using 8 points converted from the 2 primary landmarks.

Collateral analyses. Fat Analysis. As an alternative confirmation that fatty tissue was present in fleshy fin bases, a series of tissue samples from the fleshy portions of the anal fin and second dorsal fin bases were examined qualitatively by visual and microscopic examination. Tissue samples from 40 *C. temensis* individuals distributed through the range of color and pattern variation were obtained through donations of sacrificed specimens from local fishing guides.

Height/SL analysis. As a parallel test and simple confirmation of the shape changes measured by geometric morphometrics, we compared the ratio of height/SL in 200 previously collected specimens distributed among the four CPV grades using raw data collected a priori for Reiss *et al.* (2012).

Gonadosomatic and hepatosomatic index. The specimens used for gonadosomatic and hepatosomatic index measurements were the same 200 previously collected specimens described above (see Reiss *et al.*, 2012). All were weighed then dissected. Liver, testes and ovaries were weighed on a digital scale to within 0.1 gram (Sartorius model GE7101 – precision 0.1 gram). GSI was then calculated following Le Cren, (1951) as GSI = 100 (wet weight gonads in g)/(total body wet weight in g – wet weight gonads in g). HSI was calculated as HSI = 100(wet weight liver in g)/ (total body wet weight in g – wet weight liver in g).

Statistical Analysis. The freeware software suite based on and supporting tpsDig2, available from the Rohlf website at SUNY, Stony Brook, NY (http://life.bio.sunysb. edu/morph) (Rohlf, 2000) was used to define landmarks, analyze the specimens and quantify results. To determine whether the amount of variation in the shape data set was

appropriate to permit statistical analyses to be performed, tpsSmall (Rohlf, 2000) was used to assess the linear tangent space approximate to the non-linear Kendall's shape space. The landmarks were converted to shape coordinates by Procrustes superimposition (Rohlf, 2000). This procedure standardizes each specimen to unit centroid size, a size measurement computed as the square root of the summed squared Euclidean distances from each landmark to the specimen's centroid and removes information about location and orientation from the raw co-ordinates.

Multivariate analyses were used to examine body shape differences. The thin-plate spline interpolating function was used to analyze residuals from the superimposition, producing principal warps. The distribution of mean shapes of each CPV grade in shape space was evaluated with Principal Component Analysis (PCA), called Relative Warp Analysis (RWA) when applied to Procrustes shape coordinates, using tpsRelW (Rohlf, 2000). Linear regression ($\alpha = 0.05$) tested the relationship between the relative magnitude of shape divergence and the grade of color and pattern variation in tpsRegr using Monte-Carlo permutation tests for differences between mean shapes.

Results

Geometric Morphometric Analysis and Thin Plate Spline Deformation. Shape differed significantly among the four CPV grades and correlated in magnitude with the rank of assigned CPV grade. Three shifts in body shape were most apparent: (1) overall body depth; (2) relative form of the fleshy area immediately below the soft-rayed second dorsal fin; (3) relative form of the fleshy area immediately above the anal fin. Body depth and fin base enlargement was greater in the açu form than the paca form. This shift

produced the block-like body shape described in the açu form by deepening the central and posterior portion of the body, compared to the more fusiform shape of the paca form (Fig. 2).

The second dorsal and anal fin bases, as defined by bordering landmarks and sliding semi-landmarks, increased significantly in area coinciding with increase in CPV grade. Deformation of the outlined fin bases demonstrated an increase in total area where reserves are known to be deposited and contributed to the overall shape change evidenced by the initial analysis.

Relative Warps Analysis. A large fraction (20.59%) of the variance in biologically significant individual body shape differences can be explained by the first principal component of the relative warps analysis (Table 2; Fig. 3). The effect of CPV grade on the first principal component was significant (Roy's Maximum Root statistic = 3.4993, $F_s = 10.323$ $df1 = 20$, $df2 = 59$, $P < 0.001$) as it was on the total variance for which it accounted (Pillia's trace statistic = 0.7777, $F_s = 10.323$, $df1 = 20$, $df2 = 59$, $P < 0.001$). Individuals with positive amplitude on the first relative warp are increasingly deep bodied and blocky. The trajectory of shape change with increasing CPV grade is significant (Table 2). CPV Grade 1 (paca) specimens had a relatively slim overall body with a fusiform shape. Grade 2 and 3 specimens were progressively blockier. CPV Grade 4 (açu) color specimens were characteristically deep-bodied, with a relatively squared off torso. Fig. 2 shows a visualization of shape differences among variants in terms of deformation grids.

Collateral Analyses. - Fat. Examination of tissue samples from the fleshy portions of the anal fin and second dorsal fin bases showed the presence of fatty tissues. Microscopic

analysis showed the presence of adipose tissue and heterogeneous adipocytes containing fat droplets in all samples (Dettmeyer, 2011). Macroscopic examination assisted by heating of fin base tissues showed the presence of a mixture of muscle and fatty tissues.

Height/SL analysis. The average height/SL ratio increased significantly with CPV grade; 0.2694 for Grade 1 (paca), 0.2725 for Grade 2, 0.2771 for Grade 3 and 0.2822 for Grade 4 (açu) (ANOVA df = 119 , F = 5.323, p < 0.002). This result supports the shape change trend shown by geometric morphometric analysis (Fig. 4).

Discussion

Geometric morphometric analysis confirms a progressive shape change in *C. temensis*. Much of the variation was due to fatty reserve deposition in specific areas known to be potential reserve storage sites (Arrington, 2006) and which were observed in this study to contain adipose cells, implying that increased energy storage in these tissues was responsible for the change. Shape change is correlated with the degree of CPV progressing from the relatively slim, hydrodynamic form of the non-reproductive paca (CPV Grade 1) extreme to the increasingly deeper body form of specimens as they approach the açu (CPV Grade 4) extreme of color pattern variation. GSI is at its maximum in the açu form. The correlation of shape change with CPV grade indicates that maximal reserve deposition occurs immediately prior to spawning and the ensuing post-spawning period of fry guarding when adults cease feeding for extended periods. Spawning occurs once per year in lowlands flood pulse rivers (Jepsen *et al.*, 1996; Winemiller, 2001; Hoeinghaus, 2006), indicating that these linked changes are cyclical.

They correspond to the temporal patterns of large-scale habitat variation in the Amazon as described by Junk (1989) and have a close relationship to the flood pulse system's cyclical water level changes (Jepsen *et al.*, 1999), suggesting functional morphological changes linking the mechanical aspects of anatomical form to organismal behavior.

Many fish species exhibit marked morphological changes during sexual maturation cycles (reviewed in Robinson & Wilson, 1994; Smith & Skúlason, 1996; Taylor, 1999; Jonsson & Jonsson, 2001). *Cichla temensis* changed shape along the CPV gradient and CPV grade is directly correlated with GSI in both males and females (Reiss *et al.*, 2012). Gonad size is a relatively small component of total size in *C. temensis*, especially in males, where maximum gonadosomatic index remains below 0.35%. Female GSI can reach 2.5% (Reiss *et al.*, 2012). Considering the demonstrated relationships between CPV, shape change and reserve deposition, the resulting inference that reserve deposition and GSI proceed in relation to each other is supported. This is in keeping with the description of *C. temensis* as a "capital" breeder. It also supports the likelihood that the shape changes as *C. temensis* matures toward spawning during the yearly flood pulse cycle reflect the addition of reserves that will be depleted during the parents' extended post-spawning fast. Jepsen *et al.* (1999) has described this period as a "physiological winter". *Cichla temensis'* relatively low investment of reserves in gamete volume (*i.e.* as compared with egg scattering species) contrasts with the relatively high investment of reserves expended during the long fry-guarding period. These linked cyclical changes, occurring in correspondence to temporal patterns of habitat change, demonstrate a close affinity with the flood pulse system's cyclical water level changes. Further analyses might include an assay of how the specific amount of fat deposited

relates to specimen size or age, or perhaps a comparison of how this effect appears in other flood pulse Cichlids. Ultimately, a synthesis of this data and other information related to the timing of environmental, physiological and behavioral changes may help to more clearly elucidate this effect.

Liver and reserves. Although the liver is recognized as an important site of lipid deposition and reserve storage in fishes (Larson, 1991; Helfman *et al.*, 1997), data analyzed during this study pointed to a dichotomy between reserves used for gametogenesis and for maintenance, justifying further analysis of the phenomenon. This study initially focused on the effect of corporal fatty reserve deposition (in the muscles, mesentery and fin bases) in specimens without direct consideration of liver reserves for several reasons; first, the geometric morphometric analysis in this study was based on the use of uniformly positioned photographs acting as specimens. Photographic specimens, of course, do not lend themselves to dissection and analysis of internal organs. Second, although patterns of reserve deposition (specifically regarding liver reserve versus alternate corporal reserve deposition) vary with species and life history parameters, the literature shows that fishes whose reproductive period does not coincide precisely with periods of low food availability or high energy requirements may have offset periods of reserve deposition and consumption between the liver and corporal deposits. When reproductive maturation is disengaged from migration, maintenance or periods of minimal food availability, increases in hepatosomatic index (HSI) are not necessarily synchronized with increases in corporal reserve deposition or utilization (Larson, 1991; Schaan *et al.*, 2009). Further it is suggested that materials essential to gametogenesis are stored in the liver (Craig *et al.*, 2000; Regost *et al*., 2001) and that body fat is not

metabolically essential for reproduction; rather it is used primarily for maintenance during low food or high energy consumption periods, such as winter in temperate zone fishes (Larson, 1991). This may be the case in *C. temensis* during fry guarding.

In *C. temensis* in a flood pulse environment, gametogenesis generally occurs during the period of greatest food abundance while maintenance needs (the period of fasting associated with fry rearing) occurs only after gametes have been expended (Jepsen *et al.*, 1999; Winemiller, 2001). Thus, liver and corporal reserves are not utilized in a synchronized manner; rather they are disengaged and complementary. Inhamuns *et al.* (2009) indicated that *Cichla* sp. contained different ratios of lipid components at different times during the flood pulse and attributed this to differing metabolic and reproductive needs. Although Arrington *et al.* (2006) found a significant relationship between lipid liver content and the mesenteric fat index, analysis of the relationship between HSI and GSI in *C. temensis* segregated by CPV grade, shows that after an initial period of increase in both indices, HSI stabilizes and then drops as GSI increases to its maximum. This effect is even more pronounced in females and is similar to that shown in another Neotropical flood pulse river species, *Brachyhypopomus draco* (Schaan *et al.*, 2009). In summary, as *C. temensis* individuals approach the açu form and gametes and gonads mature, HSI begins to decrease while corporal reserves continue to increase. Açu specimens (CPV Grade 4) with the highest mean GSI showed the lowest mean HSI (Fig. 5).

Like gonad size, liver mass is a relatively small component of total mass in *C. temensis*, with hepatosomatic indexes ranging from 0.4% to 1.6% (Reiss *et al.*, 2012). Since these organs are contained within the body cavity, liver volume and gonad volume

both contribute to the overall torso shape as analyzed herein and are thus effectively incorporated into the gross shape change. Since their size increases are disjunctive and negatively related when at maximum, they likely tend to balance each other's contribution to shape change as spawning approaches. Jepsen *et al.* (1999) also noted that specimens collected with mature gonads never contained food in their stomachs, essentially eliminating engorged abdomens as a factor in increase in body depth in the açu (CPV Grade 4) form. Hence, all of these considerations are indirectly included in the gross shape analysis. They are irrelevant to the secondary analyses of fin base fat deposition sites.

Nuchal hump. Fontenele (1948) described the nuchal hump that occurs in sexually mature *C. temensis* males. Gomiero & Braga (2004) noted its appearance in males shortly prior to spawning. Their histological examination showed that it contained large quantities of lipid in the form of oil droplets that were depleted after spawning. Barlow (2000) and Bleick (1975), however, found that the hump in other Cichlids is caused by local edema, diluting preexisting fat. In either case, this seasonal secondary sexual characteristic is not addressed in this analysis for several reasons. Because of the hump's irregular form and a lack of bordering or defining primary landmarks, it proved impractical to assess consistently with geometric morphometric techniques. Additionally, it occurs only in CPV Grade 4 specimens and is essentially restricted to mature males, leaving it a somewhat sporadically appearing factor in the overall body shape of specimens. It is, however, an interesting phenomenon and in light of the timing of its appearance merits consideration as a component of the entire suite of *C. temensis* cyclical physiological changes.

Cyclicality. The data used in this study were collected during and around regional dry seasons. Due to access, water level and humane treatment considerations, data of this type is not readily available from high water periods (Reiss *et al.*, 2012). Further, *Cichla* have been shown to modify their behavior and reproductive strategies in reservoirs and non-flood pulse conditions (Brafield & Llewelyn, 1982; Chellapa *et al.*, 2003), making it likely that studies in artificial environments may be inapplicable (Zaret, 1980). Therefore, it cannot be known precisely how shape variation proceeds during the high water period until a study is performed that observes individuals throughout a full year's cycle in a flood pulse environment.

In spite of the lack of shape data collected in high water, several considerations identified regarding the CPV cycle (Reiss *et al.*, 2012 ; Chellapa *et al.*, 2003), *Cichla* reproductive patterns and fry-guarding fasts (Jepsen *et al.*, 1999; Gomiero & Braga, 2004) and the large seasonal variation in dorsal muscle lipid content found by Arrington *et al.* (2006) make it likely that the block-like shape of the açu individuals returns to the hydrodynamic shape of the paca after spawning and rearing is completed. Considered together with the larger shape variance found in CPV Grades 1 and 4, this information supports a cyclical process returning the shape characteristics of açu variants to the paca form.

The cyclical morphological, behavioral and physiological changes demonstrated by *Cichla temensis* in its native habitat appear to be closely associated with the timing of predictable environmental changes. The capability of expressing and utilizing these characters in temporal accord with cyclical environmental fluctuations is likely an adaptation to the species' natural flood pulse environment.

Acknowledgements

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Figure Captions

Fig. 1. Configuration of landmarks (red dot within yellow circles) as applied to all color pattern variation (CPV) grades of *Cichla temensis*. (1) the anterior point of the skull; (2) the anterior edge of the base of the first dorsal spine; (3) the center of the base of the 10th dorsal spine; (4) the base of the first long ray of the second dorsal fin; (5) the base of the 9th ray of the second dorsal fin; (6) the posterior point of termination of the second dorsal fin; (7) the posterior base of the anal fin; (8) the base of the 7th anal fin ray; (9) the anterior insertion of the anal fin; (10) the cloaca; (11) the ventral termination of the scale row emanating from the insertion of the 10th dorsal spine; (12) the ventral termination of the scale row emanating from the anterior point of the insertion of the pelvic fin.

Fig. 2. Thin-plate-spline deformation grids depicting (a) overall body shape differences between extremes of CPV grades (magnified 3x); (b) the increase in area at the bases of the second dorsal and anal fins between extremes of CPV grades (magnified 3x).

Fig. 3. Biplot of the uniform components in each direction (UniX and UniY) of morphometrical differences in 80 specimens of *Cichla temensis* in 4 color variation patterns (CPV) as measured by 9 Thin Plate Spline (TPS) distortion variables (V1-V9). Colored numbers indicate the CPV grade of individuals. The total spread of scores among individuals of each CPV are indicated by an envelope (solid line polygon) calculated as the minimum convex hull for that group. Position in the plot relative to other individuals indicates the degree of similarity in morph. Vectors point in the

direction of gradient change for that TPS variable and the magnitude indicates the

strength of the gradient. Angles between vectors indicate the TPS interset correlations.

Fig. 4. Height to length ratio of color pattern variants of *Cichla temensis.* The average

height/SL ratio increased with CPV grade from a low of 0.2694 for CPV Grade 1 (paca),

0.2725 for CPV Grade 2, 0.2771 for CPV Grade 3 and 0.2822 for CPV Grade 4 (açu).

Fig. 5. Relative mean GSI *vs*. relative mean HSI of color pattern variants of *Cichla*

temensis. Points for GSI represent the mean value for each CPV grade as compared to

the range encountered. Points for HSI represent the mean value for each CPV grade

compared to the range encountered.

Table 1. Guide to color pattern variation (CPV) grades and local names of *Cichla temensis*.

CPV grade	Grade 1	Grade 2	Grade 3	Grade 4
Common name	paca	n/a (called paca)	n/a (called açu)	açu
Description	Cryptically patterned, dark grey/brown background, prominent series of light spots in four loosely delimited horizontal rows. Total absence to trace of gold/brass on ventral edge of operculum and torso. Dorsal fins dark with white spots, recapitulating body pattern. Ventral fins dark brown to dark red. Postorbital markings and vertical bars 1, 2 and 3 almost invisible against dark background.	Significant gold/brass color radiating from lower edges of operculum extends to torso, encompassing pelvic fins and ventral lateral region anterior to vertical bar 2. Dorsal and posterior torso dark grey/ brown. Dorsal fins dark, ventral lower fins become red. Black vertical bars 1, 2 and 3 equal in prominence to background of white spots. Postorbital markings emerge from background.	Torso dominated by gold/ brass color below lateral line. Dorsal fins remain dark while ventral fins become a lighter, brighter red. Black vertical bars become visibly dominant on torso. Light lateral spots remain visible, but contrast against torso diminished. Postorbital markings now clearly visible.	Brightly colored 3-barred variant. Torso bright yellow - gold color except for dorsal extremes. Dorsal fins and upper portion of caudal fin blue ranging to bright, electric blue. Ventral fins bright red to orange. Vertical black bars 1, 2 and 3 sharply demarcated, dominating pattern. Light spots mostly absent or trace. Postorbital markings sharp and distinct.

Table 2. Relative Warp Analysis of *Cichla temensis*. Singular values (SV) and percent explained for each relative warp (principle axis). The first relative warp was significantly different from random (v2 ¼ 7.6539, 2.1387; $P < 0.05$), indicating that much of the biologically significant individual body shape differences can be explained on this component

Relative warp (principle axis)	Singular value (SV)	% explained	Cumulative %
1	0.17896	20.59%	20.59%
2	0.17634	20.00%	40.59%
3	0.15128	14.72%	55.31%
4	0.12616	10.24%	65.54%

Figure 1.

530 mm TL
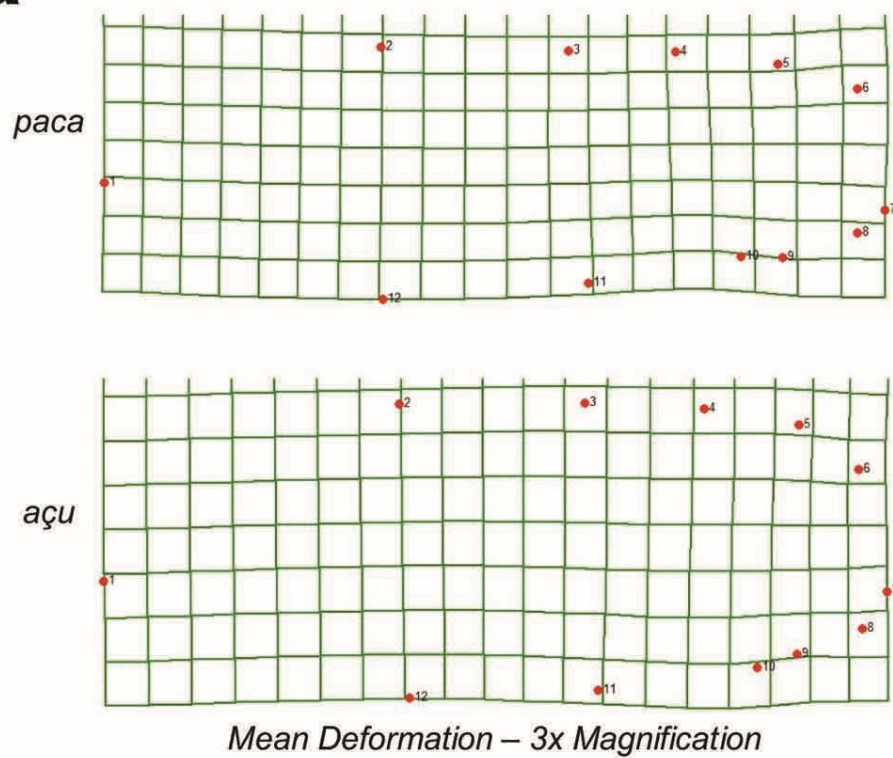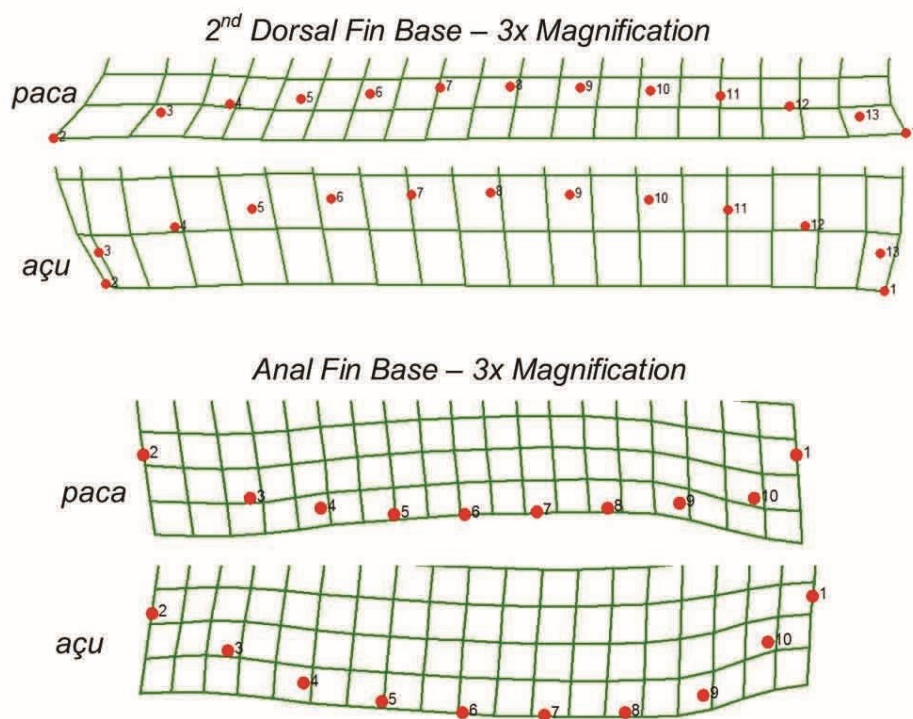
Figure 2.

Mean Deformation – 3x Magnification

2nd Dorsal Fin Base – 3x Magnification

Anal Fin Base – 3x Magnification

Figure 3.

Figure 4.
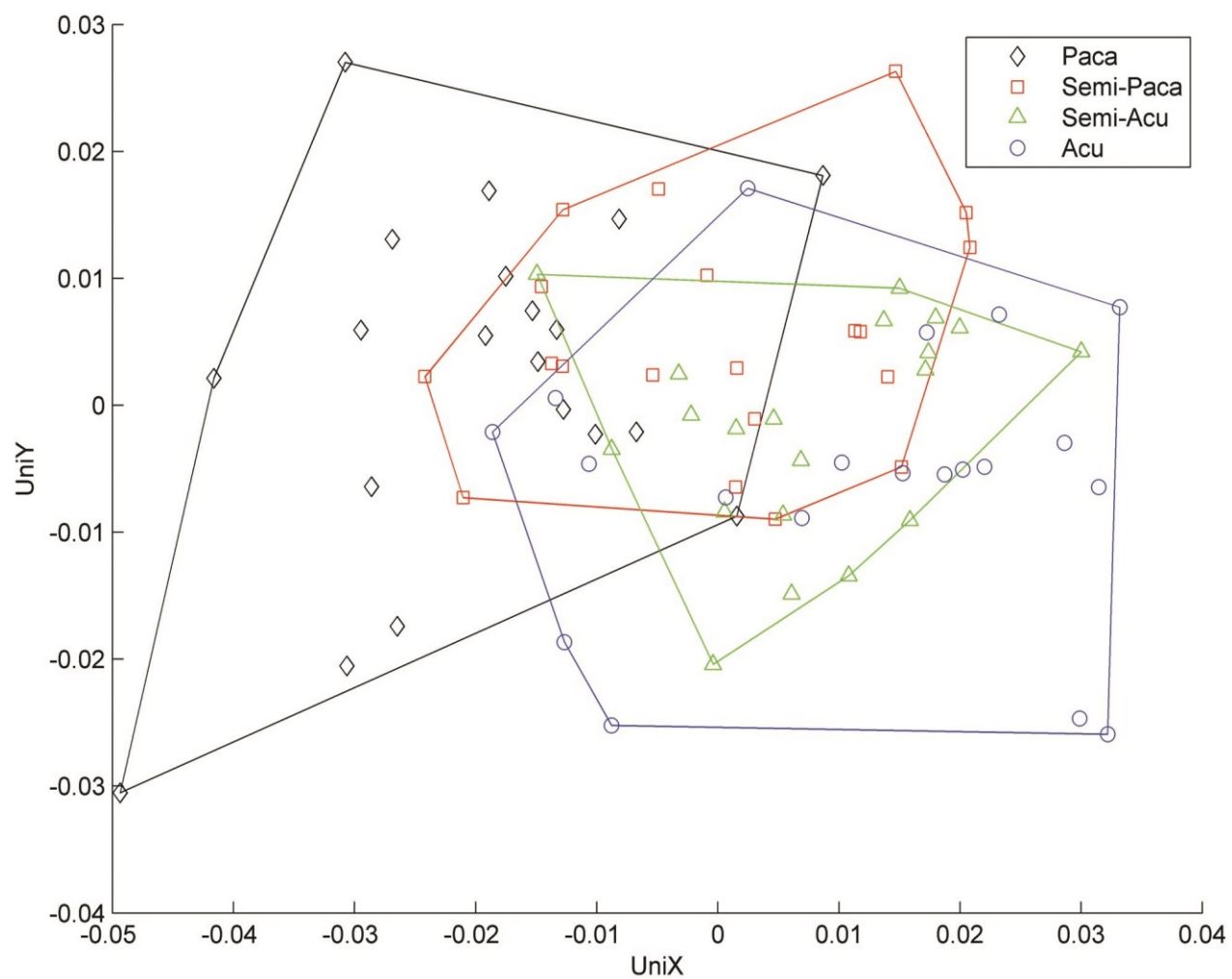

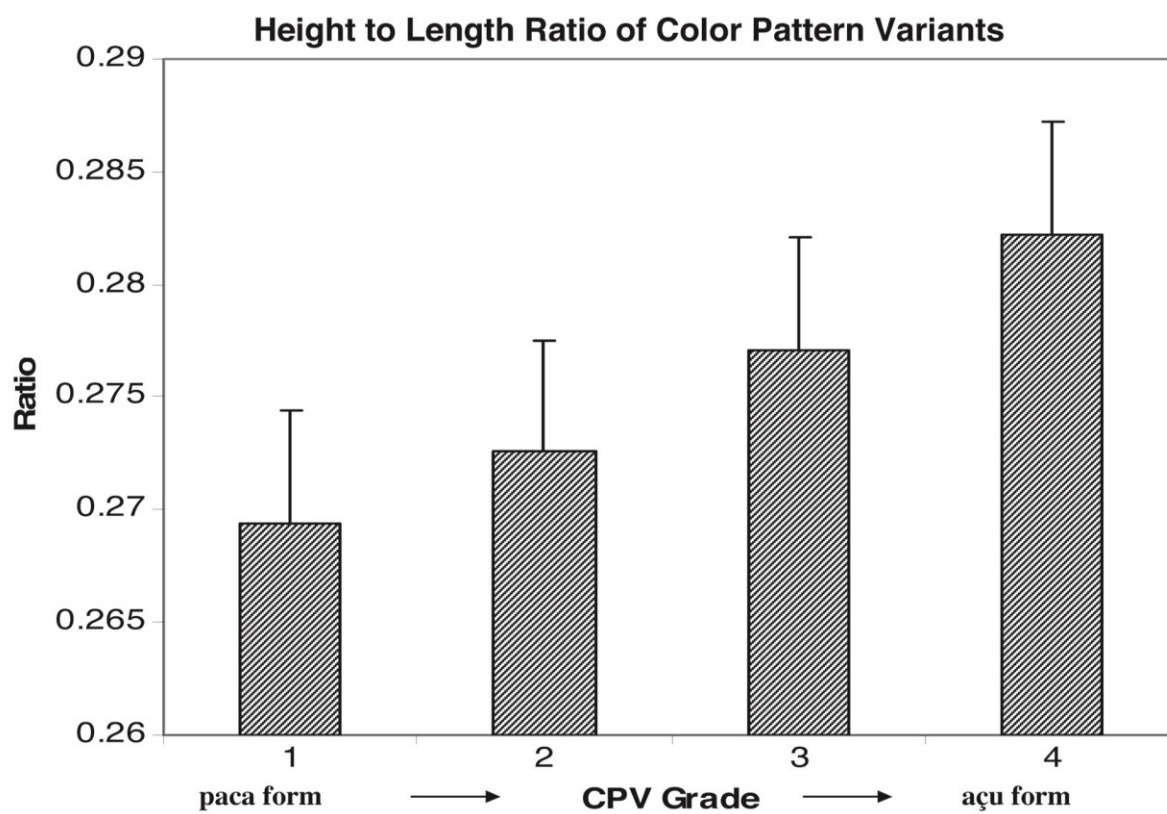**Height to Length Ratio of Color Pattern Variants**

Figure 5.
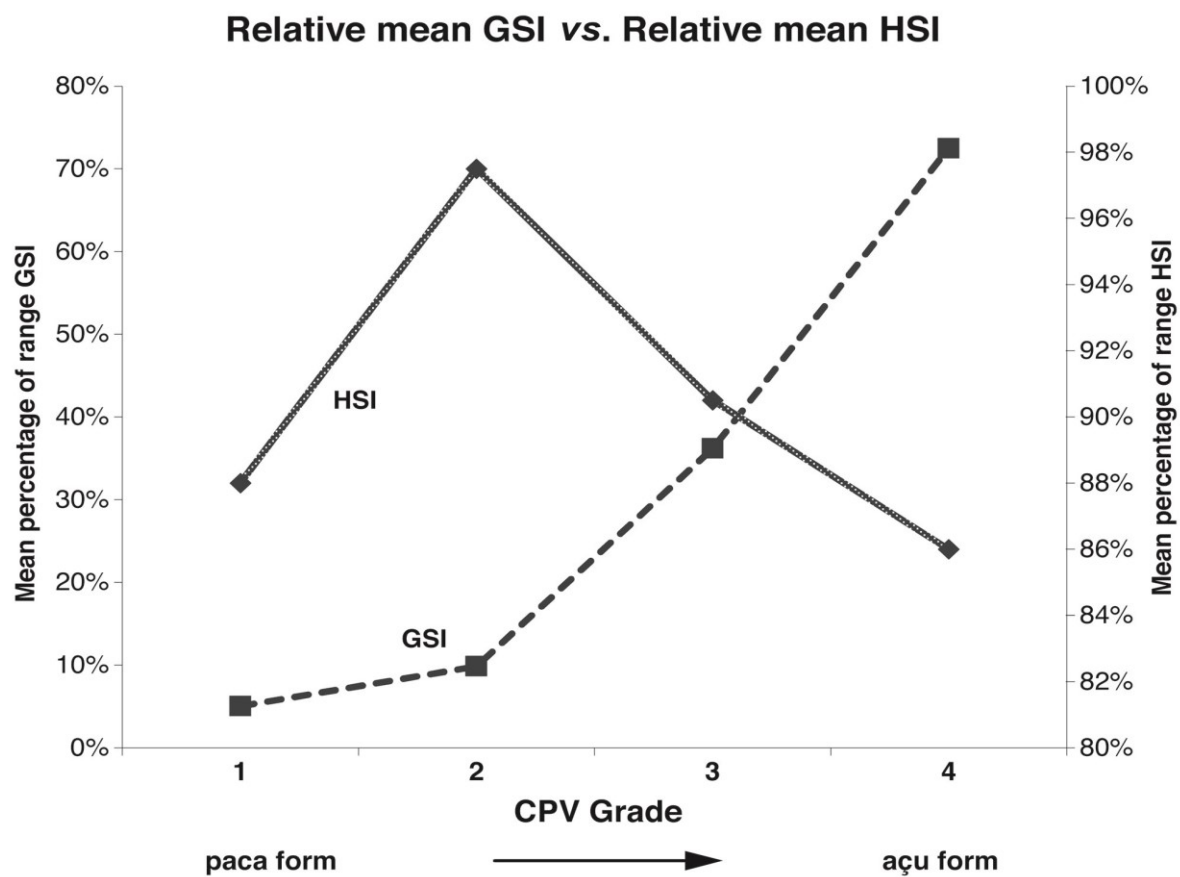
Chapter 3

A periodic environment yields a cyclical life history;

How *Cichla temensis* adapts to its flood pulse habitat

Abstract

An annual cycle of macro-scale environmental changes affects *Cichla temensis* in its natural, Amazon flood pulse habitat. In this context, enormous variations occur in water level, food availability and habitat structure. Although some prior findings address seasonal and cyclical environmental conditions, many studies were performed in isolated portions of the flood pulse cycle or outside of the flood pulse habitat entirely (i.e. reservoirs, pisciculture). Review of the literature on *C. temensis* suggests that aspects of the species' life history are temporally and spatially correlated with the flood pulse. Recent work supported by numerous observations made over many years reinforces this connection and suggests that cyclical flood pulse conditions drive many of its behavioral and physiological adaptations. Therefore, further synthesis is useful to refine these inferences. This review attempts to integrate the characteristics of the flood pulse and aspects of *C. temensis'* life history in a comprehensive and defined manner for the first time. The findings of two new studies included herein provide evidence for a relationship between *C. temensis* habitat use in relation to the flood pulse and demonstrate that *C. temensis* spawning frequency is negatively correlated with water level. These findings

complement the results of Chapter 1 and 2 of this dissertation to improve our understanding of the life history of *C. temensis*.

Introduction

Konrad Lorenz pointed out that an important function of biology, ecology and ethology is to provide insights into the relationships between an organism and its environment (Lorenz, 1950). Thereafter, Niko Tinbergen (1963) affirmed that animals possess behavioral characteristics in the same fashion that they possess structural and physiological characteristics. Maynard Smith (1966) then suggested that habitat-specific selection can maintain adaptations to different habitats. These perceptions asserted that aspects of an organism's life history may be correlated with environmental factors.

The River Continuum Concept (RCC) (Vannote & Minshall 1980) demonstrated that these theories are applicable to aquatic life; however, the Flood Pulse Concept (FPC) elucidates their applicability to Neotropical fishes (Junk *et al.,* 1989). The FPC indicates that physiological, morphological and ethological adaptations occur in response to the distinctive mechanical, energetic and trophic characteristics of rivers that experience cyclical changes in water level. Hydrological pulsing is a driver for patterns of ecological processes and predictable pulsing favors the adaptation of organisms to these patterns (Junk *et al.,* 1989). Water level fluctuations are major determinants of conditions such as food and habitat availability (Saint-Paul *et al.,* 2000). The timing of the flood pulse and the stage of the life cycle of the organisms affected can be a deciding factor in whether or not an organism can profit from the resources or environmental changes delivered by the flood pulse (Junk & Wantzen, 2004).

With specific reference to Amazon blackwater flood pulse fishes, certain life history patterns have been interpreted as being adaptive, considering the predictability and relative intensity of both temporal and spatial variation in abiotic environmental parameters (Winemiller, 1989). Recent studies have investigated the hypothesis that the predictable nature of the Amazon flood pulse is a driver of accommodating adaptations for other Amazon fishes (Crampton, 2008; Schaan *et al.*, 2009). Thus, a detailed synthesis of an organism's characteristics in relation to its environment can be instrumental in elucidating its life history and the forces that drive its adaptations (Azevedo *et al.*, 2011).

The central Amazon basin exemplifies an environment under the influence of a flood pulse (Junk *et al.*, 1989; Junk & Wantzen, 2004). A generally predictable seasonal progression of rainy and dry periods generates a cycle of extreme flood and ebb conditions. Water level changes typically range from 6 to 12 meters above low water (Crampton, 2008), but can vary as greatly as 15 meters within a single yearly cycle (Goulding, 1980). This enormous volumetric change triggers a series of secondary effects such as changes in pH, ionic concentration, nutrient availability, primary production, food availability and habitat accessibility (Melack & Forsberg, 2001).

The Perciforme fish family, *Cichlidae,* is widely distributed throughout the Amazon basin. Cichlids are among the most morphologically diverse and phenotypically plastic of fishes (Barlow 2001). It has been suggested that many species may be physiologically capable of adjusting aspects of their life history to conditions in their environment (Val & Almeida-Val, 1995). Within this family, the genus *Cichla* contains 15 species, several of which are native to flood pulse environments (Kullander &

Ferreira, 2006). Although a significant body of work exists on the genus, many earlier

studies were plagued with confusion regarding taxonomy, species range and behavioral

characteristics. Because of the logistical difficulties associated with studying these fishes

in remote rivers, many studies were performed in artificial environments outside of

natural flood pulse habitats, and often with the species misidentified or *incertae* (Willis *et*

al., 2007). In 2006, a morphologically based taxonomic revision (Kullander & Ferreira,

2006) allowed for better resolution in all aspects of the study of *Cichla,* especially for the

taxonomy of *Cichla temensis* (Reiss *et al*., 2012).

      Cichla temensis is an ideal example of an organism under the influence of a flood

pulse. The Amazon flood pulse's large scale cyclical environmental changes occur

throughout *C. temensis* natural distribution in tannin-stained, acidic, blackwater systems

(Winemiller, 2001). This species inhabits lowlands flood pulse rivers in regions of the

Amazon River basin including the Rio Negro and Uatuma drainages and blackwater

tributaries of the Rio Madeira and Rio Branco (Kullander & Ferreira, 2006; Holley, *et al*.,

2008; Reiss *et al*., 2012), as well as Venezuelan and Columbian flood pulse tributaries of

the Orinoco (Kullander, 2003). With its generally predictable pattern of ecosystem

changes, the flood pulse is a key factor affecting fish communities in aquatic ecosystems

(Saint-Paul *et al.*, 2000). A flood pulse can have different characteristics in different river

systems and water types**.** It can be monomodal or polymodal, predictable or unpredictable

and have a high or low amplitude (degree of water level change) (Junk & Wantzen,

2004). *Cichla temensis' * distribution consists primarily of monomodal, predictable

regions with a medium to medium-high amplitude, typically 5 to 10 meters difference in

water level (Wittman *et al.*, 2004). These conditions confer upon aquatic organisms a

"physiological and phenological window of susceptibility" to the benefits and disturbances of the flood pulse (Junk *et al*., 1989).

Cichla temensis' complex cyclical suite of physiological and behavioral characters (Reiss *et al*., 2012) serve to make it an ideal species with which to examine such ecosystem interactions. Thus, in consideration of what is currently known about *C. temensis,* efforts to understand its life history are best considered within the context of its habitat's flood pulse cycles. This chapter provides a synthesis of the ecology of *Cichla temensis*, based in part on the prior summary by Winemiller (2001), in the context of the flood pulse environment and incorporates more recent studies (Reiss *et al*., 2012; Reiss & Grothues, 2015). Further, I synthesize new information on the annual cycle of physiological changes, habitat use, feeding patterns, and spawning periodicity in blackwater systems. Understanding the interactions between these factors can help provide a basis for management of this economically important species and suggest future investigations. Furthermore, it may help elucidate the evolutionary and ecological forces that have led to the genetic and phenotypic adaptations to a cyclically changing environment in this, and perhaps other, Amazon species.

Materials and Methods

Study Area. The Amazon River and its associated basin is the largest river system in the world (Smith, 1981). Although the bulk of the system is in Brazil, it is also a major drainage for Venezuela, Columbia, Peru, Ecuador and Bolivia (Sioli, 1984). The study area considered herein is located entirely in northwestern Brazil, in the central Amazon

basin, and is focused on three regions within *C. temensis'* natural distribution, all of which contain blackwater river-floodplain systems (Figure 1).

The Rio Negro is the largest northern tributary of the Amazon, extending for over 1300 miles. It is the world's fifth largest river (by discharge) (Winemiller & Willis, 2011) and the world's largest blackwater river (Sioli, 1984). *Cichla temensis* is widely distributed throughout the river and its blackwater tributaries (Willis *et al*., 2007). The Rio Negro is described in three sections due to its massive size and differing regional, geological and fluvial characteristics (Lima & Ribeiro, 2011). The upper Rio Negro reaches from its headwaters to the city of São Gabriel da Cachoeira, Amazonas (AM), Brazil. The middle Rio Negro extends to slightly downriver of Barcelos, AM, Brazil, at its confluence with the Rio Branco, (Figure 1, between points N and CC) and spans a region with numerous dendritically patterned tributaries (tree-like, with v-shaped confluences). The lower Rio Negro continues to its confluence with the Solimões (Amazon mainstem) at the city of Manaus, AM, Brazil. All three sections are affected by the flood pulse and contain tributaries and floodplain structures typical of *C. temensis* habitat. The middle Rio Negro is categorized as a "periodically flooded forest wetland" (Junk *et al*., 2014). With its readily navigable waters, numerous tributaries, predictable high amplitude flood pulse, and readily accessible *C. temensis* habitat, this region has become an internationally recognized sport fishery (Holley *et al*., 2008). This has facilitated access for the author (owner of a sportfishing operation) and made this region a focal point of this study. Data collection was concentrated primarily on the Rio Negro's tributaries and not on the main channel, although *C. temensis* occur throughout.

Two other regions with accessible populations of *C. temensis* include the lower Rio Madeira basin, a "river-floodplain wetland", and the Rio Branco basin, a "periodically flooded savanna wetland", (Junk *et al*., 2011). Both are whitewater trunk rivers (waters carrying suspended sediment) that do not hold *C. temensis* in their main channels, however their blackwater tributaries do. In the Rio Madeira basin, *C. temensis* can be found in a region extending roughly from the city of Borba, AM, Brazil, to the Madeira's confluence with the Amazon. Data were collected in the Igapo Acu complex of this region (Reiss *et al*., 2012). Many of this study's observations were also made in blackwater tributaries in the Rio Branco basin (Vasconcelos *et al*., 2005) (Figure 1).

Blackwater floodplain structures in the Amazon basin differ morphologically from whitewater systems. This is due largely to the lack of sediment load and deposition (Goulding *et al*., 1988), to the differing substrate, and the differing flooded forest surroundings in blackwater systems (Sioli, 1984; Walker, 1985, Junk *et al*., 2011). Although these floodplain structures have not been clearly cataloged for blackwater systems, local names and descriptors are consistent throughout the region (personal communication - Flávio Ruben P. Oliveira Júnior; engenheiro fluvial). Since these structures vary substantially and since the limnological terminology used in whitewater systems is not precisely applicable, this synthesis will use the local names. An endeavor to correlate these native terms with technical nomenclature used elsewhere is included.

Permanent, transitional and ephemeral aquatic habitats exist in the study region during the flood pulse cycle and all were sources of *C. temensis* specimens, data and observations. Several types of lacustrine structures are encountered in the study area. Most are lentic at water levels below bankfull (the level that just fills the channel to the

top of its banks and below the point where the water overflows onto a floodplain (Leopold *et al.*, 1964)) and are generally permanently connected to the main river channel.

Using local terminology, permanent structures include; "igarapés" (creeks and creek-like blocked valleys, structurally similar to small ria lakes whose flow stops and becomes lentic in the absence of rains); "lagoas" (generally channel shaped lagoons, likely resulting from river bed migration); "voltas mortas" (oxbows or "lagos de ferradura" - semicircular or circular lagoons where the main stream of a meandering river has cut a new channel, isolating a portion of its old bed); and "lagos" (large, dish-shaped, lake-like depressions that may have independent water sources) (Wright, 1982; Jepsen *et al.*, 1997; Esteves, 1998) (Figure 2).

Transitional lentic areas are temporary and created by falling water levels in normally fluvial areas. They include "ressacas" (deep shoreline pockets) and "paranás secas" (secondary channels or braids whose flow is interrupted by emerging sections of river bottom) (Esteves, 1998). Ephemeral structures are created by changing hydrodynamics in fluvial shoreline or open-water areas, particularly in periods of falling water. These areas are often conducive to *C. temensis* feeding and may persist for days or weeks. They include; "pontos" (shoreline points with flow on the upriver side and interrupted flow downriver; "saídas" (the downriver outlet of draining flooded areas); "praias" (beaches); "barrancas" (steep banks downriver from lagoon mouths); "bocas" (the mouths of lagoons, creeks and small streams); "aguas paradas" (interfaces between flowing and still water or still sections within or between eddies) (personal and guide observations) (Figure 3).

The flood pulse cyclically inundates or exposes terrestrial areas bordering the river and its aquatic structures. Described as the Aquatic Terrestrial Transition Zone (ATTZ), these areas are correspondingly exploited or abandoned by aquatic biota (Junk *et al.*, 1989) (Figure 2). Igapó, a low-diversity, seasonally flooded forest lines the banks of blackwater flood pulse systems (Goulding, 1980). Lower in gradient, are areas of standing live or dead timber and "galhadas" (branch and root tangles of fallen trees) (Crampton, 2008), often interspersed with terrestrial shrubs. Grasses are mostly absent in nutrient poor blackwater systems (Goulding *et al.*, 1988), leaving the moving littoral's final stop at the water's sand or clay bottom (personal observation) (Figure 4). The floodplain is surrounded by "terra firme" forests, outside of the ATTZ, which are not inundated.

Sources. This synthesis, including a review of the literature and new studies compares aspects of *C. temensis'* life history and the Amazon basin's flood pulse environment. The attributes of the FPC are from the existing literature. However, specific aspects of the flood pulse occurring within blackwater habitat, as well as *C. temensis'* responses, were obtained from a combination of sources. Sources include the existing literature and recently reported results that are presented in Chapter 1 and 2 of this dissertation. Additional information comes from previously unpublished experimental data, accumulated statistics compiled from 2001 through 2014 in Brazil's central Amazon lowlands by a commercial sportfishing operation (Acute Angling, Inc.), and personal observations made from 1994 to the present (Table 1) in 42 Amazon lowland flood pulse rivers (Figure 1).

The commercial sport fishing operation (owned by the author for over 20 years), is focused on catching *C. temensis* in the central Amazon lowlands. The operation's mobile mothership and floating camp facilities move from the southern central Amazon basin to the north, in a yearly progression from September through March. The route is designed to follow the progression of cyclical weather patterns that generate the flood pulse, allowing the operation to remain in productive fishing conditions. It hosts approximately 30 groups of 8 to 14 anglers per group, using 4 to 7 guides piloting individual aluminum bass boats over a 26-week season each year. It operates primarily during falling and low water, with or without the author's presence. The operation typically captures (and releases) 30,000 to 40,000 *C. temensis* per season, with each capture recorded. The data reported serves to connect measurable and observable environmental effects of the flood pulse with habitat use, feeding and spawning activities.

Additional observations used were made over a period of 20 years on 42 rivers by the author and a team of experienced fishing guides. All guides were trained by the author in assessment of CPV grade, data recording and morphometric measurement techniques (Reiss *et al*., 2012) (Table 1, Figure 1).

Color Pattern Variation and Habitat use Study. Observations and catch data from the sportfishing operation were used to analyze relative location and the ratio of açu to paca in the late FW and early LW periods in the Rio Igapo Açu/Lago Tacquia complex, a river-floodplain system. This region is south of the Amazon mainstem, separate from the Rio Negro basin, and influenced by the Rio Madeira's flood pulse (see Figure 1 map inset and point labelled "IG"). Therefore, the flood pulse periods occur approximately four to six weeks earlier than the flood pulse periods described for the Rio Negro basin. The

system is, nonetheless, typical of *C. temensis'* blackwater habitat. Data collection occurred over a four-week period in September and October of 2008, during which conditions corresponded to an idealized period. During this time, the sportfishing operation ran a weekly route from the outlet to the headwaters of the system and back. The operation's guides were supplied with waterproof notebooks to document the number of fish in each CPV grade (as described in Reiss *et al.*, 2012) caught daily by the two anglers fishing with each of the guides for a total of 6672 observations. This data was compared to overall water levels in the system (measured in a fixed location) along with the relative water level of the area fished (a function of gradient within a river or system), during the progression along the flood pulse periods. Statistical analysis of these data by multiple linear regression was calculated using Matlab.

Spawning Frequency. An analysis of spawning frequency in relation to water level was made over a five week period on the upper Rio Caures in late October and early November of 2012 (Figure 1, point C). During these months, water levels dropped quickly and consistently from just prior to bankfull in the FW through to the beginning of LW. In this season, the naturally occurring period was essentially oscillation free and corresponded to an idealized period.

The sport fishing operation's trained and experienced fishing guides regularly scan surface waters to identify schools of fry guarding parents and their broods (since they provide their angling clients with excellent opportunities to encounter their quarry). Anglers commonly call these "fry balls". The term "fry" is used throughout this synthesis to describe young *C. temensis* under parental guarding. In total length (TL) they range

from free-swimming larva at approximately 6 mm TL to small juveniles, up to approximately 60 mm TL (Rutledge & Lyons, 1976; Zaret, 1980). During the study period, guides were instructed to record only *C. temensis* fry sightings that satisfied a specific requirement. Since a small percentage of viewable fry are attributable to *Cichla orinocensis*, a sympatric congener who similarly guards its brood, fry sightings were accompanied by angler attempts to induce a guarding parent to strike a lure designed to appear to threaten a brood. When a strike by *C. temensis* occurred, guides used a clicker to note those sightings. The strike observation requirement also helped to minimize possible cumulative effects since parents rarely struck again after an initial capture. One additional step was taken to avoid potentially confounding effects. Every seventh day was a transition day when anglers were changed, thus angling effort was reduced during these days. In order to minimize this effect, transition day variability was neutralized by considering it identical to the previous day. Statistical analysis of this data by Pearson's product-moment coefficient was calculated using Microsoft Excel.

Results

Synthesis of Flood Pulse Characteristics. The FPC describes a lateral exchange between a river channel and its associated floodplain due to a mostly consistent, cyclical change in water level of the parent river that instigates massive environmental changes. The focus here is on the effects of the cyclical drought and inundation that characterizes the flood pulse and is a major influence on the ecology of the organisms occupying the connected "river-floodplain system" (Junk & Wantzen, 2004). In lowland regions of the Amazon basin, the annual hydrological cycle causes periodic coupling and decoupling of

heterogeneous floodplain areas from the parent rivers (Junk *et al.*, 1989). In effect, the annual flood pulse creates a slowly but continually moving land–water interface that forces littoral zone fishes to accommodate constantly changing habitats in a non-linear but generally predictable cycle (Arrington & Winemiller, 2006). This floodplain area is described as the aquatic/terrestrial transition zone (ATTZ) and its inshore edge is termed the "moving littoral" (Junk *et al.*, 1989) (Figure 4).

Four hydrological periods (Figure 5) are recognized, as derived from those defined by Arrington and Winemiller (2006) in reference to a blackwater *C. temensis* habitat in Venezuela and congruent to those described more generally in Columbia by Munoz-Sosa (1999). These periods present distinct differences in factors affecting *C. temensis* such as food availability, spawning sites, brood and juvenile food supplies and shelter from predation. A generalized location corresponding to several tributaries in the middle Rio Negro basin is used to define a typical calendar of the hydrological periods. This region is offset by roughly two months earlier from a region in Venezuela (Arrington and Winemiller, 2006). The yearly flood pulse cycle in *C. temensis'* blackwater habitat occurs at different times in different regions of the Amazon basin. This is due to differing weather patterns and hydrological effects that may be temporally disconnected from local rainfall by the impounding effects of trunk rivers (Goulding *et al.*, 1988) and by water stored in the floodplain (Richey *et al.*, 1989a) (Figure 6). The cycle in rivers with divergent directions of flow as close as 50 km apart may be offset by weeks or months.

Falling Water (FW) typically occurs in the middle Rio Negro basin from September through November. Heavy rainfall in the area and to its south typically ceases

in July (Junk *et al*., 2014). Water levels in the Amazon mainstem drop, allowing the Rio

Negro and its tributaries to begin to empty (Forsberg *et al*., 2001). Water leaves the igapó

(seasonally flooded forest), begins to expose lacustrine floodplain structures and drops

from its peak level through bankfull level, continuing until reaching its low point.

Low Water (LW) typically occurs from December through February. Water levels

reach their yearly low point and remain there for several weeks, reducing most aquatic

habitat to a "spatial mosaic" (Junk *et al*., 1989). In a typical tributary system feeding a

trunk river, like the Rio Negro, this consists of the main channel, side channels and lentic

floodplain remnants such as igarapés, lagoas, voltas mortas, lagos, ressacas, and paranás

secas.

Rising Water (RW) occurs from March through May. Counterclockwise-rotating

Coriolis forces occur south of the equator (Pianka, 1978) and drive cyclical weather

patterns throughout *C. temensis'* habitat, resulting in a south to north progression of the

flood pulse periods (Bastos *et al*., 2007). Water levels begin to rise, often without rain

occurring in that area. Earlier rains in the southern basin may affect the headwaters of

north flowing Rio Negro tributaries and fill the Amazon mainstem, causing impounding

effects in the Rio Negro. Rains typically begin in April on the Rio Negro itself, and

contribute further to the rise. Water overflows banks and begins to fill the igapó.

Tributaries flowing from south to north and experiencing both rain in the headwaters and

impounding effects from the Rio Negro, may rise sooner than the mid and upper reaches

of north to south flowing rivers, where headwaters remain dry longer.

High Water (HW) occurs from June through August. Water levels peak, generally

5 to 10 meters above LW, and may remain high, trapped by the impounding effects of

trunk rivers and the Amazon mainstem, even when local rains have ceased. In some regions, an overflow from whitewater systems may deliver pulses of nutrient to otherwise blackwater systems while neighboring systems may create transitory interfluvial marshes connecting them (Forsberg *et al.*, 2001).

Large river flood pulse phenomena are considered to be consistent and predictable on a macro-scale (Richey, 1989b; Bayley, 1995), however, there are often substantial day-to-day, week-to-week and year-to-year oscillations within the larger cycle (Junk & Wantzen, 2004) (Figure 6b). The larger, macro-scale cycle is likely the primary driver of the associated *C. temensis* life history characteristics examined herein (Junk & Wantzen, 2004). Therefore, in order to more clearly describe the scale and consistency of this larger cycle, flood pulse water levels are schematically represented throughout this synthesis as a series of sinusoidal curves composed of positive and negative values using bankfull level as the ordinate. The variability pattern of these waves taken in a temporal sequence constitutes the flood pulse cycle (Neiff, 2001). The macro-scale phenomena are characterized without the inclusion of unpredictable and inconsistent short-term oscillations (Figure 5). Such "idealized" schematic depictions are used in the literature to describe macro-scale flood pulse phenomena (Smith, 1981; Wright, 1982; Junk *et al.*, 1989; Tockner *et al.*, 2000; Arrington & Winemiller, 2003; Junk & Wantzen, 2004). The specific pattern used in this synthesis follows that of Junk *et al.* (2014) for the middle Rio Negro. Consistent with this approach, seasons, conditions and behavior patterns are also "idealized". Since different areas within a single system can vary greatly in depth and gradient, all depictions are based on effects that would occur at a single point or location within a larger system.

The Flood pulse in Blackwater Systems. The flood pulse affects each of the Amazon basin's three primary water types, whitewater, clear water (sometimes called blue water) and blackwater. However, the flood pulse in blackwater systems differs substantially from that of other water types (for detailed descriptions of white and clear water see Junk, 1973; Sioli, 1984; Henderson & Crampton, 1997; Saint-Paul *et al*., 2000). Unlike whitewater river-floodplain systems with high loads of inorganic nutrient transported in suspended sediments, blackwater river-floodplain systems have low concentrations of suspended sediments and produce relatively infertile floodplains (Goulding *et al*., 1988; Junk *et al*., 1989). They are stained with chromatic dissolved organic compounds and along with low pH (ranging from 3.6 to 5.8) and low conductivity w (<10 μS cm $^{-1}$), they have low dissolved inorganic nutrient content (Goulding, *et al.,* 1988: Wilson, *et al*., 1999; Sioli, 1984).

Blackwater occurs when acidic, wet, anaerobic soils permit the slow decay of organic matter. Sourced primarily from vascular plant material submerged during high water in the igapó and further contributed to by runoff from forest sources, blackwater is generated by continual leaching of humic, fulvic and tannic acids along with a complex mixture of other dissolved organic matter (DOM) (Ertel *et al*., 1986). Humic acids can make up 40% to 80% of the DOM in blackwaters. Fulvic acids, phenolics, lignin and other organic acids, comprise the bulk of the balance (Ertel *et al*., 1986). The blackwater Rio Negro drainage in the Brazilian Amazon is darkly stained by its high content of DOM (Ertel *et al*., 1986), giving the trunk river its name, which translates as Black River.

Colored and light absorbing humic and fulvic acids also generate high dissolved organic carbon (DOC) concentrations in surface waters (Roelke *et al*., 2006). Nutrient

poor soils underlain by high kaolinite clays (or podzols in the Rio Negro basin) sustain

these concentrations (Sarmiento & Pinillos, 2001). Despite the presence of this

allochthonous carbon in the DOC content, dissolved nutrient levels remain quite low.

Lack of alluvial sediment from the Andean uplands further limits productivity in

lowlands, blackwater systems (Sarmiento & Pinillos, 2001), creating substantially

different ecosystem characteristics than in other Amazon flood pulse waters.

Highly acidic and ion-poor waters are generally associated with low abundance,

low diversity and low biomass of aquatic animal life. Fish biodiversity and abundance,

however, are very high in the Rio Negro basin (Goulding *et al*., 1988). The ability of fish

to thrive physiologically in these highly acidic conditions has led to speculation that

DOM might be protective against the ion-poor acidic conditions (Gonzalez, *et al*., 2002).

The acid tolerance exhibited by native blackwater fishes leaves water level fluctuations as

a key environmental factor affecting these fishes. Water level in turn, strongly influences

food and habitat availability (Saint Paul *et al*., 2000). The particular characteristics of the

flood pulse in blackwater systems are important aspects of the environment affecting *C.*

temensis, differing substantially from conditions affecting many of its allopatric

congeners in other water types.

Cichla temensis is found almost exclusively in blackwater systems. Although

isolated observations of individuals encountered in whitewater in the Rio Branco basin

during RW and HW have occurred, this is presumed to be the result of temporary

flooding between systems or simply fish passing from one system to another when such

transitory connections exist. *Cichla temensis'* are not normally observed in clear waters

(also called blue waters in some regions) occurring within their natural range (such as

headwaters streams) (Winemiller, 2001; personal observation). There is evidence that *C. temensis* is not as physiologically well adapted as other *Cichla* to variations in water type (Winemiller, 2001).

Color Pattern Variation and Habitat use Study. With the exception of water level, the physical and chemical water parameters of the Rio Igapo Açu/Lago Tacquia system (Figure 7) remained stable throughout data collection. Water levels were consistent with a blackwater river transitioning from FW to LW mode. The mean water conditions were; temperature 30.34C (SD 0.83), pH 5.41 (SD 0.59), dissolved oxygen 5.56 (SD 0.74) and conductivity 0.007 s (SD <0.001). Water levels, measured at a single point, dropped 216 cm over the 4-week period.

Angler skill levels varied greatly, and guides were afforded flexibility in their daily operations, thus individual guide results and daily totals did not demonstrate trends when examined in isolation. However, since anglers (n = 40), guides (n = 7) and angling target locations (n = >1000) changed constantly throughout the total four weeks (214 angler/days) observation period, the variability of individual skill levels and specific daily fishing effort was submerged within the large amount of pooled data (Figure 8). Pooled data included 107 individual sampling events from which 6672 measurements were taken. A multiple linear regression model was calculated, where; Percentage Acu = (WaterLevel x β_1) + (RelativeGradient x β_2) + b. Relative gradient refers to the phenomenon of water levels being relatively lower in upriver locations, thus creating a relatively more advanced stage of the flood pulse cycle as compared to downriver locations. Both water level and relative gradient had a significant effect on the percentage

of açu versus paca (Table 2). During FW and LW, there was a consistent overall increase in the percentage of açu as overall water levels fell in the river-floodplain system (p = <0.01) (Figure 8c). The relative gradient increases for each of the locations from 1 through 5, thus relative water level decreases. Each location represents approximately equidistant upriver movement (Figure 7). The percentage of açu relative to paca increased as the relative gradient increased (p = <0.001) (Figure 8d).

Spawning Frequency. Spawning frequency, as measured by fry sightings, increased steadily during the five week study period on the Rio Caures, reaching a maximum when water levels reached a minimum (Figure 9). There was a significant negative correlation (Pearson's r = -0.9205; p = <0.0001). Water levels, measured at a single point, dropped a total of 147 cm during this period.

Response of *C. temensis* to the Amazon Flood pulse Cycle

Falling Water (FW) –

Habitat availability and use. In pulsative environments, the pattern of flooding or ebb may influence the manner in which organisms utilize a variable ecosystem (Rozas, 1995). The changing water level and moving littoral experienced by *C. temensis* through the flood pulse periods modifies the type and abundance of available habitat, consequently mediating the availability of prey, protective cover and functional spawning structure.

Early FW creates productive feeding habitat while later FW creates more spawning habitat. Thus, during FW, habitat use becomes nonrandom (Arrington & Winemiller, 2006). As water levels fall and approach bankfull level, large-scale changes

in surface area, volume, and the ATTZ occur rapidly. The moving littoral redefines the available habitat types and triggers an array of behavioral and physiological responses in *C. temensis*. Two distinct habitat types begin to become available in close proximity to each other, with a tendency to be selectively occupied by individuals in differing CPV grades.

Newly exposed, ephemeral structures particularly conducive to feeding, such as "pontos", "saídas", "praias", "barrancas" and "bocas" (Figure 3) provide effective foraging opportunities for *C. temensis* (personal and guide observations). These sites, generally associated with interfaces between lotic and lentic areas, yielded a higher percentage of fish in the paca form (individuals not yet prepared for the spawn (Reiss *et al*, 2012)) (Figure 8). Their foraging during this period precedes the process of depositing the fatty reserves that will be used for gametogenesis and the extended fast occurring in association with the spawn and subsequent fry guarding period (Arrington *et al*., 2006; Reiss & Grothues, 2015).

Some individuals may begin this maturation process earlier or proceed more successfully than others do, thus, a continuum of CPV grades begins to coexist at this time in the flood pulse (Reiss *et al.,* 2012). In contrast to seasonally immature paca variants, açu variants are prespawn, actively spawning, or fry guarding individuals. As water level continues to fall, additional lentic area forms, exposing increasing numbers of potential spawning sites (primarily in "igarapés", "lagoas", "voltas mortas", "lagos", "ressacas" and "paranás secas") (Figure 2). The sportfishing operation's data showed that the percentage of açu variants captured increased relative to paca variants as these lentic areas became more available. While conditions suitable for foraging and feeding tend to

aggregate paca, spawning site availability tends to aggregate açu within a system (Figure 8b and 8d). In addition, angler catch statistics demonstrate that the overall percentage of fish in the paca form caught by angling is higher early in FW while açu become more prevalent as water drops (Figure 8c). During FW, both overall water level and relative water level (lower relative water levels occur in higher gradient positions) (Figure 7) and based on angler catch are inversely correlated to the percentage of açu versus paca. (Figure 8d). Thus, habitat use by individual *C. temensis'* in different CPV grades is linked to the type of physical space made available during each of the hydrological periods of the flood pulse.

Feeding. Prey availability is at a maximum and prey are more easily accessed by predators during FW because the receding moving littoral isolates or drains floodplain bodies such as interfluvial marshes, disconnected lagoons and igapó (Figure 10) (Saint-Paul *et al*., 2000; Luz-Agostinho & Agostinho, 2008). The reduction in water volume and floodplain surface area concentrates all forms of aquatic life in a reduced space (Lowe-McConnell, 1979) and drastically diminishes habitat for prey (Crampton, 2008). Both prey and predators begin to move into the river channel, its connected lagoons, and permanent low-water bodies as they are forced to leave the draining igapó. In addition to the increased availability of local prey species, the transport of primary production from whitewater rivers that are rich in nutrient into oligotrophic blackwater systems by migratory fishes appears to be a general phenomenon within Neotropical river basins (Hoeinghaus *et al.*, 2006). Important migratory prey species in the genera *Semaprochilodus*, *Prochilodus* and *Brycon* enter many blackwater systems during FW,

importing biomass from outside and further contributing to food availability (Hoeinghaus *et al*., 2006).

As a result of these changing conditions, feeding during FW is intense. Angler catch is most productive with food-imitating baits at this time (personal and guide observations). Observation of feeding fish caught by angling shows that they are often found in small groupings (5 to 35 fish), in proximity to areas providing ready access to prey. The groupings tend to consist of similarly sized individuals. The number of individuals in a group appears to be inversely correlated to the group member's individual sizes. For example, a grouping of young adults (approx. 320 to 400 mm TL) may consist of 25 or more individuals; while a group of larger adults (approx. 500 mm or more TL) feeding together rarely exceeds 10 individuals (personal observations, n = >100). *Cichla* are extremely effective predators with the ability to significantly affect prey populations (Winemiller, 2001). They typically occupy the top of the piscine component of the aquatic food chain and have been described as potential keystone predators (Winemiller, 2001). Thus, as the FW progresses, prey populations begin to diminish due to the intense predation (Jepsen *et al*., 1997).

Spawning. The availability of sites suitable for *C. temensis* spawning increases during FW (Figure 11). Permanent flood plain structures cease to be inundated by high waters, allowing downstream flow to become blocked by newly emerged land area and making newly lentic transitional aquatic structures such as "ressacas" and paranás secas" available (Jepsen *et al*., 1997). Within these lentic floodplain remnants, the littoral moves from the densely forested and shaded igapó to the sparser, newly exposed edges, creating

increasing amounts of sites appropriate for spawning. Based on over 300 nest

observations, these are typically in sunlit areas, away from the shaded forest. Sites are

centered on woody structure or debris on a bottom consisting of suitable (generally

sandy) substrate at depths between 1 and 1.5 meters. As spawning sites become more

available, an increasing number of mature *C. temensis* (açu with deposited fatty reserves

(Reiss *et al*., 2012)) begin to spawn, as shown by the results of the spawning frequency

study (Figure 9) and as indicated in the literature (Jepsen *et al*., 1997).

Early life history. As spawning activity proceeds, post-spawn adults will begin to enter

open, lentic lagoon waters guarding recently hatched, free-swimming broods. Fry

guarding in *C. temensis* is a well-observed phenomenon in our sport fishing operation.

Fishing guides typically encounter fry guarding adults several times per day because they

are vulnerable to capture by angling and are sought after. In addition, the author has

observed (n >1000) guarded broods of *C. temensis* in over 30 rivers during a 20-year

period. Guarding parents are always in the açu form and the most visible early stage

broods feed primarily on zooplankton at the surface. Although the study area's

blackwater is heavily tannin-stained, it is relatively transparent (> 1 meter Secchi depth

(Junk, *et al*., 2011)), making these brightly colored, CPV grade 4 individuals readily

observable. They range in size from just over 300 mm TL (the smallest sexually mature

individuals (Jepsen *et al*., 1999)), to approximately 1 m TL (the largest sizes encountered,

(Holley *et al*., 2008; personal observation)). Adults always appear positioned in relation

to their foraging fry. The fry maintain a compact school, directly below the surface, and

slowly move in a coordinated direction. They visibly dapple the surface as they feed on

zooplankton that are concentrated in the photic zone (Zaret, 1980). The male is generally

visible immediately below the brood and slightly ahead of its leading edge, making it

appear as though the male is the school's focal point and leading the school. The female

can be seen (or detected indirectly by lures), within 2-3 meters of the brood. Guarding

parents react aggressively to perceived threats. Whether presented by an artificial lure or

an actual fry predator (such as *Acestorhyncus falcatus*) moving into the brood, the male

will attack the threat violently, slapping it with his mouth, head, side, or tail. The female

will also attack if a threat is detected appropriate to her position, particularly if the male

has already responded or been captured. Fry are guarded for as long as 2 months and can

reach sizes > 60 mm (Jepsen *et al*., 1999; personal observation).

Low water (LW)

Habitat availability and use. LW creates new conditions where protection from

predation (Jepsen *et al*., 1997) and access to food become priorities for individuals not

engaged in spawning or fry guarding. Although the maximum extent that water recedes

from year to year and the duration of LW can vary greatly (Melack & Forsberg, 2001), in

most circumstances, the moving littoral entirely retreats from the igapó. This leaves

"galhadas" and submerged small woody debris to provide cover against predation along

lagoon banks (Figure 4). Without access to cover, small to medium sized *C. temensis*

individuals can be exposed to predation by freshwater dolphin (*Inia geoffrensis*) and

otters in daytime, and by caiman, snakes, and subsistence fishermen at night, when they

are essentially inactive (Goulding *et al*., 1996; personal observation). Thus, non-

spawning *C. temensis* individuals will often be concentrated near cover providing

structures during LW (personal and guide observations). Although most lagoons will

retain a connection to the main channel, even if only a shallow or narrow neck, some floodplain lakes and features may become entirely disconnected. Unless they are large and/or have a secondary source of water input (such as springs or creeks), fish populations tend to not remain in disconnected features (personal observation). Non-spawning individuals may retreat to the river channel, often in substantial numbers as LW progresses. There, they tend to be concentrated in "bocas" and "ressacas" with adequate cover and access to prey (personal observation).

Feeding. LW instigates a period of maximal resource limitation for aquatic biota and feeding, along with food availability, becomes reduced (Lowe-McConnell, 1964; Winemiller, 1989). Fish captured by the sportfishing operation during LW are increasingly in the açu form as spawning activity (and its associated extended fast) moves toward its maximum (Figure 8). Individuals with mature gonads do not have food in their stomachs (Jepsen *et al*., 1999). Feeding for nest and brood guarding piscivores is likely constrained since pursuit of prey would leave the eggs or fry vulnerable to predators (Jepsen *et al*., 1999). Feeding during this period may also be hormonally constrained, since nest guarders have been observed to refuse prey, even when readily available without requiring pursuit or immediate risk to the eggs (Jepsen *et al*., 1999). The author and fishing guides also observed this behavior with nest guarding individuals (n = >1000); however, post-spawn fry guarders will opportunistically attempt to feed if a food-imitating lure is presented in a manner that allows engulfment without leaving their fry guarding position (n = >1000 observations). This behavior is distinct from the non-engulfing, violent protective attacks shown toward threats to fry.

Spawning. During LW, spawning sites reach their maximum availability and *C. temensis* arrives at the peak of its spawning period (Figure 11) (Jepsen *et al*., 1999). Most *Cichla* species are capable of multiple clutches each year in reservoir and artificial habitats with reduced or irregular variability, however, the predictable resource fluctuation in natural flood pulse habitats likely drives reproductive seasonality in *C. temensis* (Jepsen *et al*., 1999)*.* The extended fast that begins with spawning, and continues through post-spawn brood care, acts as a "physiological winter" (Lowe-McConnell, 1964) for *C. temensis*. The likelihood that *C. temensis* is restricted to a single spawn per year in its natural flood pulse habitat is further supported by examination of otoliths showing annuli that demonstrate a once-yearly period of reduced food intake and growth (Jepsen *et al.,* 1999).

Morphological changes occur in *Cichla* spp. before spawning. Nuchal hump formation occurs in *C. temensis* prior to spawning (personal observation), similar to that described for an introduced population of *Cichla* in Lake Gatun Panama (Zaret 1980) (originally described as *C. ocellaris*, this population has since been identified as *C. pleiozona* (Kullander & Ferreira, 2006)). Varying degrees of intensification of coloration have been observed in prespawn individuals of other *Cichla* spp. (Zaret, 1980; Winemiller, 2001; Chellappa *et al*., 2003; Gomiero & Braga, 2004), although none are as distinct as the CPV grade changes that occur in *C. temensis* prior to spawning (Reiss *et al.*, 2012).

Based on personal observation of *C. temensis* and similar to the described behavior of other *Cichla* spp. (Zaret 1980; Jepsen *et al*., 1999), the spawn typically begins with a pair bonded male and female preparing a site containing a woody or rocky

object that permits egg adherence at depths ranging from 1 to 1.5 meters. Nesting observations (n = >300), and fry guarding observations, have shown the male to be the larger individual, ranging from approximately 20% to more than 300% of the estimated (or measured) weight of the female. Bottom substrate varies from region to region. In sandy bottom areas such as the Rio Negro basin, pairs will typically select an exposed piece of wood on the bottom and clear the surrounding area of detritus. The male digs several small pits adjacent to the wooden egg substrate where he will later position the newly hatched larvae. The author has observed female spawners to make a pass over the substrate, depositing a linear row of self-adhering eggs, followed closely by the male, ostensibly fertilizing the row of eggs. This process is repeated for up to two hours, similar to that reported for other *Cichla* (Braga, 1952; Zaret, 1980). Fecundity ranges from < 2,000 eggs up to > 10,000 eggs, and as in other batch-spawning species, is probably dependent on female size (Winemiller 2001; Gomiero & Braga, 2004). Once egg deposition and fertilization is complete, the male will position himself directly next to or above the eggs, ostensibly to create circulation and guard against egg predators. The female will occupy a more remote and variable position, analogous to a perimeter guard. *Cichla pleiozona*, however, was described as behaving with mostly reversed (although sometimes overlapping and interchanging) roles between the sexes during egg and fry guarding (Zaret 1980).

Early life history. Eggs begin to hatch after approximately 48 hours. The male picks up the newly hatched larvae in his mouth and deposits them in one of the pits he has dug in the sand surrounding the woody substrate (personal observation; Zaret, 1980). He then

positions himself over the pit, fanning the location, while both parents guard against

predators, primarily small characins, who are typically positioned in close proximity.

After an additional 2 to 3 days, the yolk sacs are absorbed and the larvae become free-

swimming fry that are capable of feeding (Zaret, 1980).

Parents and brood then leave the nesting area together. The newly free-swimming

fry gather around the head and nuchal hump of the male (personal and guide observations

(n = >300)). At this time, the young may number in the thousands and are approximately

6 mm in TL (personal observation; Zaret 1980). The male, with the brood still clustered

around his head, will slowly swim from the nesting site into lentic open water, while the

female continues to provide perimeter duty (personal observation) during this transition

and for some time beyond. The author's observations suggest that the female leaves the

brood well before the male, possibly as soon as two weeks after fry guarding begins. The

brood and parent(s) remain in this manner, with either both, or only the male, guarding

the fry for up to two months, or until they reach a size of > 60 mm TL (Zaret, 1980). Fry

grow at approximately 1 mm per day (Rutledge & Lyons, 1976). Observed later-stage

broods with large fry appear to consist of individuals numbering in the hundreds

(personal observation).

Rising Water (RW)

Habitat availability and use. Rising water causes large-scale movement of the littoral in

the ATTZ (Figure 2, Figure 4) and fishes respond. Once bankfull level is exceeded,

surviving prey species, largely in the families *Prochilodontidae, Anastomidae and*

Characidae, return to the flooding igapó, seeking cover from predation (Saint-Paul *et al*.,

2000) and food (periphyton, zooplankton, detritus and fallen vegetation) (Forsberg *et al.*, 1993). *Cichla temensis,* likely following its prey, is now rarely encountered in the FW and LW structures it recently occupied. Postspawn, fry-guarding fish are still observed in open water, although many will enter the igapó, presumably for forage for their broods and possibly for protective cover.

Feeding. Feeding opportunities are now at a minimum for *C. temensis*. Diminished prey populations make the ephemeral feeding locations created in FW relatively unproductive when they reappear in RW. Water movement is reversed relative to FW, now flowing into, rather than out of, the floodplain. Remaining prey fishes are disappearing into the igapó and migrators begin returning to flooded whitewater systems. Fry guarding parents continue to fast.

Spawning. Rapidly diminishing lentic areas diminish the number of potential spawning sites; consequently spawning frequency drops. Simultaneously, minimal prey availability for adults and greater food availability for fry and juveniles coincides with the period of post-spawn fry guarding and parental fasting. This suggests that *C. temensis*' reproductive timing is adaptive and serves to improve fitness, as described for other flood pulse fishes (Lowe-McConnell, 1979) (Crampton, 2008). *Cichla temensis* parents, as capital breeders, have built up their reserves prior to spawning and are physiologically prepared to endure the extended fast instigated by their fry guarding reproductive strategy coinciding with the reduced availability of prey (Arrington *et al.*, 2006; Reiss & Grothues, 2015). The effect of this period of extensive resource expenditure, minimal

resource regeneration and reduced prey availability leaves most *C. temensis* in significantly reduced condition, as estimated from the condition factor, K (K=W/L3) (Jepsen *et al.*, 1997; Hoeinghaus *et al.*, 2006).

Early life history. *Cichla temensis* continue guard their broods in both remaining lentic areas and parts of the newly accessible igapó (personal observation). Fry may benefit from the additional surface area of water, which enables increased primary production, and the newly available submerged leaf and stem structure that offers algal periphyton (Putz & Junk, 1997); both are food sources for the zooplankton the fry feed on (Zaret, 1980). Tropical fishes possess life history adaptations to flood pulse environments, in particular, reproductive seasonality that coincides with rising water (Hoeinghaus *et al.*, 2006), allowing offspring to access a richer food source in the floodplain (Goulding, 1980). As fry mature to independent juveniles, their diet changes to small fishes and crustaceans (Braga, 1953; Zaret, 1980). Access to the igapó during this period, and the increased availability of prey fishes' offspring and other small prey, likely provides greater access to *C. temensis* juveniles' changed food source. No longer guarded by parents at this time, juveniles are also likely to benefit from the additional protective cover in the igapó, much as their parents' similarly sized prey does. Thus, for *C. temensis*, spawning during LW and prior to RW likely ensures that progeny are able to maximize their benefit from the changing conditions driven by the flood pulse.

High Water (HW)

Habitat availability and use. The igapó is flooded once more (Figure 2c) and *C. temensis* individuals are now difficult to encounter. Except for rare fry guarders, almost no individuals are observed in the open areas of the resubmerged river-floodplain complex. Although little is definitively known about *C. temensis* habitat use and movement during HW, studies have shown that tagged *C. temensis* can move up to 40 km in a year before recapture (Holley, *et al*., 2008). Conversely, other work indicates that they are relatively sedentary and rarely move far from their initial tagged location during the course of a year (Hoeinghaus *et al*., 2003). It may be possible that both things are occurring differentially in HW in response to an individual's size and food type availability.

Feeding. The feeding behavior of *C. temensis* during this period is not well known, although it is likely that foraging tactics change due to the vastly increased water volume and dense cover that prey can now access (Bayley, 1995). In some regions, an overflow from whitewater systems may deliver pulses of elemental nutrient to blackwater systems, increasing primary production and ostensibly increasing food availability for prey fishes. In other regions, adjacent blackwater systems may become connected by transitory interfluvial marshes (Forsberg *et al*., 2001). Many prey species reproduce in the flooded igapó and marshes during HW, while others migrate (Hoeinghaus *et al*., 2006). There is speculation among anglers and some researchers that small *Cichla* individuals may gravitate to the flooded areas (such as interfluvial marshes), where smaller prey may be more readily available (personal communications with Bruce Forsberg). It is possible that

larger individuals move longer distances into the lower reaches of tributaries and trunk rivers to avail themselves of larger migratory prey species (personal communications with Mario Thomé Souza). Numerous accounts from indigenous peoples living in *C. temensis* habitat support both of these speculations, however no definitive information is available.

Spawning. Since fry-guarding individuals are occasionally observed in open water well beyond two months after LW, some part of the *C. temensis* population spawns even during HW (personal observation). This has been observed with other *Cichla* (Zaret 1980). However, high water levels make appropriate spawning sites rare and HW fry sightings are not common (although they may also be more difficult to identify in these conditions). HW lacks the confluence of favorable conditions that begin in FW and initiate the broader population's change in CPV (Figure 8), ultimately leading to spawning (Figure 11).

Early life history. Broods spawned during LW are now independent juveniles. With a minimum of spawning activity, there are few fry guarding parents. A spawn occurring in HW suggests that fry guarding would end in FW. Although food availability may be adequate for newly piscivorous juveniles in FW, their smaller size would also make them subject to predation in the concentrated conditions of FW, just as the prey population that is displaced from the igapó as the cycle begins anew.

Discussion

Limitations of the Study. The purpose of this synthesis is to help determine how the

flood pulse influences *C. temensis'* life history and habitat use in its natural environment

(Figure 12). It attempts to integrate environmental factors with morphological, behavioral

and physiological characters. Therefore, some components of the literature based on

studies from artificial or transplanted habitats were not applicable and were not

considered.

Further, it is clear that there is a lack of detailed information during RW and HW

in both the literature and this synthesis, leaving substantial questions unanswered. The

previously published CPV study cited in this synthesis formed a series of snapshots of *C.*

temensis color pattern variation taken during late FW and LW (Reiss *et al*., 2012). The

new data presented here provides a more continuous picture by extending the field of

view to include early FW and early RW. However, this is not a complete cyclical set, and

it remains uncertain precisely how CPV and feeding proceed during HW. Post-spawn

adults, particularly males, are thin (personal observation n = > 100) and in lower

condition following fry guarding (Hoeinghaus *et al*., 2006). Although gathering

information during RW and HW periods presents logistical challenges, observation of the

post-spawn recovery and high water behavior of these adults would add to our

understanding of *C. temensis* life history.

The sport fishing operation's movement, outside of its pre-planned season-long

south to north migration, impacts the patterns of catch data. The operation reacts to

oscillations in the flood pulse and periodically makes large-scale movements to change

location when conditions are not optimal for angling. Large-scale movements may

involve moving hundreds of kilometers and changing river systems. These movements
determine the base locations from which the individual fishing boats operate and are
selected to ensure daily access to productive fishing waters for the anglers while
providing secure anchorage for the operation's mothership and floating camp. The studies
described in this synthesis used data collected only during periods where the sportfishing
operation was able to remain in a single region for an extended time. This ensured
correspondence to actual local conditions and eliminated potential confounding factors
due to short-term hydrological variations and long distance movement.

Further, individual guides, who are instructed to maximize the daily catch of their
angling clients, effect small-scale movements. All guides had discretion to use their
fishing boat's mobility within the general area of each day's base location. The scale of
the guides movements during a given day are typically in the range of 15 to 25 km from
the mothership and are contained within the same level of the flood pulse and the same
relative gradient. Thus, small-scale movements were not likely a confounding factor. The
specimens and data used in this study were collected by angling, therefore they do not
reflect all life history stages and are gathered entirely from adult fish and, rarely, large
juveniles. Larvae, fry and juveniles under 200 mm are not normally caught by angling
and were not recorded in the habitat use study.

Due to the substantial difference in observability of nests in different regions and
hydrological periods, attempting to correlate nest sighting frequency with water level
may be confounding. Although nests consistently contain a central component of
submerged wood or stone onto which the eggs are adhered and nests are generally located
in sunlit areas, conditions that allow dependable observation of nests are not always

present (personal observation). On beaches or sandy bottoms in LW, as in the Rio Negro basin, *C. temensis* will select a nest site and clear the surrounding area of detritus in a 1 to 2 meter radius (personal observation). These nests are readily visible and observable. However, in higher water levels and in rivers south of the Amazon main stem where the bottom substrate is primarily oligotrophic clay, *C. temensis* will often select live or dead standing trees as spawning sites. The woody bases provide egg adhesion sites and the cavities formed by exposed root sections likely serve as substitutes for the pits dug in sandy bottomed rivers (personal observation). Nesting on woody structure directly on the bottom is less common in these conditions. Such nests are far more difficult to find and see. Therefore, as a measure of spawning frequency, counting observed nests with the naked eye is not a dependable indicator.

Counting observed assemblages of surface feeding broods and fry guarding parents is, however, easily and consistently performed in all regions and during all water levels. Brood-guarding *C. temensis* utilize open water lentic habitat where fry, organized into easily seen, surface-dappling schools, can feed on photic-zone-oriented zooplankton (Zaret, 1980). Only transitory windy or rainy conditions affect the ease of sighting and counting these assemblages that anglers commonly call "fry balls".

The observation of a protective strike by *C. temensis* not only served to identify the species, but also minimized the likelihood of a brood being counted twice. Since one or more parents stay with the brood for up to two months, the incidence of fry sightings could effectively become cumulative during FW and LW. Additionally, different guides may encounter the same brood. However, after being captured in this fashion once, or very rarely twice, individual parents stopped responding to attempts to induce a strike

(personal and guide observations) and hence were unlikely to be counted again for this analysis. In fact, we observed cases where a guarding male with his brood persisted alone in a lagoon for almost the entire study period and, after an initial capture, resisted all further attempts at inducement to strike. The strike observation requirement, therefore, minimized duplicate observations of fry guarding parents, however, there is no way to verify that the same brood was never counted twice.

Despite these concerns, I believe these observations have provided important insights. Direct observation was the first human method of gaining knowledge and it continues to provide scientists with much of the empirical evidence they obtain (Bogen, 2009), particularly in behavioral ecology (Rohwer and Rohwer, 1978). Data for this synthesis used empirical research techniques based on both experimentation and observation. The nature of the experimental data described in this text are readily understood and designed to be repeatable. Observational data and results, however, are not only difficult to collect, but they can be difficult to present. They are less intuitively interpreted by the reader and can be subject to observer's bias, misperception, anecdotal effects, and suffer from lack of access (Shapere, 1982).

This study focused on *C. temensis* in its natural, uncontrolled aquatic habitat, where observation was one of the tools necessary to collect behavioral information. The most useful observations are made by trained, unbiased observers; have large numbers of repetitions; and are comparable among multiple observers (Hult, 2005). This study met those conditions. All observers had 10 to 20 years of experience as guides for anglers pursuing *C. temensis* in the Amazon, and were trained in basic fisheries techniques. Observations were made over a period of 21 seasons in a broad range of locations and

with hundreds or thousands of repetitions by each observer. Several observers routinely compared, discussed and reviewed observations. Every effort was made to maximize observational accuracy, however, confirmation of these observations by other investigators will be important to reinforcing their validity.

The Amazon Flood pulse relative to other pulsed systems. Since its first mention in the literature, the FPC (Junk *et al*., 1989) has proven to be an effective integrative tool for the study of ecological processes in river-floodplain systems (Junk & Wantzen, 2004). However, it has been modified, extended and criticized by various authors and is not the only model applicable to pulsed systems. Pulsed systems of many types and frequencies exist and have been shown to have extensive effects on their respective biota. There are twice-daily tidal zones (Rozas, 1995), irregularly timed small stream pulsations (Walker, 1985) and twice-yearly inundations of temperate zone rivers (Wetzel, 2001) in addition to the large scale, monomodal and predictable Amazon flood pulse of *C. temensis*' habitat. Pulsing systems occur worldwide and are not limited to the tropics (Junk *et al*., 1989). Additionally, the focus of much central Amazon FPC research is on whitewater river-floodplain systems, while the habitat of *C. temensis,* the object of this synthesis, is limited to blackwater systems, with substantially different characteristics. Nonetheless, the FPC remains the most applicable hydrodynamic descriptor and theoretical superstructure for this synthesis.

Each of the flood pulse periods discussed in this synthesis are far more variable and complex than their "idealized" description indicates. Factors contributing variability include unpredictable weather from one season to the next and differences in gradient, geology and biota between regions. Rains may arrive early or late in the season, and

hydrological periods may be shorter or longer than usual. In spite of this short-term, within-year variability, the overall, long-term characteristics of the flood pulse are consistent (Richey et al., 1989b; Tockner *et al*., 2000). The hydrological period descriptions are well-fitted to the long-term characteristics of the FPC, making these idealized descriptions useful as a timekeeping mechanism.

The response of *Cichla temensis* to the flood pulse can vary. When short-term variability occurs during a hydrological period, it results in oscillations to the ecosystem, and consequently affects the fishes' behavioral and physiological responses (Junk & Wantzen 2004). A common short-term pulse of rain in FW (called a "repiquete" in Brazil) may cause water levels, in rivers that have already dropped below bankfull, to rise back up into the igapó before dropping again (Goulding *et al*., 1988). The effect on prey availability is immediate as they quickly seek the denser protective cover of the flooded forest. As a result, the feeding, fattening and spawning behavior of *C. temensis* associated with FW may be temporarily interrupted, and then later restarted.

The flood pulse may also display year-to-year variation (Goulding *et al*., 1988). Such larger-scale anomalies are coupled with the El Nino-Southern Oscillation (ENSO) phenomenon (Richey *et al*., 1989b). Droughts may occur, as in 2006 in the Rio Madeira basin, lowering water levels to the extent that lakes, lagoons and floodplain structure become completely dry (personal observation). *Cichla temensis* then becomes confined to the lotic environment of the river and is forced outside of its regular LW environment. Conversely, the normal FW and LW period may never fully occur in a particular region affected by unseasonable rain or the impounding effects of flood in other areas. An area may never drop below bankfull, thereby diminishing the availability of food and

spawning structure as was observed in tributaries of the Rio Branco, where water levels remained in the igapó throughout the cycle in 2008. Since inclusion of these oscillations would not add to the clarity or usefulness of this synthesis, the same form of idealized description and schematic graphical depiction used for water levels is also used to describe prey availability, spawning structure availability and spawning frequency. These idealized depictions are representative of the longer-term behavior of the flood pulse, the ecosystem and the fishes based on my 20 years of experience and observation. They do not represent what may occur in a given region at a specific point in time.

Water level is not the only environmental factor that can affect *C. temensis*. Changes in water level in RW, cause the moving littoral to trace the same path through the ATTZ as in FW, only in reverse. Similarly a reversed movement of the littoral occurs during a repiquete in FW. In both cases *C. temensis'* response to rising water is different than to falling water, even though absolute water levels may be at very similar levels. Feeding activity, in particular, is greatly diminished and habitat use changes (personal observation). The results of the habitat use study showed that during FW, water temperature, pH, dissolved oxygen and conductivity remained essentially stable as water levels fell. During RW, however, larger variations in water characteristics can occur, such as an abrupt drop in temperature and, if rain is associated with the rise, a substantial decrease in water clarity (unpublished data). These factors have profound effects on the behavior of fishes of all kinds (Helfman *et al*., 1988). *Cichla temensis* short-term response to these factors suggests that the direction of the moving littoral may be as important as the absolute water level. These factors have not been integrated into this synthesis, but could be the focus of future studies.

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Table 1

Year	Season	Rivers	# Weeks	Avg. # Guides	Avg. fish /angler /wk	Total Fish Observed by Author	Total fish Caught by anglers	Falling Water	Low Water	Rising Water	High Water
1	1994-95	A	1	4	100	100	1000	1			1
2	1995-96	M,C,T	8	4	233	933	1000	6	2		
3	1996-97	M,C,CU,X	8	4	200	800	1000	6	2		
4	1997-98	MM,MAM,C,AU,UB,T,X	14	4	200	1400	2000	10	4		
5	1998-99	M,IG,C,J,X,T	15	4	217	1625	2000	8	5	2	
6	1999-00	MM,M,C,J,MS,UB,UX	16	4	157	1257	2020	14	2		
7	2000-01	M,C,UQ,N	15	4	162	1215	2020	12	2	1	
8	2001-02	M,C,UQ,JT,T	16	4	173	1383	2800	8	5	3	
9	2002-03	M,C,CU,X,JT,T	15	4	179	1339	2800	8	7		
10	2003-04	M,C,X,T	11	5	210	1155	2000	7	4		
11	2004-05	M,C,T,JT	10	4	210	1050	2000	8	2		
12	2005-06	UA,I,T,JT	5	4	117	292	1400	3	2		
13	2006-07	IG	9	4	300	1350	2500	4	3	2	
14	2007-08	J,T	8	4	175	700	2800	2	6		
15	2008-09	M,C,CU,X,JT,T	15	4	179	1339	2800	7	6	2	
16	2009-10	M,C,N	9	6	210	945	19800	7	2		
17	2010-11	M,C,N	9	6	210	945	19800	7	2		
18	2011-12	M,C,N	9	6	210	945	19800	9			
19	2012-13	M,C,N	9	6	210	945	19800	6	3		
20	2013-14	M,C,N	9	6	210	945	19800	9			
21	2014-15	M,C,N	9	6	210	945	19800	9			
Exploratory trips		CC,TE,AR,PN,PR,S,MY,JR, TF,P,BL,AL,PN,AR,AU,TU	20	0	70	1500	1500	6	6	4	4
Totals and Averages		42 River systems	Total 240	Average 5	Average 194	Total 23109	Total 397100	Total 156	Total 66	Total 14	Total 4

River Name	Symbol	River Name	Symbol
Caures	C	Urariquera	UQ
Matupiri	M	Jaupiri	JP
Igapo Ac u	IG	Araca	A
Quini	CU	Demeni	D
Amapa	AM	Araraha	AR
Preto do IgAcu	PR	Paduari	PN
Negro	N	Preto de Norte PR	
Tapera	T	Sepuke	S
Xeriuini	X	Marmelos	MM
Massaui	MS	Mamori	MR
Uneuini	UX	Mamiya	MY
Urubaxi	UB	Jurua	JR
Marie	MA	Tefe	TF
Curicuriari	CC	Paru	P
Uapes	UA	Trombetas	TB
Ira	I	Branco Lakes	BL
Urvini	UN	Alegria	AL
Lago Taquia	LT	Pauini	PN
Tea	TE	Arara	AR
Jufari	J	Akiana	AU
Jatapo	JT	Turi	TU

Table 1. Summary of Amazon basin rivers visited, fish observed and flood pulse at right identifies rivers. A total of 42 rivers were visited during a 20 year period. *temensis* specimens were observed.

Table 2. A multiple linear regression model, where Percentage Acu = (WaterLevel x β_1)
+ (RelativeGradient x β_2) + b was calculated to assess the effects of two variables on the
ratio of fish in the açu form to fish in the paca form. Statistical analysis for the resulting
values (Percentage Acu = (WL x -7.06E-05) + (RG x 0.042813) + 0.19571) including
standard error (SE), tStat and the p value are expressed for the variables, Relative
gradient refers to the phenomenon of water levels being relatively lower in upriver
locations, creating a relatively more advanced stage of the flood pulse cycle as compared
to downriver locations. Water level refers to the amount of change in depth. Both water
level and relative gradient had a significant effect on the percentage of açu versus paca.

Percentage Acu = (WL x -7.06E-05) + (RG x 0.042813) + 0.19571

	Estimate	SE	tStat	pValue
Intercept	0.19571	0.03925	4.9862	2.47E-06
Water level	-7.06E-05	2.62E-05	2.6988	0.008122
Relative Gradient	0.042813	0.010928	3.9178	0.00016

Figure Captions

Figure 1. Locations of 42 rivers in the Amazon basin that were accessed during 21 flood pulse seasons, from 1994-2014 (Table 1). Data and observations were collected from rivers in three major basins (Rio Negro, Rio Madeira, Rio Branco). The middle Rio Negro region corresponds to the area between the points N and CC.

Figure 2. Schematic representation of the flood pulse effect on a section of a Rio Negro basin river-floodplain tributary system depicting an overhead view of the Aquatic Terrestrial Transitions Zone (ATTZ) with a compressed sampling of permanent and transitory floodplain structures. Darkened areas show the position of the moving littoral. (a) in high water (HW). (b) in falling water (FW) and rising water (RW) at bankfull level. (c) in low water (LW). Taller treetops in the igapó protrude from the flooded area. Floodplain structures shown include 1. "igarape", 2. "lago, 3. isolated "lagoas", 4. "parana seca", 5. "ressaca", 6. "lagoa", 7. "volta morta", 8. "praia".

Figure 3. Schematic representation depicting an overhead view of a section of a Rio Negro basin river-floodplain tributary with a concentrated sampling of transitory and ephemeral floodplain habitat. Structures shown include 1. "aguas paradas", 2. "boca, 3. "ponto", 4. "barranca", 5. "praia", 6. "galhadas".

Figure 4. Schematic representation of the flood pulse effect in a Rio Negro basin river-floodplain system lagoon ("lagoa"), permanently connected to the river and depicting a vertically exaggerated section of a typical lagoon bank during the four defined hydrological periods (derived from Junk *et al*., 1989).

Figure 5. Idealized depiction of minimum and maximum water level in the study area. Water levels are represented as a generalized version of the typical yearly data recorded by middle Rio Negro field stations (Forsberg *et al*., 2001)(Junk *et al*., 2014). Idealized bankfull level is depicted as an indication of the temporal portion of the flood pulse it occupies.

Figure 6. **(**a) Year-to-year variability of the amplitude of hydraulic effects during flood pulse periods and the occurrence of oscillations within a period. Water levels are shown as cm above stage of the Rio Jau main channel. (b) Precipitation measured at Capoeira Grande field station (located on the Rio Jau main channel ≈ 50 Km upriver from its confluence with the Rio Negro) (from Forsberg, B., & Castro, J. 2001). Comparison of a and b demonstrates that regional water levels in the study area can move independently of rainfall due to the impounding effects of trunk rivers and precipitation in other areas.

Figure 7. The Rio Igapo Açu complex (a) in low water (LW); b) in early falling water (FW). The arrows correspond to mothership anchorage locations, with higher numbers representing higher gradient (upriver) locations. See Fig. 1 for location of Igapo Açu (labelled IG).

Figure 8. (a) Daily percentage of *Cichla temensis* captured in the açu form of Color

Pattern Variation versus the paca form displayed in the same direct order of sportfishing

days as they occurred over a four week period. (b) Daily percentage clustered by location

as the sportfishing operation moved during the four week period. (c), The mean

percentage of açu (regardless of location) per week increased as water dropped. (d) the

mean percentage of açu per location (regardless of week or gross system-wide water

level) increased as the operation moved upgradient (upriver). Note that day 6 and day 1

are the same location. Days 7, 14 and 21 were transfer days without fishing.

Figure 9. Fry sightings versus water level. During a five week period on the Rio Caures

from October 20, 2012 to November 23 (see Fig. 1 for location), *C. temensis* fry-ball

sightings were recorded daily as water level dropped from the late Falling Water (FW)

period to Low Water (LW). The number of fry sightings increased steadily.

Figure 10. Schematic representation of prey availability for *Cichla temensis* during

falling water (FW). The moving littoral in the Aquatic Terrestrial Transition Zone

(ATTZ) concentrates aquatic biota in a diminished water volume and increases prey

availability for *C. temensis*. As levels drop below bankfull and access to protective cover

decreases, predation reduces prey populations to a minimum in low water (LW).

Figure 11. Schematic representation of the relationship between idealized environmental

conditions and the behavioral and physiological response of *C. temensis*. Prey availability

peaks in FW after bankfull level is reached. Spawning structure availability increases as

water levels drop and reaches a peak during low water. Spawning activity is inversely

related to flood pulse water levels. A period of intense feeding precedes maximum

reproductive readiness, so the spawning activity peak is temporally offset from bankfull

by the progression of CPV and the deposition of reserves. These environmental and

behavioral correlations coincide with study results and observations made throughout the

flood pulse cycle.

Figure 12. Summary of characteristics of idealized flood pulse periods and *Cichla*

temensis' behavioral and physiological response during each water level period in its

blackwater flood pulse habitat. See Fig. 8 and 9 for detailed description of prey

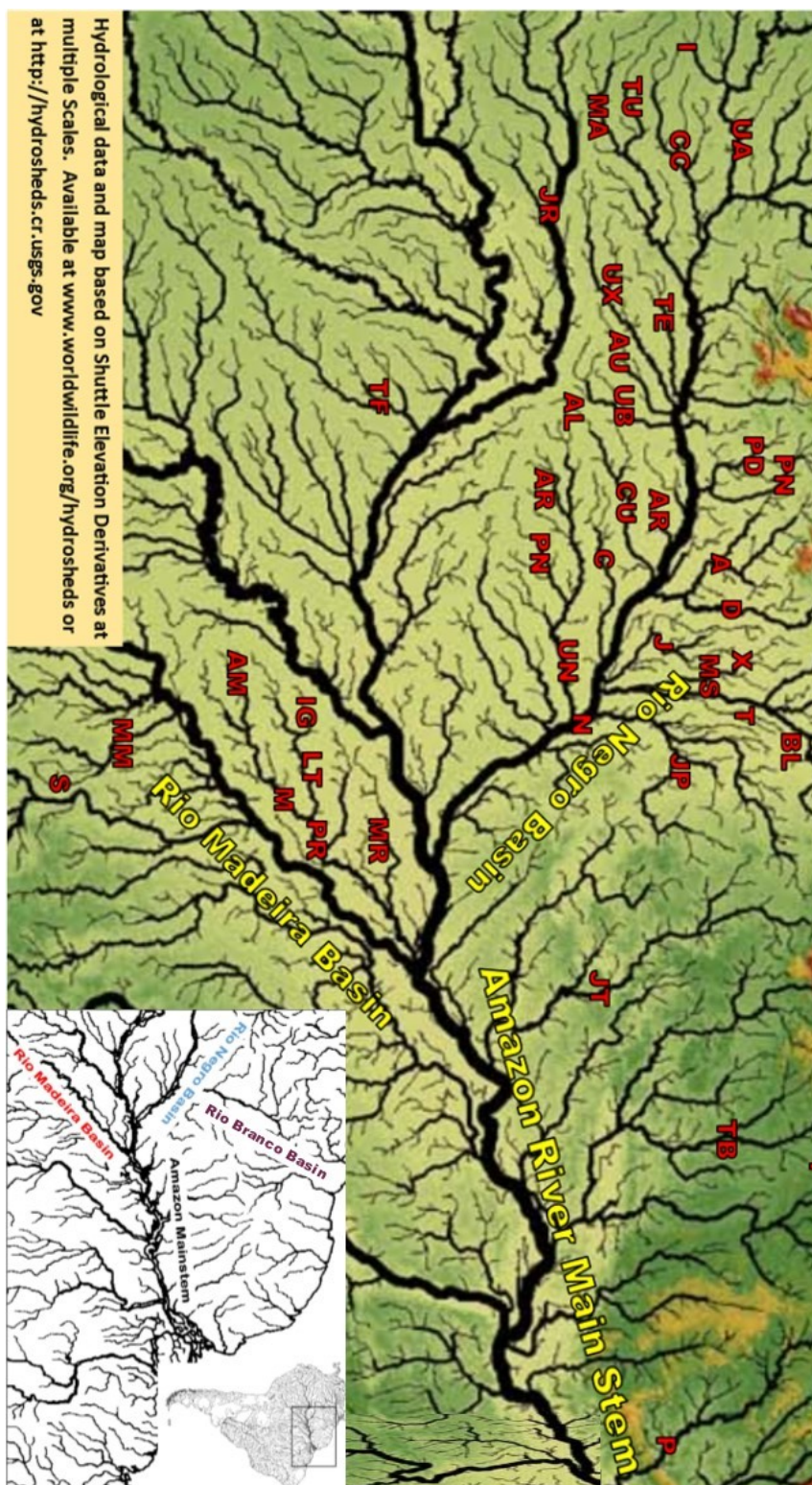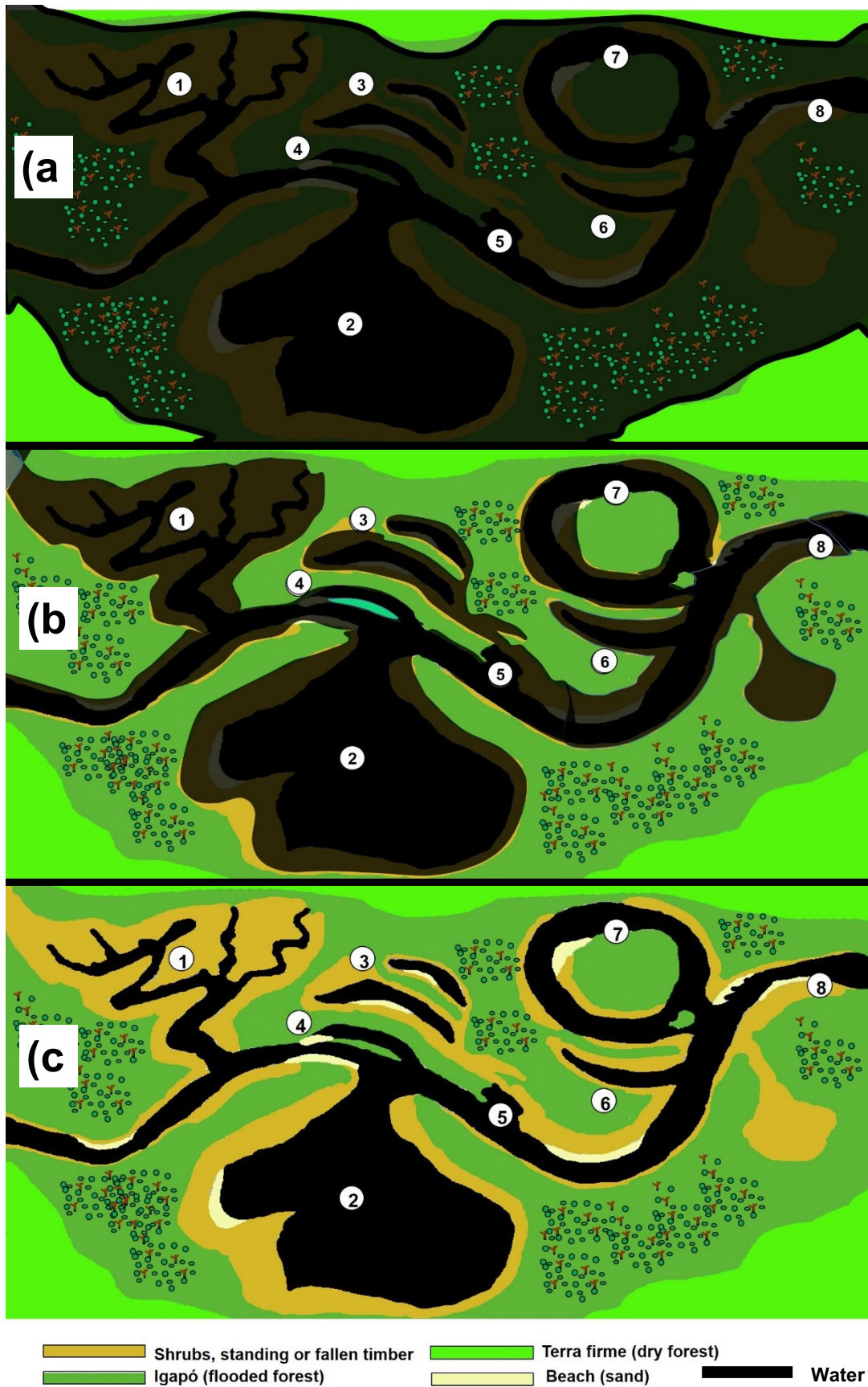availability and spawning activity.
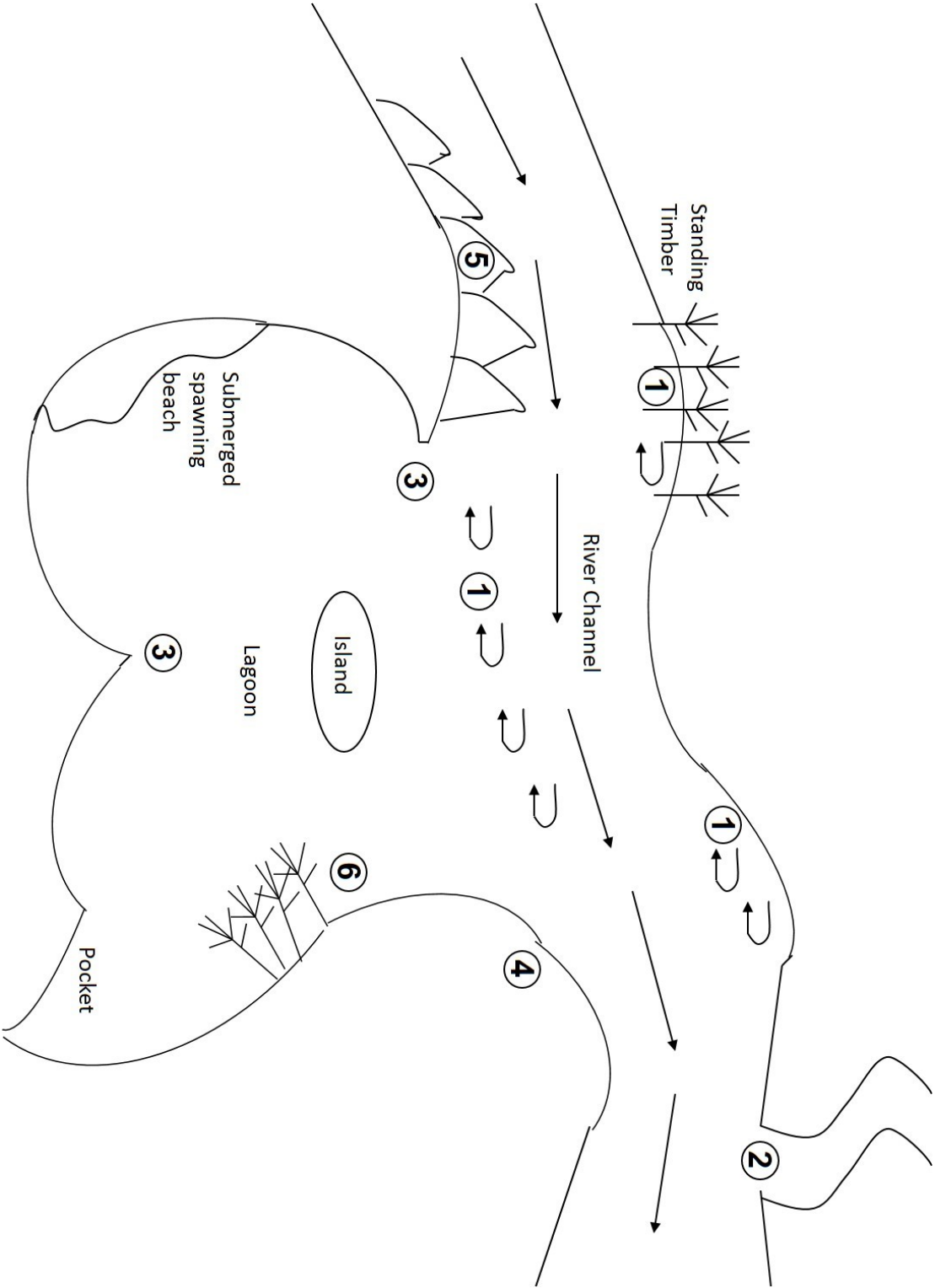
Figure 1.

Figure 2.

Figure 3.

Figure 4.

≈ 10 meter variation

High Water (HW)

Rising Water (RW) →

← Falling Water (FW)

Low Water (LW)

Moving littoral

Sandy Beaches or Bottoms

Fallen timber (galhadas)

Terrestrial Shrubs and/or standing timber

→ Approximation of bankfull level in river
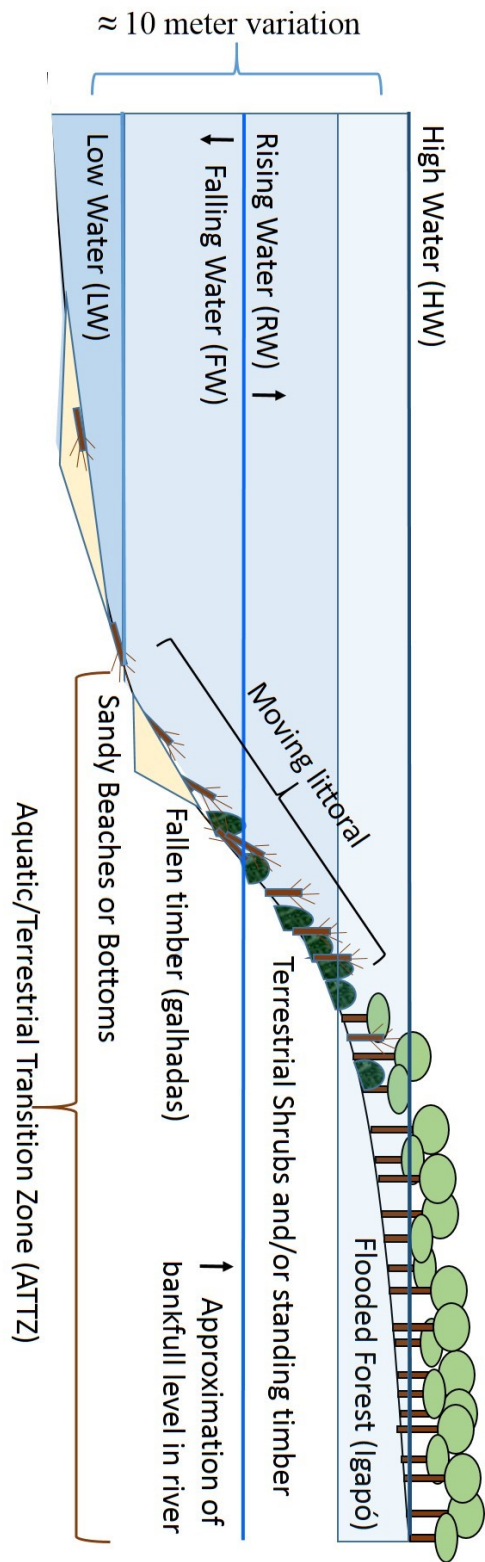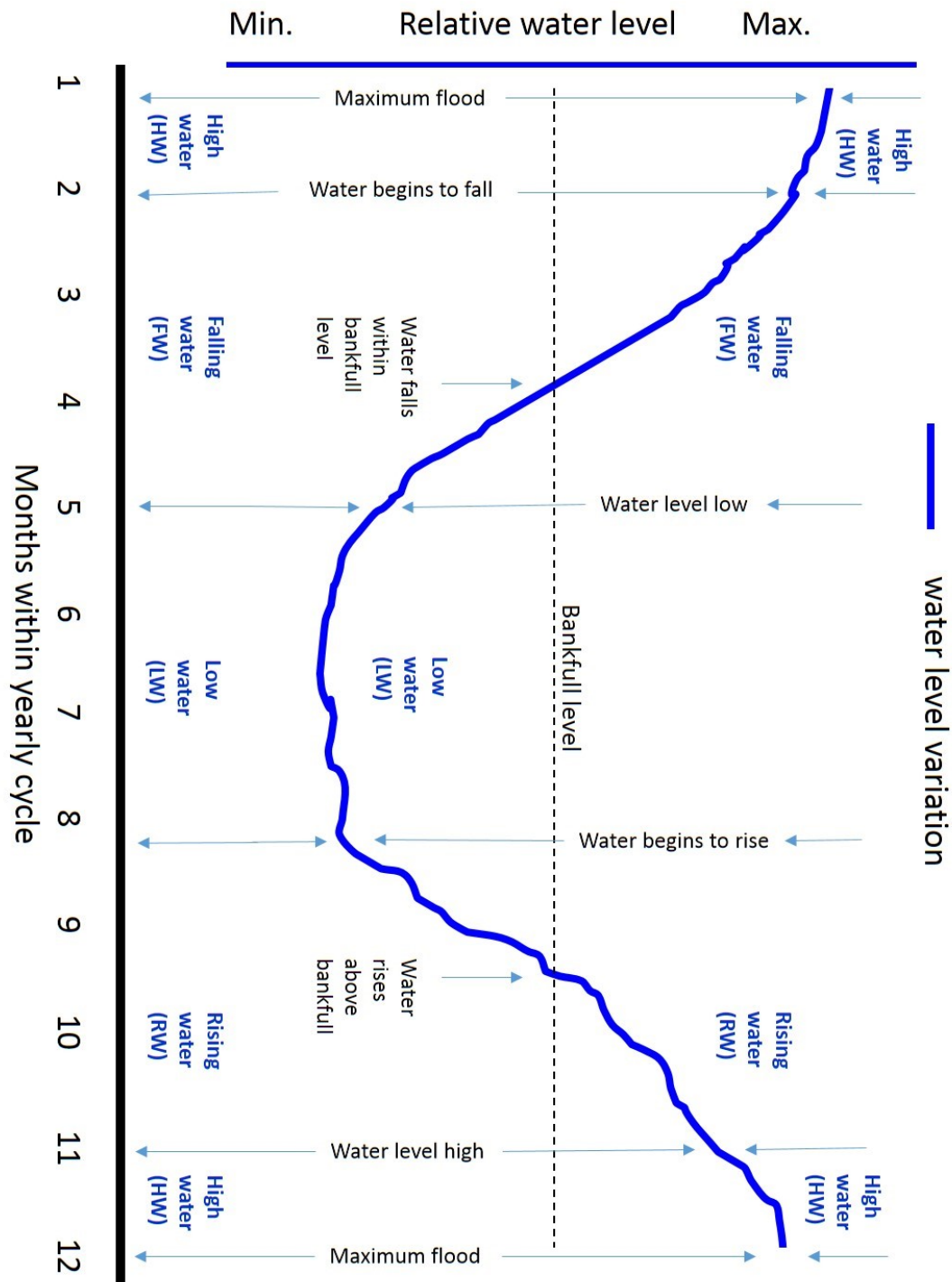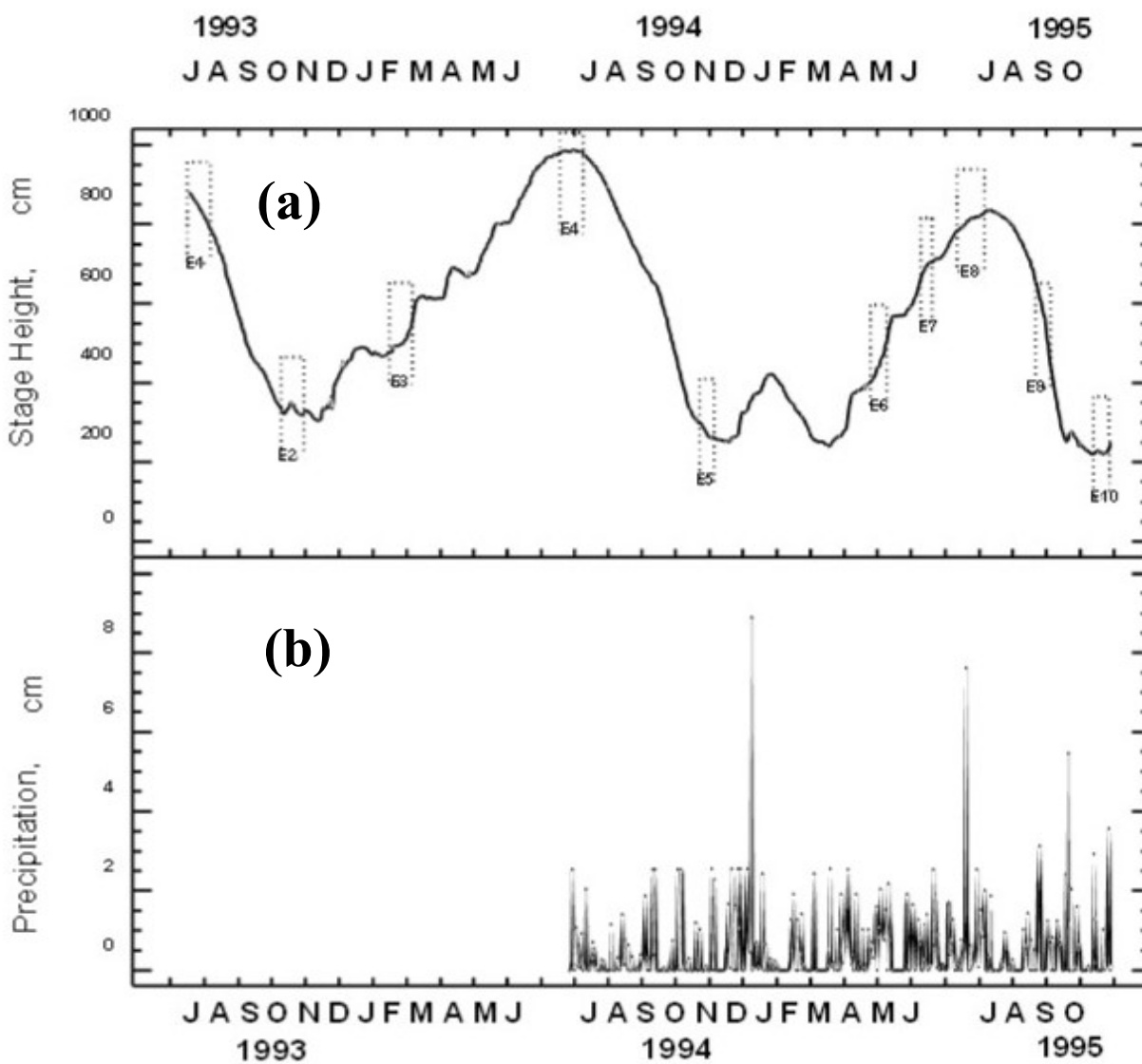
Flooded Forest (Igapó)
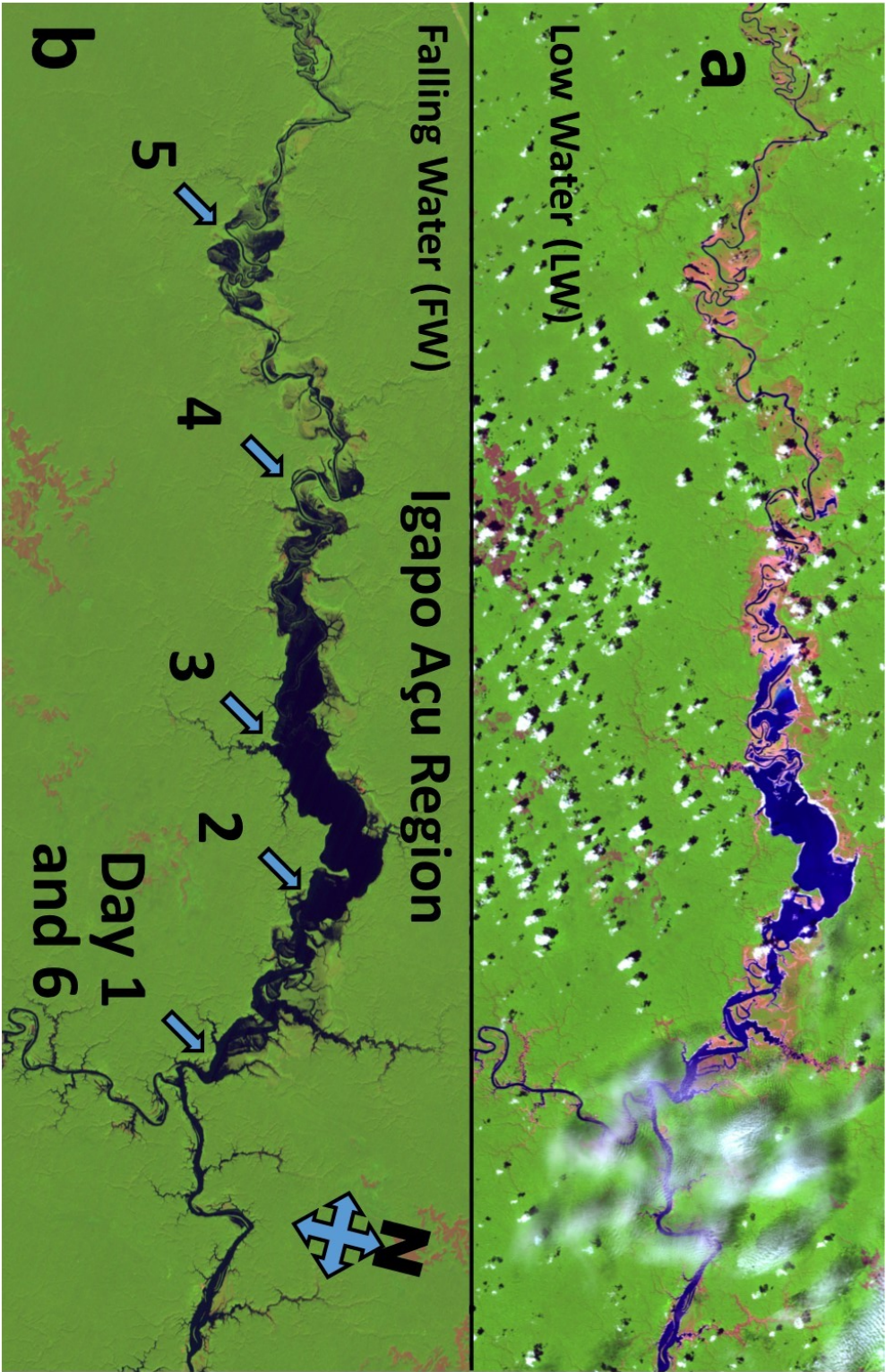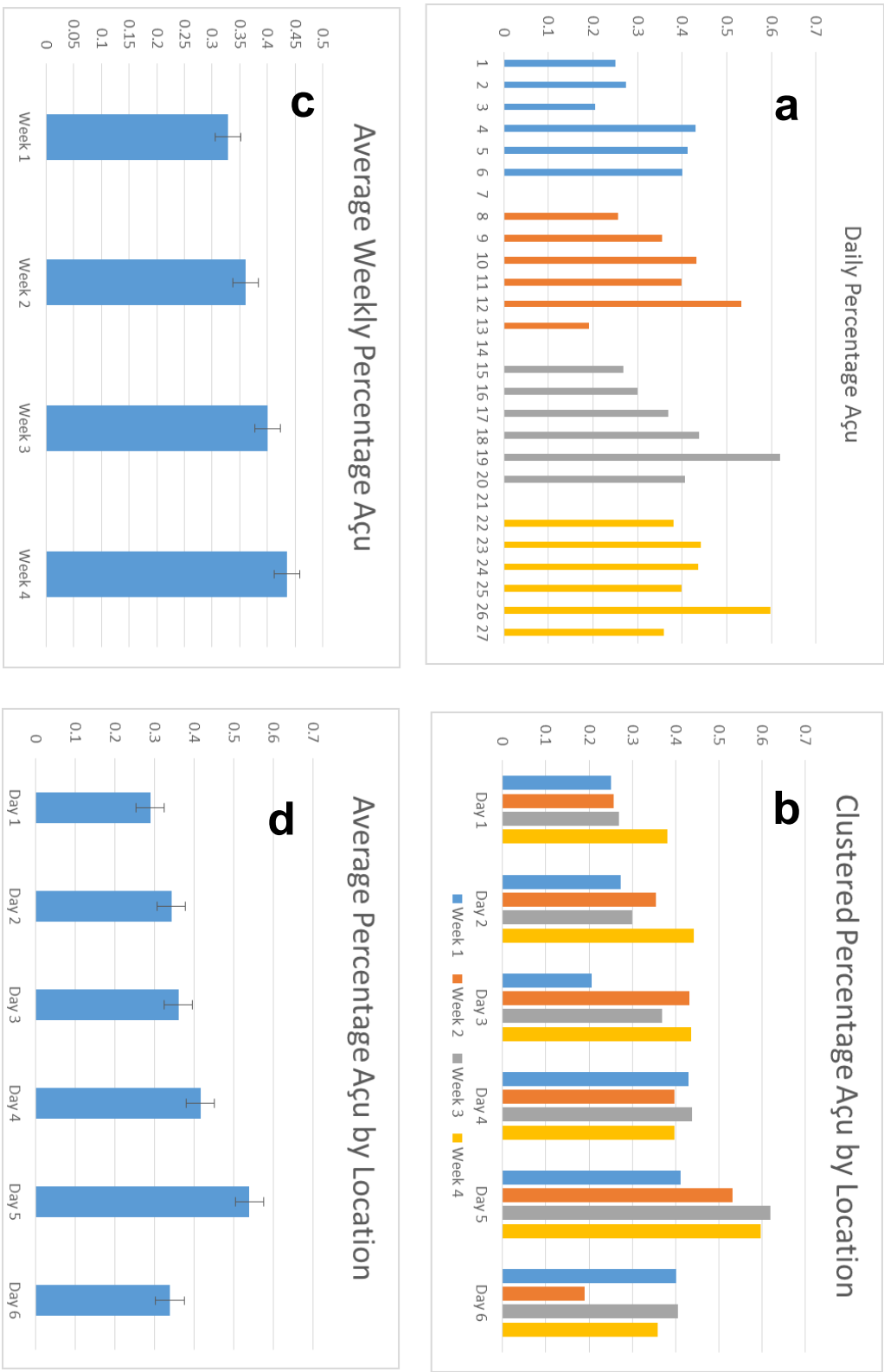
Aquatic/Terrestrial Transition Zone (ATTZ)

Figure 5.

Figure 6.

Figure 7.

Figure 8

Figure 9.
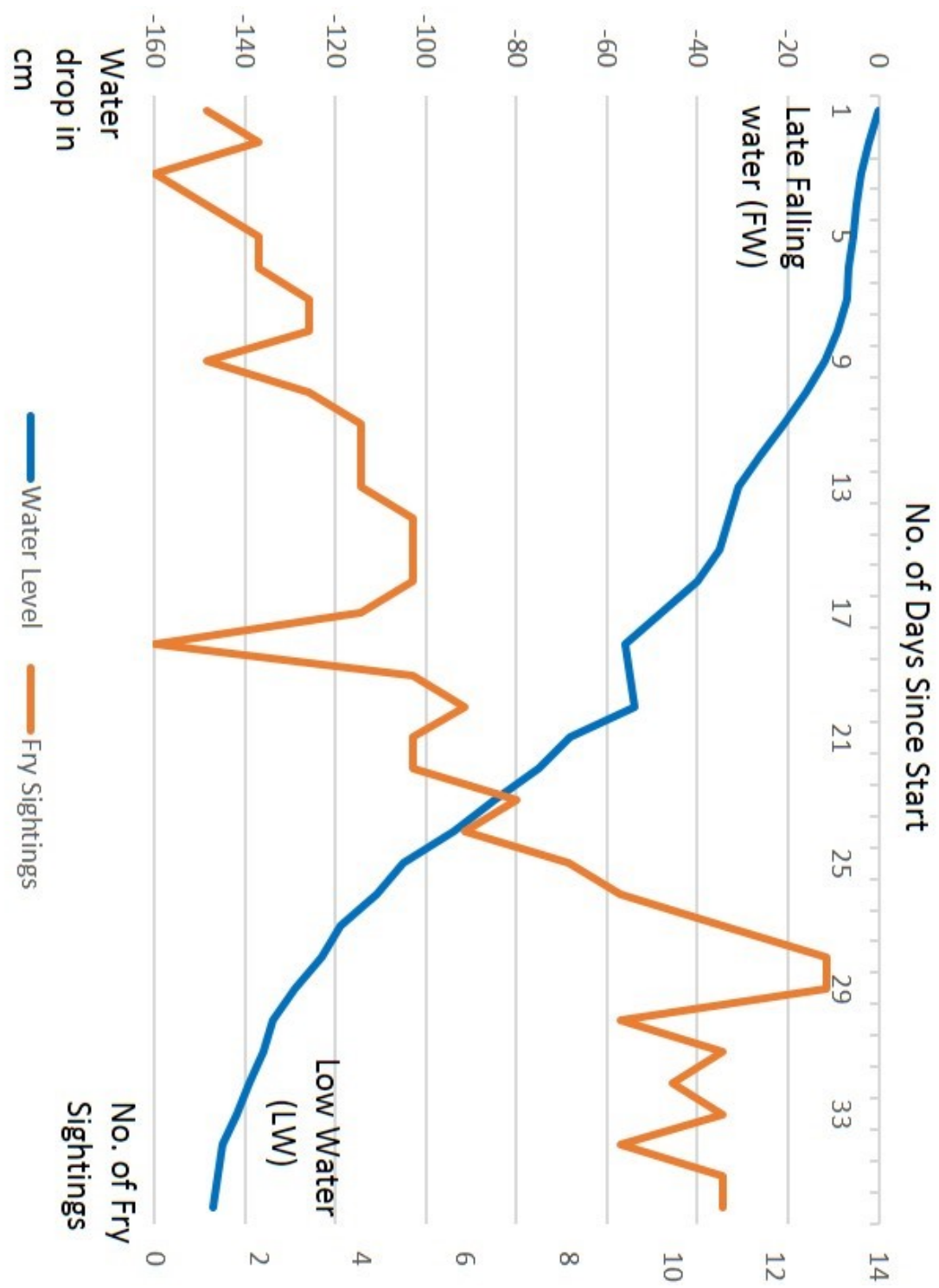
Figure 10.

Figure 11.

Figure 12.

FALLING WATER	LOW WATER	RISING WATER	HIGH WATER
Igapó emptying	Igapó emptied	Igapó flooding	Igapó flooded
Prey concentrated	Prey diminishing	Prey at minimum	Prey in Forest
C. temensis feeding at maximum	C. temensis feeding diminishing	C. temensis feeding at minimum.	C. temensis must hunt to feed.
CPV change begins	C. temensis mostly in açu form	C. temensis mostly guarding fry	C. temensis mostly in paca form
All grades present	Maximum spawning sites available	Water rising - spawning sites disappearing	Rare spawning sites available
Spawning sites becoming available	Spawning at maximum	Spawning reduced to a minimum	Maximum fry forage available
Spawning activity begins			

Months within yearly cycle

1 2 3 4 5 6 7 8 9 10 11 12

CPV Progression
Reserve Deposition

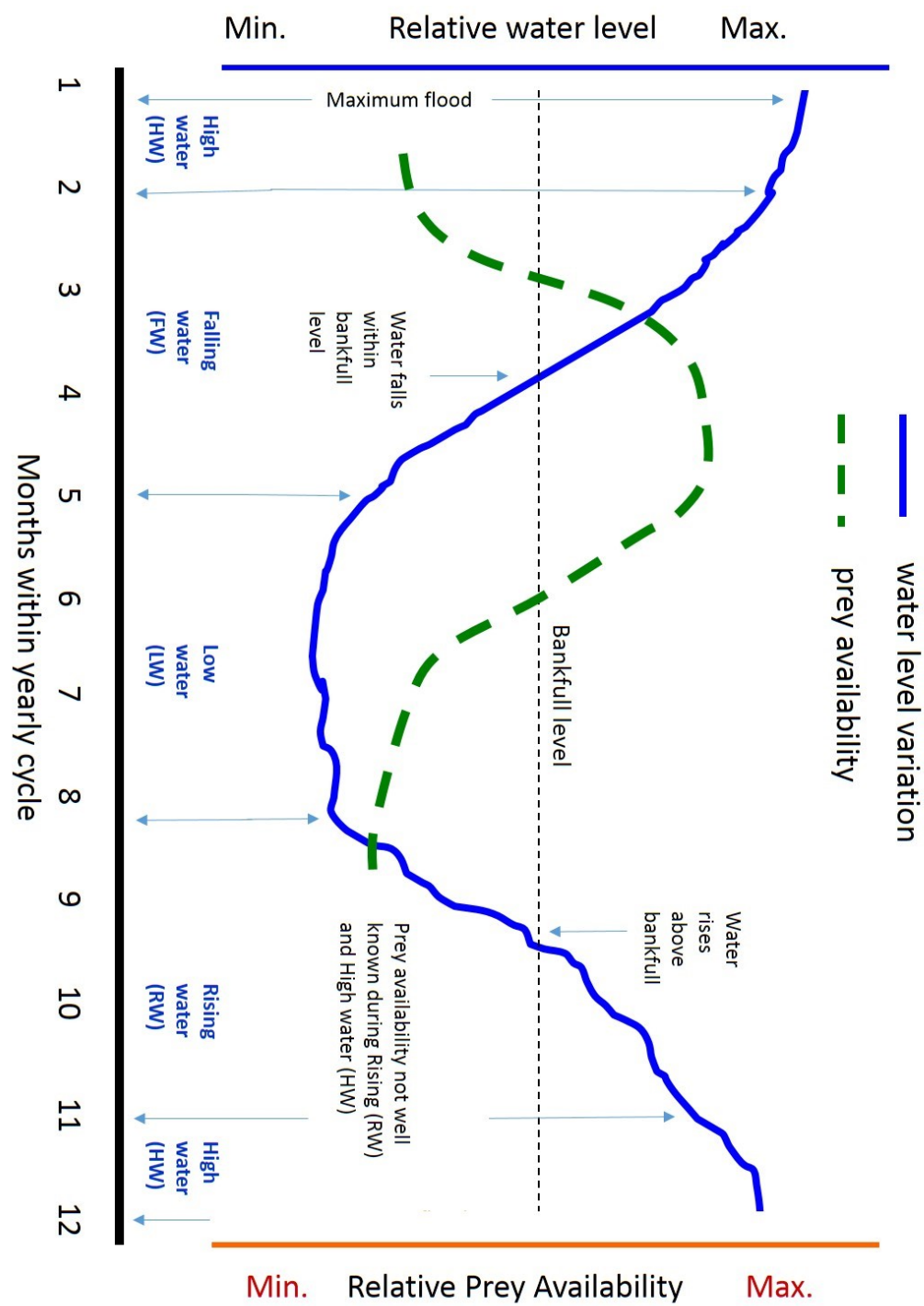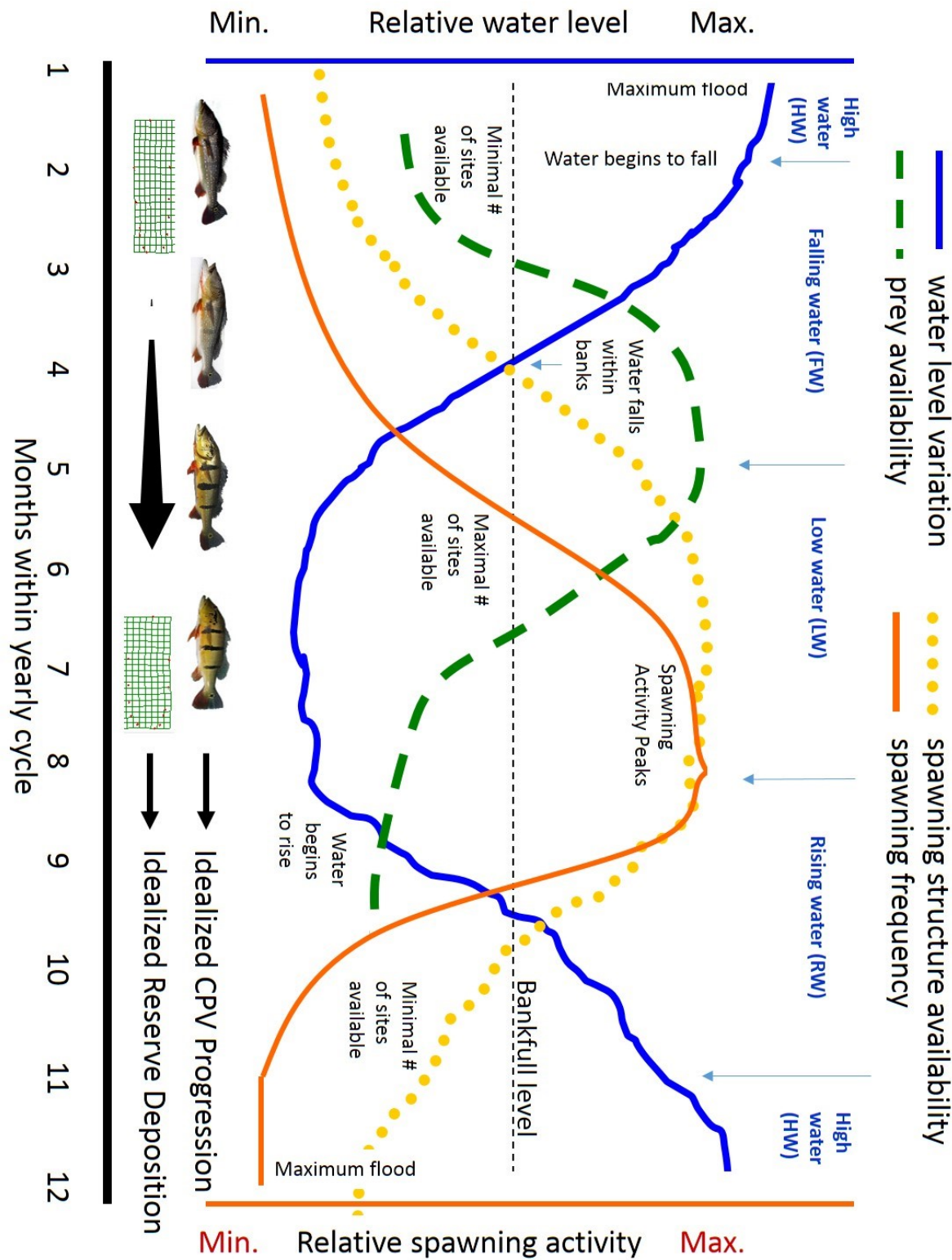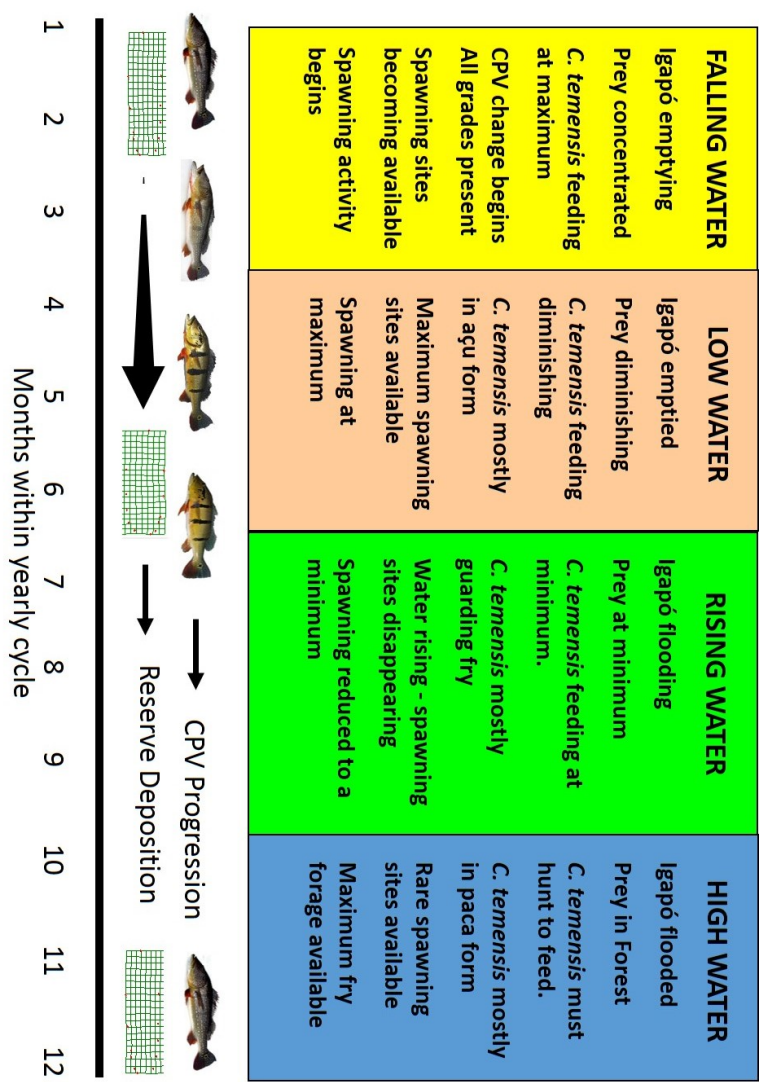**Conclusions**

The work presented in this dissertation was performed in the central Amazon

basin, the natural habitat of *Cichla temensis.* This aquatic environment is subject to a high

amplitude flood pulse, likened to a once-a-year tide. Because the study area forced

consideration of the region's characteristics together with the fish's response, it helped

clarify certain aspects of the species' life history. Thus, it serves to augment prior work

on the species, mostly performed in artificial habitats, with later work, mostly focusing

on aspects of its natural life history in certain segments of the flood pulse cycle. Taken

together with the most recent studies correlating many of its behavioral and physiological

characteristics with the cyclical nature of the flood pulse, a clearer picture of the species'

life history is available.

In Chapter 1, I was able to resolve lingering taxonomic questions relating to the

existence of strikingly different color and pattern variants and demonstrate that all of the

variants are a single species (Reiss *et al*., 2012). The study showed that *C. temensis'*

striking variations in color and pattern (CPV) are directly correlated to an individual's

gonadosomatic index (GSI) and represent that individual's cyclically changing state of

reproductive maturity. The results led to a change in both the sportfishing arena and

among various entities responsible for regional fisheries management. It also sparked, in

me, an enhanced awareness that aspects of the species' life history are likely adaptations

to the pulsative nature of its environment.

Therefore, in the second chapter, I examined apparent shape changes in

individuals as they progressed from the paca to the açu form during the falling and low

water periods of the flood pulse (Reiss & Grothues, 2015). The shape changes proved to be correlated to CPV. The study further demonstrated that they were related to the deposition of fatty reserves for gametogenesis and the period of fasting associated with the spawn and ensuing brood care. Considered together with the timing of the seasonal modulation of *C. temensis* flood pulse habitat, a pattern emerged, suggesting that the flood pulse concept (FPC) (Junk *et al*., 1989) and *C. temensis'* life history in its natural environment were inextricably intertwined.

In the third chapter, I undertook to synthesize the species' life history with the cyclical nature of its pulsative environment and in light of the FPC. Additional research was necessary to support some of the assumptions raised by this hypothesis and was performed in two studies included in the chapter. They demonstrated, respectively, that *C. temensis* in various CPV grades use different portions of their habitat nonrandomly and that spawning frequency is negatively correlated to water level. This allowed a framework to be erected that describes aspects of C. temensis life history in relation to its pulsing environment for the first time. The resulting synthesis may provide useful information for future management and conservation policies for this important species.

Large gaps remain in our knowledge of this enigmatic creature and its extraordinary environment as outlined by the work of this dissertation. A good portion of *C. temensis'* response to the rising water and high water periods of the flood pulse is poorly understood. Studies to elucidate its feeding behaviors and its movements and habitat use during the peak of the flood are needed. In addition, many segments of its life history are also poorly known. For example, there is little information about its early life

history and juvenile phase after the period of fry guarding ends. Clarification of the precise nature of its return to the paca form is also absent.

As they say, "a little knowledge can be a dangerous thing". In my case it can lead to dangerous speculation, and with *C. temensis*, there is plenty to speculate about. The nuchal hump is a particularly fertile focus for speculation. Although it is common among cichlids and present in other Perciform families, when considered with *C. temensis'* observed behaviors it raises some interesting questions. Is it an "honest signal" for sexual selection? Do broods gather around the male's head because the hump provides a chemical or visual signal? As males progress through the fry-guarding period, the hump appears to become less solid, appears to ooze and show signs of wounding or irritation. Does the hump provide a food substance or supplement, as in other Neotropical Cichlids (i.e. *Symphysodon* spp.)?

Catch data and other unpublished data from the studies in this dissertation suggest that there may be a disparity in male/female sex ratio, with females being more common. If not a capture mechanism bias, this bears investigation, and if shown to be factual raises other questions, in particular, why? The author has observed many spawning pairs and nests. There have been occasions when a third, smaller fish was present nearby and not attacked by the male, who would normally violently attack an unwanted interloper (such as a sneaker male). Could this be a second female, adding to the clutch? Does a possible excess of females indicate possible multi-parent broods? Do males with shapes displaying substantial fat deposition or particularly large males attract additional females?

Furthermore, there is variation in the literature regarding *Cichla* spp. parental roles and male and female fry guarding behavior in lakes and reservoirs (Zaret, 1980;

Gomiero and Braga, 2004) and those described herein pertaining to *C. temensis* in natural flood pulse rivers. These observations were made between *C. temensis* and other *Cichla* species (*C. pleiozona, C. ocellaris, C. monoculus*) and may be simply species differences. However, the existence of these differences in closely related congeners leads one to examine the question of what might be the most likely explanation for the particular reproductive behaviors observed in *C. temensis*. Parsing this question suggests that both the concepts of parental investment (Trivers, 1973) and sexual selection might be considered in seeking an explanation (Andersson & Simmons, 2006) and may present an interesting topic for further investigation.

Cichla temensis' modest GSI at maturity (Reiss *et al*., 2012) corresponds with its K type reproductive strategy (Pianka, 1970). Male gonads range up to 0.25% while females' will reach 2.5% of their body weight (Reiss *et al*, 2012). This tenfold larger material investment by the female, suggests that the male's larger investment in brood care may balance the scales of parental investment, avoiding an imbalance between sexes that might reduce either parent's ability to invest in other offspring. It may even justify speculation that the female's earlier departure from brood care and her observed ability to maintain ova in various stages of maturity (Chellappa *et al*., 2003; Gomiero & Braga, 2004) could enable her to distribute her offspring among more than one male during FW and LW.

This possibility resonates with descriptions of *Cichla* pair bonding behavior (Zaret, 1980) and the author's observations of *C. temensis* in the field, suggesting that there may also be a component of sexual selection inherent in mating. The author has encountered (n = >10) groupings of mature males arrayed in lagoons and aggressively

defending their location, possibly engaging in lekking (male display) behavior. Larger males appear to consistently dominate these aggregations (personal observation). Their size may be a factor in determining access to more visible, more defendable or earlier appearing spots. Aspects of size, coloration, shape, and perhaps even the nuchal hump, may thus be potential honest signals for sexual selection (Winemiller 2001). Sexual selection can remain stronger in males than females even in cases where males alone foster the brood as is the case in sticklebacks (*Gasterosteus aculeatus)* (Andersson & Simmons, 2006). Selection of a competent male(s) to guard her offspring may improve female fitness, while being able to attract a larger, more fecund egg provider for his brood may improve male fitness.

There are other questions. Nests are often in close proximity. Is this because a specific area's character simply provides more spawning structure, or is there a fitness benefit when potential egg predators have a larger gamut to run before accessing a centrally located nest? Aggregations of spawning ready males are encountered from time to time. Are they lekking? Are they all together because good nesting sites are all together? Perhaps speculation of this sort may provide ideas for future research. Answers to these questions could lead to better understanding of the species.

My goals in this pursuit have been, from the beginning, to provide useful tools for better management and conservation of this species. With this dissertation, I have laid a framework for better understanding of this important species. I would measure my success in this endeavor by the future creation of effective, sustainable management and conservation tools, designed to maintain the integrity of *C. temensis* populations in the Central Amazon basin, while maintaining its accessibility to anglers and local residents

alike. I hope my grandchildren can have the pleasure of experiencing this creature in its native environment.

Appendix 1. Sexual dimorphism in *C. temensis*

Papillae. Male and female *C. temensis* both possess reproductive papillae that can provide a reliable method of determining sex. In addition to their functional reproductive differences, they differ visibly in morphology in adult individuals in all stages of sexual maturity. In all CPV grades, the female papilla is relatively larger and substantially wider than the male's and is recessed in a broad and deep indentation in the fish's ventral surface, located between the cloaca and the anterior insertion of the anal fin (Fig. 1). There is a clear separation between the female recess and the indentation of the cloaca. The indentation associated with the male papilla is shallower and contiguous with the cloaca. Both the external surface and underside of the male papilla is smooth, whereas both surfaces are heavily grooved in the female (Fig. 2).

The differences are further exaggerated in actively spawning individuals, demonstrating that form appears to follow function in this organ. The male's papilla must dispense sperm over rows of eggs previously affixed to a nest surface by the female, while the female's must accommodate the passage of 3 mm long x 1 mm wide, oval shaped eggs. In spawning individuals, the female papilla is distended, substantially unfolding the grooves, expanding beyond the indentation and angled further outward from the torso than at other times. This enlargement ostensibly allows for passage of the sizable eggs. The male organ is likewise angled outward, however only slightly distended and continues to appear smooth on its external surfaces.
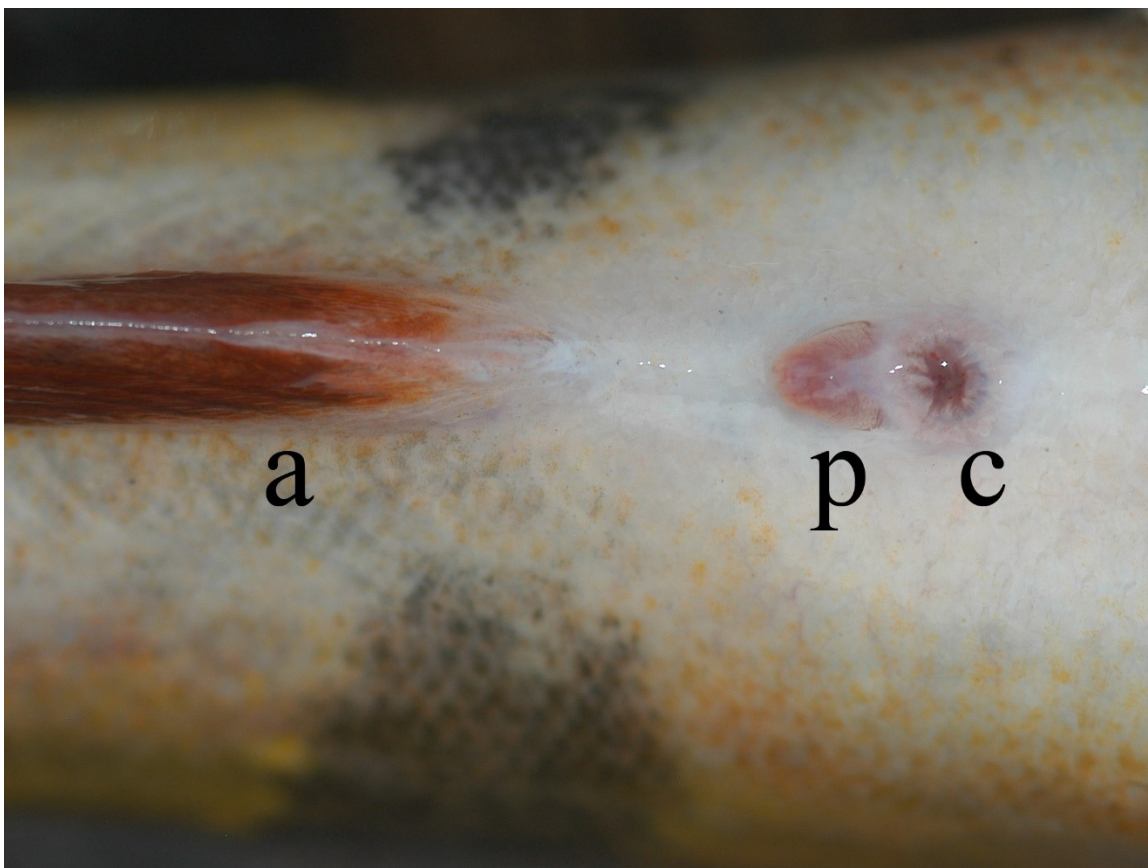
Figure 1 – Location of *Cichla temensis* external papilla, shown in a live individual captured in the Rio Caures, Amazonas, Brazil. (a) anal fin. (p) papilla, anterior to anal fin, posterior to cloaca. (c) cloaca.
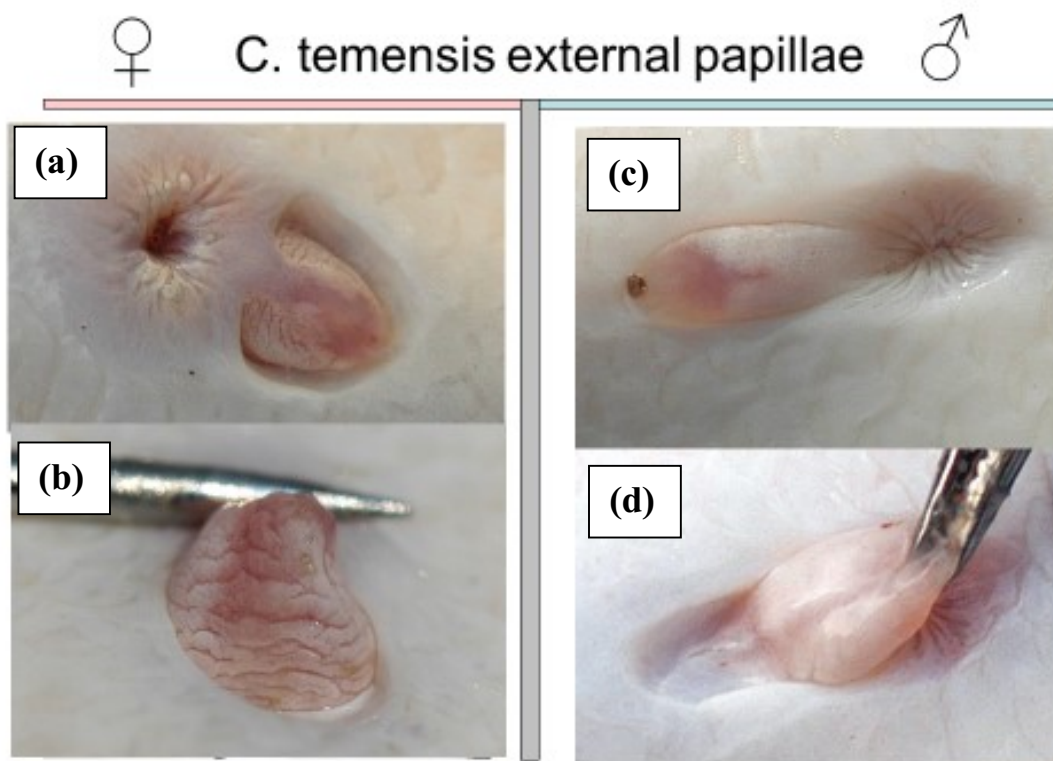
Figure 2. Variation in female and male papilla in live specimens approximately 400 mm in Standard Length, collected on the Rio Caures, Amazonas, Brazil. (a) Female papilla in non-spawning individual. b. Underside of female papilla. c. Male papilla. d. Underside of male papilla. These photos are representative of non-spawning individuals in all CPV grades and demonstrate the clearly visible differences in morphology.