

Air-breathing and movement ecology of *Arapaima* sp. in the Amazon

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Abstract (Academic)

The annual hydrological cycle of floodplains supports fishes that are uniquely adapted to optimize resources throughout the year. Such adaptations to changing environments include air-breathing for seasonally hypoxic waters and directed movements to best utilize habitats as they become available. This study examined the environmental, temporal and body-size influences on air-breathing behavior and movement ecology of *Arapaima* sp., one of the most economically and ecologically significant species in the Amazon. Acoustic (n=15) and radio (n=12) telemetry was used to study the influences on air-breathing and movement ecology of arapaima in the Central Amazon. Generalized additive mixed models showed that temperature was the most influential predictor of air-breathing intervals, followed by body size. The shortest breathing intervals were associated with consecutive “aggressive” breaths while the longest breathing intervals had consecutive “calm” breaths. Generalized linear mixed models showed that flood stage was the most important predictor of residency time, directional movement, and rate of movement. Fish moved faster in the flood and dry stages than the rising and falling stages, and spent longer in one place in the rising and falling stages than the flood and dry stages. Findings of this study may be used to inform management decisions for arapaima conservation, such as protected habitat and population counts, with applications to fishes across river-floodplain ecosystems globally.

Air-breathing and movement ecology of *Arapaima* sp. in the Amazon

Gretchen L. Stokes

Abstract (Public)

The annual hydrological cycle of floodplains supports fishes that are uniquely adapted to optimize resources throughout the year as their environment changes. Such adaptations include air-breathing for seasons with low oxygen levels and directed movements (i.e. upstream, downstream) to best utilize habitats as they become available during the year. This study examined the environmental, temporal and body-size influences on air-breathing behavior and movement ecology of *Arapaima* sp., one of the most economically and ecologically significant species in the Amazon. Acoustic and radio telemetry was used to study the influences on air-breathing and movement ecology of arapaima in the Central Amazon. Model results showed that temperature was the most influential predictor of air-breathing intervals, followed by body size. The shortest breathing intervals were associated with consecutive “aggressive” breaths while the longest breathing intervals had consecutive “calm” breaths. Generalized linear mixed models showed that flood stage was the most important predictor of how long a fish spent in a given area, the direction in which it travels and the rate at which it moved. Fish moved faster in the flood and dry stages, and spent longer without moving in the rising and falling stages. Findings of this study may be used to inform management decisions for arapaima conservation, such as protected habitat and population counts, with applications to fishes across river-floodplain ecosystems globally.

Dedication

To my parents, Bill and Nancy Stokes, and my sister, Sarah
for their continued love and support,

and

To the fishermen
for teaching me much of what I know about arapaima and
keeping me alive in the field.

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This work was made possible with the help of many people, and the culmination of hundreds of hours of sweat, hard work and perseverance, for which I will be forever indebted with gratitude.

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Preface

The chapters that follow have been written with the intention of submission for publication in peer-reviewed ecology journals. They have been formatted according to specifications of targeted journals and the committee chair.

All work in the chapters that follow is my original work. Consultation with several co-authors and committee members has been made and will be made with the remaining co-authors following submission of this thesis and prior to the submission to journals.

Chapter 2, *Environmental and Body Size Influences on Air-Breathing Characteristics of Arapaima sp.*, will be submitted for publication to the *Journal of Fish Biology*, with co-authors Leandro Castello, Eduardo Martins, Thiago Petersen, Steven Cooke, Michael Power and Jansen Zuanon.

Chapter 3, *Movement ecology of Arapaima sp. in a transitional river-floodplain in the Amazon*, will be submitted for publication in the *Journal of Animal Ecology* with co-authors Leandro Castello, Eduardo Martins, Thiago Petersen, Steven Cooke, Michael Power and Jansen Zuanon.

Chapter 1. Introduction

River-floodplain ecosystems with a natural hydrological regime support some of the most productive fisheries and most diverse ichthyofauna in the world (Welcomme 1985, Oberdorff 2011). They also face increasing threats from habitat modification and degradation, leaving very few rivers with full connectivity and natural flooding cycles (Bayley 1995). In North America alone, over two-thirds of rivers have at least one dam (Paukert & Galat 2010). Modified river systems do not allow for a full understanding of fish movement, behavior and ecology, thus the only way to fully understand freshwater fisheries ecology is to examine river-floodplains with a natural hydrological regime and native vegetation.

The dynamic river-floodplain process that drives fish ecology in large rivers is best described as the “flood pulse” (Junk et al. 1989). Much different from the widely accepted River Continuum Concept, the principal idea of the flood pulse cycle is that a river, its catchment and its floodplain comprise an ecological unit. Nutrient cycling occurs laterally within a floodplain rather than nutrient spiraling along a longitudinal dimension (Junk et al. 1989, Galactos et al. 2004). The flood-pulse creates a moving littoral zone and as floodwaters rise, the inundation of vegetation releases nutrients, which increases production and decomposition. When water levels are highest, decomposition exceeds the rate of production, resulting in decreased dissolved oxygen levels. As waters recede, aquatic life becomes concentrated to fewer suitable habitats, which often have high temperatures and low oxygen levels (Junk et al. 1989, Goulding 1980).

The structural complexity of floodplains supports a high diversity and unique distribution of fishes that move as habitats becomes available throughout the year. Lateral connections to

floodplains and connective channels play an important role for fish movements, as fish often move with the flood-pulse to maximize available resources and increase fitness as environmental conditions change (Borcherding et al. 2002). Unlike theories based on longitudinal movements in the main channel, fish in river-floodplains also move from the main channel into adjacent lakes or the floodplain, known as lateral migration (Daget 1960, Welcomme 1979). Several studies have detailed the patterns in habitat use and lateral migration across flood stages, however the specific timing and triggers of such movements remains unknown (Fernandes 1997, Castello 2008).

The flood-pulse cycle significantly alters aquatic conditions throughout the year and these fluctuations require specialized adaptations in aquatic organisms, including those found in fishes living in seasonally hypoxic and low oxygen waters (Junk et al. 1983, Junk et al. 1989). As waters recede and fishes are concentrated in pools, lakes, or channels, they often face poor water quality and a lack of oxygen (Junk et al. 1989). To cope with anoxic conditions, tropical fishes have adapted a range of mechanisms and strategies. Among them is air-breathing in fishes. While air-breathing behavior is a relatively common attribute of fishes in river-floodplains, few studies have examined the surfacing characteristics or breathing intervals of air-breathing fishes, or the factors that influence them in a natural environment.

This study aims to address these questions of air-breathing behavior and movement ecology using one of the most obligate air-breathing fishes in the world, *Arapaima* sp. Some of the largest fishes in the world are found in the genus *Arapaima* (Class Actinopterygii, Family Osteoglossiformes), known locally as *pirarucu* (Portuguese) or *paiche* (Spanish). The genus *Arapaima* was considered to be a monotypic genus since the type species *Arapaima gigas* was described (Schinz, in Curvier, 1822) until only a few years ago. To date, five species of arapaima

have been identified based on morphological differentiation, which is likely a result of geographically distinct areas in tributaries of the Solimões and Amazon Rivers. It remains unknown how many species exist and the precise distribution of each, though it is suspected there may be as many as ten or more species (Stewart et al. 2013, Stewart pers. com.). For this reason, and due to the fact that no genetic or taxonomic work on arapaima has been done where this study took place, arapaima will be referred to as simply *Arapaima* sp. for the purposes of this study.

Arapaima are some of the most economically and ecologically significant fishes in the Amazon, reaching up to 200 kg in weight and over 3 m in length (Arantes et al. 2013). Arapaima are predatory, characterized as secondary consumers of piscivorous and omnivorous fishes (Watson et al. 2011, Queiroz 2000). Well-adapted to seasonally anoxic and hypoxic floodplain lakes, arapaima are obligate air-breathing fishes, requiring surface respirations every 5 to 15 minutes (Sánchez 1969). Arapaima move through the river-floodplains and floodplain lakes during the annual flood cycle using lateral migration strategies (Castello 2008).

Historically, arapaima were some of the Amazon's most important fishes and supported one of the largest Amazonian fisheries (Castello et al. 2015). A century ago, arapaima were abundant and harvested by the hundreds or even thousands for the sale of their meat. However, such overexploitation and lack of sustainable management regulations led to rapid declines in arapaima populations in the 1950s and 60s. In 1975, *Arapaima gigas* was listed on the Convention on International Trade of Endangered Species (CITES) Appendix II, and nearly a decade later, was listed as "vulnerable" on the IUCN Red List. It is now considered to be "data deficient" (World Conservation Monitoring Centre 1996). Recently, arapaima populations have been found on the decline, overexploited and even considered "locally extinct" in some areas,

while populations are recovering as a result of sustainable fisheries management in a few areas (Castello et al. 2015, Petersen et al. 2016).

Recently, a promising approach in arapaima conservation was developed using community-based management (Castello 2004). The method uses a system in which fishers count arapaima based on the frequency at which they surface the water to breathe air. These counts are used for population abundance, from which annual fishing quotas are determined (Castello 2004). This approach has helped restore arapaima populations in several locations but it relies on biological information, which is largely unknown in many areas (Castello et al. 2015, Petersen et al. 2016).

Like much of tropical freshwater fisheries conservation, arapaima conservation relies on an improved understanding of biological and ecological processes (Reid et al. 2013, Cooke et al. 2012). Knowledge of natural history and seasonal aspects of fish biology, particularly during the flood stage, are two factors inhibiting riverine fish conservation and restoration (Cooke et al. 2012). Further, this study addresses the influence of variables, such as temperature, that are relevant in light of climate change and the uncertainty of such influences on tropical fishes (Oberdorff et al. 2016). Thus, there is a need to understand the ecology of fishes in natural river-floodplains in order to better understand fish ecology in all parts of the world. The following chapters aim to support this understanding by examining the environmental, temporal and body-size influences of air-breathing behavior and movement ecology of *Arapaima* sp.

References

- Arantes, C. C., Castello, L., Stewart, D. J., Cetra, M., & Queiroz, H. L. 2010. Population density, growth and reproduction of arapaima in an Amazonian river-floodplain. *Ecology of Freshwater Fish*, 19(3), 455-465.
- Bayley, P. B. 1995. Understanding Large River: Floodplain Ecosystems. *BioScience*.
- Borcherding, J., Bauerfeld, M., Hintzen, D., & Neumann, D. 2002. Lateral migrations of fishes between floodplain lakes and their drainage channels at the Lower Rhine: Diel and seasonal aspects. *Journal of Fish Biology*, 61(5), 1154-1170.
- Castello, L. 2004. A method to count pirarucu *Arapaima gigas*: fishers, assessment, and management. *North American Journal of Fisheries Management*, 24, 379-389.
- Castello, L. 2008. Lateral migration of *Arapaima gigas* in floodplains of the Amazon. *Ecology of Freshwater Fish*, 17(1), 38-46.
- Castello, L., Arantes, C. C., Mcgrath, D. G., Stewart, D. J., & Sousa, F. S. D. 2015. Understanding fishing-induced extinctions in the Amazon. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 25(5), 447-458.
- Cooke, S., Paukert, C., & Hogan, Z. 2012. Endangered river fish: Factors hindering conservation and restoration. *Endangered Species Research*, 17(2), 179-191.
- Daget J. 1960. Les migrations des poissons dans les eaux douces tropicales Africaines. Proceedings of the Indo Pacific Fisheries Council 8: 79-82.
- Fernandes, C. C. 1997. Lateral migration of fishes in Amazon floodplains. *Ecology of Freshwater Fish*, 6(1), 36-44.

- Galactos, K., Barriga-Salazar, R., & Stewart, D.J. 2004. Seasonal and habitat influences on fish communities within the lower Yasuni River basin of the Ecuadorian Amazon. *Environmental Biology of Fishes*, 71, 33-51.
- Goulding, M. 1980. *The Fishes and the Forest*. University of California Press, Los Angeles.
- Junk, W.J., Soares, G.M., & Carvalho, F.M. 1983. Distribution of fish species in a lake of the Amazon river floodplain near Manaus (Lago Camaleão), with special reference to extreme oxygen conditions. *Amazoniana*, 7, 397-421.
- Junk, W.J., Bayley, P.B., & Sparks, R.E. 1989. The flood pulse concept in river floodplain systems. In: Proceedings of the international large river symposium. *Canadian Journal of Fisheries and Aquatic Sciences*, 110-127.
- Oberdorff, T., Jézéquel, C., Campero, M., Carvajal-Vallejos, F., Cornu, J. F., Dias, M. S., Duponchelle, F., Maldonado-Ocampo, J. A., Ortega, H., Renno, J. F. & Tedesco, P. A. 2015. Opinion Paper: How vulnerable are Amazonian freshwater fishes to ongoing climate change?. *Journal of Applied Ichthyology*, 31, 4-9.
- Paukert, C. P. & Galat., D. 2010. Warmwater rivers. In W. Hubert & M. Quist (Eds.), *Inland Fisheries Management in North America* (pp. 699-730). Bethesda, Maryland: American Fisheries Society.
- Petersen, T. A., Brum, S. M., Rossoni, F., Silveira, G. F. V., & Castello, L. 2016. Recovery of *Arapaima* sp. populations by community-based management in floodplains of the Purus River, Amazon. *Journal of Fish Biology*, 89(1), 241-248.
- Queiroz, H.L. 2000. Natural history and conservation of pirarucu, *Arapaima gigas*, at the Amazonian varzea: Red giants in muddy waters. PhD thesis, University of St. Andrews, St. Andrews, Scotland.

- Reid, G. McG., Contreras MacBeath, T. & Csatadi, K. 2013. Global challenges in freshwater fish conservation related to public aquariums and the aquarium industry. *International Zoo Yearbook*, 47(1), 6-4.
- Sánchez, J.R. 1969. El “paiche:” aspectos de su historia natural y aprovechamiento. *La Revista de Caza y Pesca*, 10, 17-61.
- Stewart, D. J. 2013. Re-description of *Arapaima agassizii* (Valenciennes), a rare fish from Brazil (Osteoglossomorpha: Osteoglossidae). *Copeia*, 2013(1), 38-51.
- Watson, L. C. 2011. *Ecology and conservation of arapaima in Guyana: Recovery of a giant fish in distress*.
- Welcomme, R.L. 1979. *Fisheries Ecology of Floodplain Rivers*. Longman Press, London.
- Welcomme, R.L. 1985. *River Fisheries*. FAO Technical Paper No 262, Rome.
- World Conservation Monitoring Centre. 1996. *Arapaima gigas*. The IUCN Red List of Threatened Species.

Chapter 2. Environmental and Body Size Influences on Air-Breathing Characteristics of *Arapaima* sp.

Abstract

Arapaima (*Arapaima* sp.) is an obligate air-breathing fish endemic to the Amazon basin, and one of the largest fishes in South America. *Arapaima* stock assessment depends on census counts that use air-breathing surfacing events to determine population abundance from which harvest limits are set. However, these counts rely on surfacing events and surfacing characteristics that have never previously been tested. This study examines the relationship between breathing intervals and environmental parameters (e.g. temperature, transparency) and body size for radio-tagged arapaima (n=12) in an upland river-floodplain (Lake Ayapuá, Amazonas, Brazil). Generalized additive mixed models were used to evaluate environmental and size correlates of breathing interval. Temperature was the most influential predictor of air-breathing behavior, followed by body size. The shortest breathing intervals were associated with consecutive “aggressive” breaths while the longest breathing intervals had consecutive “calm” breaths. Type of breath, size and temperature predictors revealed that breathing intervals ranged from 4 - 46 minutes and were not significantly different among life stages (\bar{x} =15.9 min for subadults - adults; \bar{x} =14.8 for juveniles). While population censuses count fish in 20-minute intervals, we found that 15% of observed breaths, and two-thirds of fish, took longer than 20 minutes to breathe. Given these findings that slower breathing occurs in colder water, it is recommended that any counts conducted in water less than 28°C be increased by five minutes to 25-minute counting intervals. Stock assessment methods that include adaptations to water temperature may help improve the accuracy of census counts for improved arapaima conservation.

Keywords: arapaima, air-breathing fishes, river-floodplains, telemetry, calm, aggressive

Introduction

Air-breathing in fishes is an ancient specialization that evolved in multiple lineages from aquatic hypoxia and seasonal flooding, among other environmental phenomena (Carter & Beadle 1931, Graham 1997). Most extant fishes that require aerial respiration occur in tropical freshwaters (Graham 1997). Changes in environmental conditions over time and space, as well as individual activity patterns, effect the air-breathing behavior of tropical fishes, such as changes in the type of surfacing event and changes in breathing intervals (Graham 1997). Some of the most important of these factors include water temperature, body size, transparency, and time of day.

Rate of oxygen consumption, reflected by breathing intervals in air-breathing fishes, ultimately depends on metabolic demands. The most important environmental factor influencing metabolism in fishes is water temperature (Fry 1971). In a normal range of physiological tolerance, temperature can have significant effects on activities, such as feeding, migration and other behaviors of fishes (Fry 1971). Fish metabolism is also influenced by body size, as oxygen consumption is a function of body mass and activity level (Winberg 1956). Larger air-breathing fishes obtain more overall oxygen from air than smaller fishes and some have a positive relationship between gill surface area or air-breathing organ size and body mass (Pauly 1981, Graham 1997). Air-breathing intervals may also be influenced by environmental conditions, such as light levels and turbidity. For example, in the case of visual predators, which are often more active at dawn and dusk, reduced air-breathing intervals may occur in response to increased energy expenditures for foraging during low light periods (Graham & Baird 1984, Graham 1983, Hawkins 1986). High turbidity is also known to reduce capture efficiency and increase energy expenditures for feeding activities of visual predators (Gregory & Levings 1998, Reid et al.

1999). The degree to which these factors influence air-breathing intervals in fishes relies on an understanding of specific respiratory responses to environmental conditions.

Air-breathing intervals may also depend on a fish's air-breathing mechanism and the way in which they breach the surface (Graham 1997). There are two classifications of aquatic air-breathing fishes: facultative and continuous, the latter which can be classified as obligate or non-obligate (Graham 1997). Continuous, obligate air-breathing fishes require frequent surface respirations for normal function (Graham 1997). Surfacing may occur rapidly to avoid predation threats, aggressively as a defense mechanism, or synchronously as a means of communication (Lima Filho 2012). Surfacing characteristics may be linked to environmental factors or human disturbance, though this remains unstudied. Here, air-breathing intervals and surfacing characteristics were examined using *Arapaima* sp. (Osteoglossomorpha, Osteoglossidae), one of the largest and most obligate air-breathing fishes in the world (Brauner et al. 2004), in their native environment.

Arapaima are native to the Amazon River basin, reaching sizes of up to 3 m and 200 kg (Arantes et al. 2013). Due to their large size and economic value, they are historically one of the Amazon's most overexploited fishes. *Arapaima* seem to be mostly piscivorous predators but display some omnivory, relying on vision to locate prey (Watson et al. 2011, Queiroz 2000). They are typically found in floodplain lakes in the dry season, then move to the flooded forest to feed and reproduce as waters rise, and then migrate back to the main channel and lakes as waters recede (Castello 2008). *Arapaima* are adapted to the oxygen-poor waters and dynamic flooding cycles in the Amazon River by relying on continuous aerial breathing (Graham 1997). Observations in the field and captivity report breathing frequencies every 5 - 20 minutes (Fontanele 1948), 5 - 15 minutes (Castello 2004), 10 - 20 minutes (Fernandes et al. 2012), or 2 -

12 minutes in captivity (Stevens & Holeton 1978); however, no quantitative or systematic study of air-breathing frequencies in wild populations has been conducted. Variability in these frequencies could be explained by body size or environmental influences, such as temperature, transparency, time of day and day of year.

Arapaima possess a highly vascularized swim bladder with many septa that support oxygen utilization and small gill structures that aid in carbon dioxide excretion and ionic regulation (Farrell & Randall 1978, Randall et al. 1978, Brauner et al. 1996, Brauner et al. 2004). Arapaima gills are so small that only one-fifth of oxygen uptake occurs across the gills (Brauner et al. 2004). Eight to nine days post-hatch, arapaima become air-breathers, at which point they rely primarily on atmospheric air for respiration. As such, air-breathing is likely influenced primarily by physiochemical factors other than dissolved oxygen, such as temperature, activity level or seasonality.

The way in which surfacing occurs depends on a fish's air-breathing organs, predatory responses (i.e. synchronous breathing), or environmental influences (Lima Filho 2012). Though never formally described, surfacing characteristics of arapaima are often described by local Brazilian fishers as *mansa* (calm), *normal* (normal) and *braba* (aggressive). Such descriptions reflect the movement used to obtain air, including the way in which an arapaima's body breaks the surface for a gulp of air, its momentary behavior at the surface and the way in which it returns to the water. The most distinct differences in surfacing characteristics are reported between calm and aggressive breathing. The change in surfacing characteristics over time may be an important indicator of disturbance to arapaima and a predictor of breathing intervals. If surfacing characteristics indicate a reaction to a stimulus, such as human disturbance or presence of nest predators during reproduction, it would be expected that calm breathing would be

associated with longer breathing intervals and aggressive breathing with faster breathing intervals. More aggressive surfacing has been observed more frequently during the reproductive season, perhaps as a potential warning sign for predators (Goulding 1980).

Influences of environmental factors and body size on breathing intervals and surfacing characteristics also have conservation relevance. Overharvest and mismanagement are causing local extinctions of arapaima, except a few hotspots in the central state of Amazonas where successes in community-based management have led to population recoveries (Castello et al. 2015, Petersen et al. 2016). Recently, a promising approach for sustainable management was developed that uses population abundance counts based on surfacing events for stock assessment. In this method, fishers count the number of fish in an area of up to 2 ha over a 20-minute interval. Direct counts are determined by fishers' audio and visual observations of surfacing events (Castello 2004). The total number of fish counted during stock assessment is then used to determine fishing quotas (Castello 2004). This counting method depends on the assumption that no fish takes longer than 20 minutes to breathe, given undisturbed natural (e.g. no wind, rain) and human (e.g. human noises) conditions. No consideration has ever been given to the variability in air-breathing intervals and the degree to which factors, such as temperature or body size, may influence counts.

This study was designed to test the hypotheses that water temperature, body size, transparency, time of day, day of year and surfacing characteristics influence breathing intervals. Results from testing these competing hypotheses were compared and the most influential factors on air-breathing intervals and air-breathing characteristics were identified. This was done using the following objectives: 1) to assess environmental and body size effects on breathing intervals, and 2) to investigate relationships between surfacing characteristics and breathing intervals.

Methods

Radio telemetry was used year-round in a pristine floodplain lake system of the central Amazon Basin to measure time interval between breaths and surfacing characteristics of individual arapaima (n=12). Each fish was given a unique tag so that the influence of physiochemical, spatial and temporal variables on each fish could be measured. Data were analyzed using generalized additive mixed models and candidate models were compared using the bias-corrected Akaike Information Criterion (AICc). Prediction plots were generated to examine the influence of each predictor variable.

Study Area

Lake Ayapuá is a lake on the floodplain of the lower Purus River, which is a whitewater river characterized by high turbidity, high pH, high productivity, warm waters and abundant surface vegetation (Junk et al. 2012). The lake body is an oblong, shallow, turbid body bordered by seasonally flooded lowland vegetation (*várzea*). It is connected downstream to the Purus River and upstream to the lake's headwaters (Figure 2.1). When inundated in the flooded season, the lake's waters become more mixed with the contrastingly colder, less turbid upstream headwaters. The headwaters of Lake Ayapuá form a narrow valley (*ria*) lake with upland habitat (*terra firme*) on both sides of the main channel and many connective side channels (*igarapes*). The lake headwaters are cooler, highly transparent, and acidic, typical of blackwater ecosystems (Gourou 1950, Junk et al. 2015). All data in this study were collected in the lake headwaters (*cabeceira*), from the mouth of the headwaters to approximately 85 – 110 (dry – flood season) kilometers upstream between 11 January and 29 November 2015 (Figure 2.1).

Fish Tagging

Sixteen arapaima [(Total length (TL) = 119 cm \pm 26 cm) (Range = 80 - 170 cm)] were captured using a seine net (140 m x 6 m) in pools of the headwaters of Lake Ayapuá in November 2014 (Figure 2.1). Upon capture, arapaima were placed in a recovery canoe filled with lake water to rest and were monitored until air-breathing intervals normalized to approximately every 5 - 15 minutes.

Two tags were placed on each fish: one radio tag was inserted surgically and one anchor tag was inserted externally. Surgical tag implantation was completed following standardized tagging procedures (Wagner et al. 2011). Two to four scales were removed over the peritoneal cavity anterior of the anal fin and a local anesthetic (topical benzocaine 20%) was applied. A radio transmitter (Lotek MCFT2-3EM at 20 ppm or Sigma Eight Inc. Pisces at 60 ppm) was inserted into the peritoneal cavity through a 2 - 3 cm incision. Incisions were closed using 2 - 3 interrupted sutures of absorbable monofilament synthetic surgical sutures. According to the discretion of the lead tagger (E. G. Martins) and based on the degree of incision exposure, some fish had 1 - 2 scales glued over the incision using VetbondTM tissue adhesive. An external anchor tag with a unique fish identification number for each fish was inserted proximal to the base of the dorsal fin. After tagging, each fish was monitored for 5 - 10 minutes until it regained sufficient strength to swim away, at which point it was released. All capture and tagging protocols were approved by the Institutional Animal Care and Use Committee (IACUC #14-085) at Virginia Polytechnic Institute and State University and the Sistema de Autorização e Informação em Biodiversidade (SISBIO #6289511) in Brazil.

Radio Telemetry

Individually radio-tagged arapaima were monitored using active tracking, performed daily by boat using a 3-element Yagi antenna connected to a portable VHF radio receiver (Biotracker, Lotek, Canada). A minimum of 150 hours per month and 15 days per month of active tracking were completed over the 11-month study period. Each month, the entire navigable study area upstream of Lake Ayapuá was searched at least twice. The receiver was programmed to continually scan frequencies of all fish at maximum gain. Once a fish was located, homing in on its specific location was accomplished by removing the antenna and reducing the gain. Telemetry searches were done using a 15-horsepower motorboat at the slowest speed possible to detect fish. A specialized spark plug was installed to reduce motor interference. To increase detection distance, the antenna was attached to a 5 m tall wooden pole. To increase detection probability in the flooded season, a second setup with a radio receiver (R410 Advanced Telemetry Systems, USA) and handheld H-antenna was used by a second researcher.

Each tagged fish that was encountered was observed for a minimum of 30 minutes or three breathing events, whichever came first. If needed, oars were used to reorient the boat closer to the location of the fish as it moved. All data collection was performed silently to avoid disturbing the fish, though the initial approach to the fish was made by motor. At the time of each surfacing event, water temperature ($^{\circ}\text{C}$) and transparency (cm) at 0, 2, and 4 m (YSI Professional Plus Multiparameter) were recorded. If any noise was created by the field team or if other human disturbance occurred, all previous breathing data were discarded and counts for breathing events began again after 10 minutes. An attempt to relocate the fish later in the day or

consecutive days was made until it was no longer detected. All observations occurred between 0800 and 2200 hours daily.

One primary knowledgeable fisher assisted in the collection of environmental data and confirmation of breathing data for the duration of the study. In the case that multiple fishers were present, we relied on our primary fisher to maintain consistency while confirming results with other fishers. The fisher counted the total number of arapaima in the area, detected breathing events and categorized surfacing events. A breathing event was detected by audible or visual observation using a telemetry fix, fish size, and our fisher's knowledge of breathing characteristics. In some cases, the external anchor tag was visible in a breathing event and aided in the identification of individual fish, especially when there was more than one fish present.

Time of breath was recorded as the time the fish was visually observed breaking the surface of the water or heard surfacing. Breathing interval was calculated as the time between consecutive breathing events. A measure of certainty was recorded to indicate whether or not the observers were certain that a breathing event was the tagged individual fish under study. If there was high uncertainty due to a large number of other fish, high amount of fish movement, or weather interference, observers recorded this as uncertain (0). Level of certainty (0,1) was later evaluated for inclusion or exclusion in the analysis; data with uncertainty were not used in the analysis.

Two types of breath were recorded according to the names used by local fishers: *mansa* (calm) and *braba* (aggressive). A calm breath referred to a small, tame surfacing event where the fish broke the surface in a gentle manner for a gulp of air. The head and dorsal area surfaced briefly before the fish dove down. An aggressive breath was distinguishable by a loud smacking noise from the tail slapping the water after surfacing. Such events can often be seen by a large

splash in the water and heard from a far distance, most recognizable as a noise similar to a gunshot. In this study we examined whether a change (or lack thereof) in surfacing characteristics of consecutive breaths influenced the time between breaths. As such, the data were organized as observations consisting of the combination of consecutive breaths. This resulted in four possible categories of two consecutive breathing events: “aa,” (aggressive - aggressive) “ac,” (aggressive - calm) “ca” (calm - aggressive) and “cc” (calm - calm).

Data Analysis

Data exploration included examination of Cleveland and box-and-whisker plots to identify outliers and influential points and a visual assessment for the correct distribution (Zuur et al. 2010). Variance inflation factors were examined to check for collinearity, and variograms were used to assess spatiotemporal autocorrelation in model residuals; however, no collinearity or correlation issues were found based on visual assessment.

a. Environmental and Body Size Effects on Breathing Intervals

Generalized additive mixed models (GAMMs) were used to investigate the relationship of environmental variables and body size to breathing intervals of arapaima. GAMMs allow for non-linear relationships between the response and predictor variables, while incorporating a random effect (Lin & Zhang 1999). The following independent variables were selected for inclusion in the global model: water temperature (°C), transparency (cm), day of year, time of day (hh:mm) and total length of each individual fish (cm), based on *a priori* justification. Day of year was used as a proxy for measures of seasonality, including water level. Averaged temperature readings from three depths (0,2,4 m) were used. All covariates were standardized

prior to analysis. The response, time between breaths, was log-transformed to meet assumptions of normality and homoscedasticity of residuals. All models included a random effect for fish ID to account for unexplained individual variation in mean breathing interval. The identity link function and the normal distribution were used (Zuur & Ieno 2016). The model has the following form:

$$\begin{aligned}
 \text{TBB}_{ij} &= s(\text{Temperature}_{ij}) + s(\text{Time of Day}_{ij}) + s(\text{Temperature}_{ij} \times \text{Time of Day}_{ij}) \\
 &\quad + s(\text{Day of Year}_{ij}) + \text{Transparency}_{ij} + \text{Total Length}_i + \gamma_i + \varepsilon_j \\
 \gamma_i &\sim N(0, \sigma_\gamma^2) \\
 \varepsilon_j &\sim N(0, \sigma_\varepsilon^2)
 \end{aligned} \tag{1}$$

where TBB_{ij} is the j th observation of breathing interval (time between breaths) for individual i , γ_i is the random deviation from the intercept for individual i , and ε_i is the error term (i.e. residual). Both γ_i and ε_i were assumed to be normally distributed with mean 0 and variance σ_γ^2 and σ_ε^2 , respectively (Zuur & Ieno 2016). The term s denotes the smoothing functions estimated with cubic regression spline defined by a modest set of knots spread evenly among covariates (Wood 2006). The number of knots (k) was set as the maximum possible for automatic selection of the optimum number of knots. Cubic regression splines were placed on temperature, time of day and day of year because of their expected non-linear relationship to breathing intervals. Models were validated by plotting residuals against all variables (Zuur et al. 2009).

All possible subsets of the global model were fit to the data and ranked by second-order AICc values using package “MuMIn” (Barton 2016). All models included in the 95% confidence set for the best model were used for generating model-averaged predictions and unconditional 95% confidence intervals (Burnham & Anderson 2002). Marginal and conditional R^2 values were calculated to determine the amount of variability explained by fixed and random effects in each of the models in the 95% confidence set (Nakagawa et al. 2013). The “zero method” was used for model averaging, which assumes the variable is included in every model but sets

coefficients to 0 if not (Grueber et al 2011). Data for each prediction were generated using the minimum and maximum observed values for the predictor of interest while holding all other values at a constant mean. The exception to this was total length, where the maximum was set beyond the measured limit within the dataset in order to predict breathing intervals for larger fish.

b. Influence of Surfacing Characteristics on Breathing Interval

To assess possible relationships between breathing intervals and surfacing characteristics, type of breath was used as a predictor variable; that is, breathing interval was expected to be a function of the type of breath at the beginning of the recorded breathing interval and the type of breath at the end of the recorded breathing interval. Type of breath was only recorded for approximately half (n=126) of the total number of observations. Type of breath was accounted for in a subset GAMM with the two most influential predictor variables from the first analysis, temperature and total length. The model has the following form:

$$\begin{aligned}
 \text{TBB}_{i,j} &= s(\text{Temperature}_{i,j}) + \text{Total Length}_i + \text{Breath Type}_{ij} + \gamma_i + \varepsilon_j \\
 \gamma_i &\sim N(0, \sigma_\gamma^2) \\
 \varepsilon_j &\sim N(0, \sigma_\varepsilon^2)
 \end{aligned}
 \tag{2}$$

where terms are the same as described for model 1. The AICc values for models with and without breath type were compared to determine the support for the effect of breath type. Marginal and conditional R² values were calculated and compared between the model with and without breath type (Nakagawa et al. 2013). Predictions were created by breath type based on the above model using the previously stated prediction methods.

All statistical analysis and computations were performed using R (version 3.3.0) software (R Core Team 2016). Models were fit using the package *mgcv* (mixed generalized additive

model computation vehicle) and model selection and model averaging were conducted using package “MuMIn” (Barton 2016, Wood 2011).

Results

A total of 281 breathing events from 12 individual fish were observed during 1425 hours of telemetry effort. The median number of breathing observations per fish was 13 (range = 2 - 65) on 67 different days. The median time spent with each fish per encounter was 27 minutes (range = 15 - 139 minutes). For all size classes, the mean breathing interval was 14.0 ± 7.1 minutes (range = 4 - 46 minutes). Mean breathing interval was 15.9 ± 8.3 minutes (range = 5 - 46 minutes) for juveniles (< 1 m total length, n = 117 breaths, n = 4 fish) and 14.8 ± 6.0 minutes (range = 4 - 41 minutes) for subadults and adults (> 1 m total length, n = 84 breaths, n = 8 fish). Of all observed breathing intervals (n = 201), 15% (n = 31) were longer than 20 minutes and were observed for 8 of the 12 fish tracked in the study. Longer breathing intervals (> 20 min) occurred throughout the day (range = 9:06 - 21:26). There was no distinct size association for fish that breathed longer than 20 minutes (range 87 - 145 cm) (Figure A.2).

Temperature and total length were the most important predictors of breathing intervals (Table 2.3). Temperature was included in all of the models in the 95% confidence set, and the presence of total length only added a small weight relative to the second-ranked model that only included temperature (Table 2.3). Models that included only temperature or temperature and either time of day or total length had at least 0.10 of the model weights; collectively the top three models with these predictors contributed to over 50% of cumulative model weights (Table 2.3). Conditional R^2 and marginal R^2 values differed by less than 0.005 and thus only marginal R^2 values are reported. Marginal R^2 values of the models in the 95% confidence set ranged from

0.059 - 0.105; such results indicate that 6 to 10% of the variation is explained by the covariates assessed. An estimated 84% of the variability in breathing intervals was unexplained by the models.

Temperature had the greatest effect on breathing interval, described by a nonlinear relationship (Figure 2.2). After a small increase between 22.5°C and 25°C, breathing intervals decreased between 25°C and 35°C (Figure 2.2). Breathing interval was predicted to decrease from 16 minutes to 9.4 minutes (Δ 6.6 minutes) between 25 and 35°C for fish of average size in the study. Visual examination of breathing intervals along the temperature gradient in the study area showed longer average breathing intervals in colder upstream waters and a higher number of shorter intervals nearer to the mouth where the warm lake waters mix with the headwaters (Figure 2.3).

Predictions for breathing interval showed a positive relationship with total length (Figure 2.2). Breathing interval was predicted to increase by 2.5 minutes between fish of lengths 80 cm (13.5 minutes) and 180 cm (16 minutes). The length relationship predicted that for every increase of 0.25 m, breathing interval will increase by approximately 0.6 minutes. Results also showed a very small decrease in breathing interval during the early morning hours, followed by a small increase in breathing intervals throughout the rest of the day and evening (Figure 2.2). There was virtually no changes in time between breaths for transparency and seasonality (day of the year) (Figure 2.2).

There was some support for the effect of consecutive breath types on breathing interval, as the model with type of breath had an AICc value (163.45) that was 3.85 lower than the model with only temperature and total length (AICc = 167.30). The addition of type of breath in the model increased the marginal R^2 from 0.12 to 0.16. Like the first analysis, the conditional R^2

value was very similar to the marginal R^2 value in both models, which indicated that there was little variability in breathing interval among individuals.

Given the support for type of breath, we used model predictions to examine its effect on breathing intervals. Eighty-five percent of surfacing events were calm ($n = 153$), while 15% ($n = 28$) were aggressive. As in the models fitted to the full data set, predicted breathing intervals for all types of surfacing events decreased with temperature and increased with body size (Figure 2.4). Predicted breathing interval was the shortest (6 - 22 minutes) for two sequential aggressive breaths, while sequential calm breaths yielded the longest (10 - 34 minutes) of the breathing interval predictions. A change in breath from calm to aggressive or vice-versa had the widest range of breathing intervals (8 - 30 minutes) (Figure 2.5). For example, a 1.5 m fish at 30°C was predicted to breathe every 12 minutes when consistently aggressive (aa), every 18 minutes when consistently calm (cc), and every 16 minutes when breath type changed (ac or ca) (Figure 2.5).

Discussion

Biological Implications

This study revealed three key findings: 1) water temperature and body size had the greatest effect on air-breathing intervals of arapaima, 2) consecutive aggressive surfacing events were associated with shorter breathing intervals while consecutive calm surfacing events were associated with longer breathing intervals, and 3) 15% of arapaima took longer than 20 minutes to breathe.

The non-linear relationship found between temperature and air-breathing intervals showed intervals increasing slightly to a maximum around 25°C, then decreasing sharply. There are two possible explanations for the nonlinear relationship: 1) a small sample size at very high

and low temperatures and 2) increased physiological exertion at very high or very low temperatures. First, 25 breaths were observed in water less than 25°C but only 1 breath was observed in water greater than 35°C; therefore, extrapolations yield weak relationships in the upper bounds. Second, we expect extreme conditions, particularly those with very warm or very cool waters, to increase metabolic function, reflected by breathing intervals. Seasonal water temperatures typically range from 27°C to 31°C in the Amazon (Castello & Macedo 2016), however our study area was cooler than average (2014 - 2015 average temp = 27.7°C). It is possible that these overall cooler temperatures caused average breathing intervals to be slower than other areas in the Amazon, particularly in the cooler, far upstream reaches of the study area.

The positive relationship found between breathing interval and body size may be a result of larger fish having a lower metabolic rate than smaller ones or that larger fish are capable of gulping more air per breath than juveniles (Tytler & Calow 1985). It may also be a result of environmental factors having a reduced effect as fish grow (Tytler & Calow 1985). While an overall linear trend was seen, this study revealed highly variable breathing intervals within individual fish. For example, the high variation of 41 minutes for an individual fish (TL = 87 cm) demonstrated the likely influence of other factors. Synchronous air-breathing behavior has been observed for juvenile air-breathing fishes, including very young arapaima, as a strategy to reduce predation (Olsen et al., in prep). Thus, breathing intervals in juveniles (< 1 m) may be driven less by temperature and more by predation and social structure (Stewart pers. com.). Mature, adult fish (> 1.5 m) energetics are likely influenced by reproductive investment at the end of the dry season (typically Nov - Dec), as they expend energy in maturation, digging pan-like nests underwater with their mouths, attentively guarding the eggs, and protecting the young (Castello 2008b).

Model-averaged predictions suggest that time of day may have a small biological importance; however, this slight variation may be simply due to outlier fish observations. The slightly faster predicted breathing intervals in the early morning hours could be related to energetics of feeding activities. As predators, arapaima hunt when environmental conditions are optimal for most efficient prey capture. In the Amazon, carnivorous and piscivorous species were found most active at night and, while omnivores, planktivores, and detritivores were associated more with the morning hours (Noveras et al. 2012). Given that arapaima are primarily piscivores, this may offer support for feeding activities in the pre-dawn hours (Watson et al. 2013).

Other measured variables (transparency, time of day and day of year) showed negligible influence; however, it is possible that these variables do not represent the most direct influencers on breathing interval. Due to the relatedness of the environmental variables (i.e. flood stage and temperature), it remains a challenge to parse apart which is the primary determinant. It is possible, however, that other measures of seasonality, such as water level or dissolved oxygen, may reveal a masked effect of the annual flood-pulse.

While we observed the strongest relationships of breathing interval with temperature and body size, these effects explain only a small amount of the variability in breathing intervals. The small among-individual variability suggests a strong metabolic or physiological constraint driving breathing intervals. While some model variability is explained by the fixed effects and a very small amount of inter-individual variability, much remains unexplained (up to 84%) and thus suggests two possibilities: 1) other influences that one may (i.e. other environmental correlates) or may not be able to be measured (i.e. interactions), and 2) observation error (e.g. mistaking the breath of a tagged fish for that of an untagged fish).

Despite high variation, 4% of variability was explained by the addition of the type of breath. This indicates the time between surfacing events can be influenced by the behavioral state of the fish. Fish with two consecutive aggressive breaths were observed breathing more rapidly. This may be explained in one of two ways: 1) the fish may expend more energy to physically break the surface and slap its tail, or 2) the fish is breathing aggressively from a stimulus, which increased metabolism levels. In many cases, aggressive breathing began suddenly, perhaps due to the onset of human presence, predators in the case of juvenile arapaima, or other boat traffic. Such a large increase in mean breathing intervals (12 to 18 min) from consecutive calm to consecutive aggressive indicate that external influences can cause rapid and highly dynamic changes in metabolism.

These findings on air-breathing characteristics and intervals are relevant to the other 33 species air-breathing fishes in the world, of which the breathing habits have been seldom studied (Graham 1997). It may be predicted that similar relationships of temperature and body size would be found in such species, particularly those found in a similar habitat, such as the other two species of obligate air-breathers in the Amazon (e.g. genus *Lepidosiren*, *Electrophorus*). However, the high individual variation found in this study suggests that the life history traits and unique habitats of each species must also be considered.

Management implications

Results of the influences of body size and environmental correlates on breathing intervals reveal two key findings relevant to arapaima stock assessment. First, the influence of temperature on breathing rates should be considered in census counts, particularly in areas with large temperature ranges, such as Lake Ayapuá. Fishers count fish in 20-minute intervals, based

on the assumption that no fish takes longer than 20 minutes to breathe. Collectively, these counts comprise the data from which harvest limits are set each year (Castello 2004). Castello (2004) found that fishers' counts were normally within 10% of mark-recapture estimates. However, the method designed to count arapaima was designed in a single ecosystem (whitewater *várzea*) with near-constant temperatures ($\mu = 30.1^{\circ}\text{C}$) across the ecosystem (Castello 2008). In Lake Ayapuá, a large difference in temperature (8-10°C) exists between the mouth of the lake body to the farthest upstream reaches. Such conditions provoked speculations from local fishers about the accuracy with which the method to count arapaima could be applied. In this study, 15% of fish took longer than 20 minutes to breathe. Our results suggest that breathing intervals may be faster closer to the lake and slower in the farthest upstream reaches, due to warmer water temperatures in the mouth of the lake and cooler temperatures upstream. When using the 20-minute protocol, fishers may be more likely to accurately count fish nearer to the lake, while they are more likely to undercount fishes in the upstream reaches of our study area.

All factors aside, the effect of temperature on breathing intervals should be considered in future stock assessment protocols. Based on the results of this study, it is recommended that if average water temperature (0 - 4 m) is less than 28°C, fishers should increase the duration of counts by five minutes to a 25-minute time for counts. According to our data, this would account for approximately 93% of fish, as only 6.8% (n breaths =18) took longer than 25 minutes to breathe.

Second, surfacing characteristics should also be considered in stock assessment, as this study shows them to have some influence breathing intervals. Consecutive aggressive (*braba*) breaths are associated with shorter breathing intervals; therefore, the most reliable counts would come from undisturbed conditions. It is recommended that fishers strive to conduct counts in

conditions without changes to surfacing characteristics. Similarly, if any change in breathing behavior is seen (i.e. aggressive to calm, or vice versa), there is more likely to be shorter breathing intervals and such change could influence counts. Further studies are necessary to determine the relationships and effects of surfacing characteristics and stock assessment.

One explanation for the large range of breathing intervals and longer intervals than previous observations in other studies may be due to predator avoidance. Fishers report that when arapaima perceive threats, they may hide under logs or floating vegetation and have longer surfacing intervals (Castello 2004). Part of the stock assessment protocol requires complete silence to count fish (Castello 2004). While a best attempt was made to minimize noise level and throw out data during which significant human disturbance occurred, it is possible that the fish heard us and that it may have caused an effect on the fish. Noise created in the boat or by researchers may have caused arapaima to reduce their surfacing intervals as a way to reduce perceived danger. While the exact effect of such disturbance remains unknown, is important to make all attempts to reduce noise and external disturbance in counts for stock assessment.

Finally, results of this study warrant research on other air-breathing fishes. To the author's knowledge, no other air-breathing fishes are managed using surfacing events but such management could be considered. Two species of relevance in North America include gar (family Lepisosteidae) and bowfin (*Amia calva*). Studies of bowfin air-breathing intervals suggest a similarly positive relationship with air-breathing intervals and temperature, and that faster breathing occurs at night (Horn & Riggs 1973). Similar to arapaima, gar have a vascularized swim bladder for respiration, though their facultative air-breathing behavior has not been widely studied (Smatresk & Azizi 1987). Further investigation would be necessary to consider the individual influences on these species as was done for arapaima here.

Conservation Implications

Most air-breathing fishes occur in the tropics, which are currently undergoing some of the most rapid changes from anthropogenic influences on the planet. Designing and implementing effective conservation strategies for tropical freshwater fisheries requires knowledge of physiological traits, such as air-breathing behavior, and their relationship to environmental characteristics. Of the species of air-breathing fishes found in the tropics, arapaima are one of the most heavily exploited species in the Amazon, and are further threatened by illegal harvest and mismanagement (Castello & Macedo 2016).

Conservation of arapaima relies on accurate and robust management practices. This study recommends adaptive stock assessment be instituted across management areas and sustainable development reserves. Such adaptations would need to account for variability in breathing times and variability depending on habitat and site-specific temperatures. In the face of accelerated climate change with predictions for warming waters in the Amazon, it is important to consider managing arapaima with these considerations to avoid risk of overharvest while still continuing to support local communities and healthy fish populations.

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References

- Arantes, C. C., Castello, L., Cetra, M., & Schilling, A. 2013. Environmental influences on the distribution of arapaima in Amazon floodplains. *Environmental Biology of Fishes*, 96(10–11), 1257–1267.
- Bard, J. & Imbiriba, E. P. 1986. *Piscicultura do pirarucu Arapaima gigas*. Embrapa-Centro de Pesquisa Agropecuaria do Tropicó Umido, Belem, Brasil.
- Barton, K. 2016. MuMIn: Multi-Model Inference. Retrieved from <https://cran.r-project.org/package=MuMIn>
- Brauner, C. J. and Val, A. L. 1996. The interaction between O₂ and CO₂ exchange in the obligate air breather, *Arapaima gigas*, and the facultative air breather, *Lipossarcus pardalis*. In V. M. F. A.-V. and D. J. R. A. L. Val (Ed.), *Physiology and Biochemistry of the Fishes of the Amazon* (pp. 101-110). Manaus, Brazil: INPA.
- Brauner, C. J., Matey, V., Wilson, J. M., Bernier, N. J., & Val, A. L. 2004. Transition in organ function during the evolution of air-breathing; insights from *Arapaima gigas*, an obligate air-breathing teleost from the Amazon. *The Journal of Experimental Biology*, 207(Pt 9), 1433-1438.
- Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: A practical information-theoretic approach* (2nd ed.). New York: Springer.
- Carter, G. S., & Beadle, L. C. 1931. Respiratory adaptations of fishes. *Journal of the Linnean Society of London (Zoology)*, 25, 48-56.
- Castello, L. 2004. A method to count pirarucu *Arapaima gigas*: fishers, assessment, and management. *North American Journal of Fisheries Management*, 24, 379-389.
- Castello, L. 2008. Lateral migration of *Arapaima gigas* in floodplains of the Amazon. *Ecology of*

- Freshwater Fish*, 17(1), 38–46.
- Castello, L. 2008. Nesting habitat of *Arapaima gigas* (Schinz) in Amazonian floodplains. *Journal of Fish Biology*, 72(6), 1520-1528.
- Castello, L., Arantes, C. C., Mcgrath, D. G., Stewart, D. J., & Sousa, F. S. D. 2015. Understanding fishing-induced extinctions in the Amazon. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 25(5), 447-458.
- Castello, L., & Macedo, M. N. 2016. Large-scale degradation of Amazonian freshwater ecosystems. *Global Change Biology*, 22(3), 990-1007.
- Farmer, C. G., & Jackson, D. C. 1998. Air-breathing during activity in the fishes *Amia calva* and *Lepisosteus oculatus*. *Journal of Experimental Biology*, 201(7), 943.
- Farrell, A.P., & Randall, D. J. 1978. Air-breathing mechanics in two Amazonian teleosts, *Arapaima gigas* and *Hoplerythrinus unitaeniatus*. *Canadian Journal of Zoology*, 56, 939-945.
- Fernandes, M. N., da Cruz, A. L., da Costa, O. T. F., & Perry, S. F. 2012. Morphometric partitioning of the respiratory surface area and diffusion capacity of the gills and swim bladder in juvenile Amazonian air-breathing fish, *Arapaima gigas*. *Micron*, 43(9), 961-970.
- Fontanele, O. 1948. Contribuição para o conhecimento da biologia do pirarucu, “*Arapaima gigas*” (Cuvier), em cativo (Actinopterygii, Osteoglossidae). *Revista Brasileira de Biologia*, 8, 445-459.
- Fry, F. E. J. 1971. The effect of environmental factors on the physiology of fish. *Fish Physiology*, 6, 1-98.
- Graham, J. B. 1983. The transition to air breathing in fishes. II. Effects of hypoxia acclimation on the bimodal gas exchange of *Ancistrus chagresi* (Loricariidae). *Journal of Experimental*

- Biology*, 102, 157-173.
- Graham, J. B. & Baird, T. A. 1984. The transition to air breathing in fishes. III. Effects of body size and aquatic hypoxia on the aerial gas exchange of the swamp eel *Synbranchus marmoratus*. *Journal of Experimental Biology*, 108, 357-375.
- Graham, J. B. 1997. *Air-breathing fishes: Evolution, diversity and adaptation*. San Diego: Academic Press.
- Gregory, R.S., & Levings, C.D. 1998. Turbidity reduces predation on migrating juvenile Pacific salmon. *Transactions of the American Fisheries Society*, 127, 275-285.
- Goulding, M. 1980. *The Fishes and the Forest*. University of California Press, Los Angeles.
- Gourou, P. 1950. Observações geográficas na Amazônia. *Revista Brasileira de Geografia*, 11, 355-408.
- Grueber, C. E., Nakagawa, S., Laws, R. J., & Jamieson, I. G. 2011. Multimodel inference in ecology and evolution: Challenges and solutions. *Journal of Evolutionary Biology*, 24(4), 699-711.
- Hawkins, A. 1986. The Behaviour of Teleost Fishes. *The Behaviour of Teleost Fishes*, 1986.
- Horn, M. H., & Riggs, C. D. 1973. Effects of temperature and light on the rate of air breathing of the bowfin, *Amia calva*. *Copeia*, 1973(4), 653-657.
- Junk, W. J., Piedade, M. T. F., Schöngart, J., & Wittmann, F. 2012. A classification of major natural habitats of Amazonian white-water river floodplains (várzeas). *Wetlands Ecology and Management*, 20(6), 461-475.
- Lawson, L. L., Tuckett, Q. M., Lawson, K. M., Watson, C. A., & Hill, J. E. 2015. Lower lethal temperature for arapaima arapaima gigas: Potential implications for culture and establishment in florida. *North American Journal of Aquaculture*, 77(4), 497.

- Lima Filho, J. A., Martins, J., Arruda, R., & Carvalho, L. N. 2012. Air-breathing behavior of the jeju fish *hoplerythrinus unitaeniatus* in amazonian streams. *Biotropica*, 44(4), 512-520.
- Lin, X., & Zhang, D. 1999. Inference in generalized additive mixed models by using smoothing splines. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, 61(2), 381-400.
- Luling, V. 1964. Sur Biologie und ökologie von *Arapaima gigas* (Pisces, Osteoglossidae). *Zeitschrift für Morphologie und Ökologie der Tiere*, 54, 436-530.
- Nakagawa, S., Schielzeth, H., & O'Hara, R. B. 2013. A general and simple method for obtaining R² from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 4(2), 133-142.
- Noveras, J., Yamamoto, K. C., & Freitas, C. E. C. 2012. Use of the flooded forest by fish assemblages in lakes of the National Park of Anavilhanas (Amazonas, Brazil). *Acta Amazonica*, 42(4).
- Pauly, D. 1981. The relationships between gill surface area and growth performance in fish: a generalization of von Bertalanffy's theory of growth. *Berichte Der Deutschen Wissenschaftlichen Kommission Für Meeresforschung*, 28(4), 251–282.
- Petersen, T. A., Brum, S. M., Rossoni, F., Silveira, G. F. V., & Castello, L. 2016. Recovery of arapaima sp. populations by community-based management in floodplains of the Purus River, Amazon. *Journal of Fish Biology*, 89(1), 241-248.
- Queiroz, H.L. 2000. Natural history and conservation of pirarucu, *Arapaima gigas*, at the Amazonian varzea: Red giants in muddy waters. PhD thesis, University of St. Andrews, St. Andrews, Scotland.
- R Core Team. (2016). R: A language and environment for statistical computing. Vienna, Austria:

- R Foundation for Statistical Computing. Retrieved from <https://www.r-project.org/>
- Randall, D. J., Farrell, A.P., & Haswell, M. S. 1978. Carbon dioxide excretion in the pirarucu (*Arapaima gigas*), an obligate air-breathing fish. *Canadian Journal of Zoology*, 56(4), 977-982.
- Reid, S., Fox, M. G., & Whillans, T. H. 1999. Influence of turbidity on piscivory in largemouth bass (*Micropterus salmoides*). *Canadian Journal of Fisheries and Aquatic Sciences*, 56(8), 1362-1362.
- Smatresk, N. J., & Azizi, S. Q. 1987. Characteristics of lung mechanoreceptors in spotted gar, *lepisosteus oculatus*. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, 252(6), 1066-1072.
- Stevens, E. D., & Holeton, G. F. 1978. The partitioning of oxygen uptake from air and from water by the large obligate air-breathing teleost pirarucu (*Arapaima gigas*). *Canadian Journal of Zoology*, 56(4), 974-976.
- Tytler, P., & Calow, P. 1985. *Fish energetics: New perspectives*. Baltimore, Maryland: Johns Hopkins University Press.
- Wagner, G. N., Cooke, S. J., Brown, R. S., & Deters, K. A. 2011. Surgical implantation techniques for electronic tags in fish. *Reviews in Fish Biology and Fisheries*, 21(1), 71-81.
- Watson, L. C. 2011. *Ecology and conservation of arapaima in Guyana: Recovery of a giant fish in distress*.
- Winberg, G. G. 1956. Rate of metabolism and food requirements of fishes. *Journal of Fisheries Research Board of Canada Translation Series No. 194* (from *Intensivnost obmena i pischevye Petrebrosti ryg. nouchyne Trudy Belorusskovo Gosudarstvennove Universiteta imeni V.I. Lenina, Minsk*).

- Wood, S. 2006. *Generalized additive models: an introduction with R*. CRC Press.
- Wood, S. 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society*, 73(1), 3-36.
- Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. 2009. *Mixed Effects Models and Extensions in Ecology with R*. *Public Health* (Vol. 36).
- Zuur, A. F., Ieno, E. N., & Elphick, C. S. 2010. A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, 1(1), 3-14.
- Zuur, A. F., Ieno, E. N., & Freckleton, R. 2016. A protocol for conducting and presenting results of regression-type analyses. *Methods in Ecology and Evolution*, 7(6), 636-645.

Figures and Tables

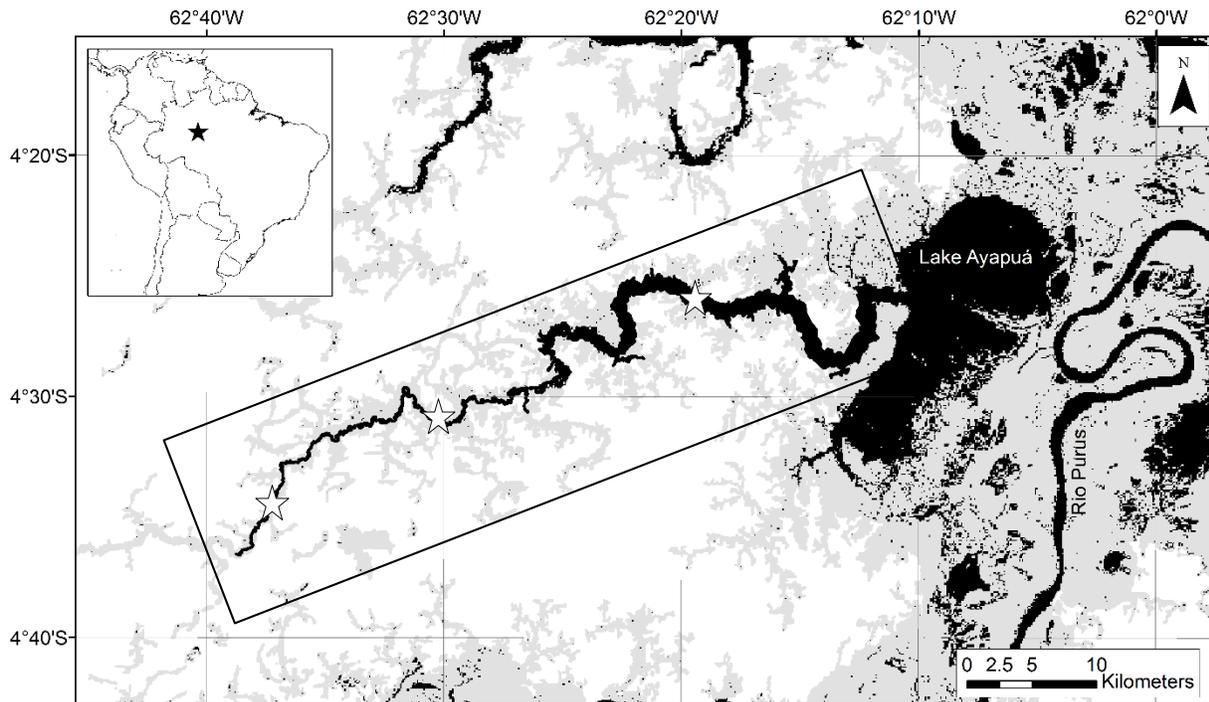


Figure 2.1. Lake Ayapúa (Purus River, Amazonas, Brazil) in dry (black) and flood (gray) seasons. Stars represent tagging sites, and the black box highlights the study area in the headwaters of the lake.

Table 2.1. Breathing classification abbreviations by breath type at time t and breath at time $t + 1$ for *Arapaima* sp. In Lake Ayapúa (Purus River, Amazonas, Brazil).

Breath at t	Breath at $t + 1$	Notation
Aggressive (a)	Aggressive (a)	aa
Aggressive (a)	Calm (c)	ac
Calm (c)	Aggressive (a)	ca
Calm (c)	Calm (c)	cc

Table 2.2. Description of physicochemical and breathing measurements collected from January to November 2015 in the Piagacu-Purus Sustainable Development Reserve.

Predictor	Description	Range	Mean \pm SD
Temperature	Mean of measures at 0, 2 & 4 m	22.8 - 35.5°C	27.7 \pm 1.79°C
Day of year	Day of calendar year from 01/01/2015	1 - 281	131 \pm 86.49
Time of day	Hour of day	8:03 - 21:43	12:30 \pm 2.90
Transparency	Water clarity from secchi disk	23 - 153 cm	92.4 \pm 22.57 cm
Total length	Total standard length of tagged fish	80 - 189 cm	103.77 \pm 18.83 cm
Type of breath	Identified by local classification system	Calm, Aggressive	--

Table 2.3. 95% confidence set of models examining the effect of total length and environmental variables on breathing interval in *Arapaima* sp. in Lake Ayapuá (Purus River, Amazonas, Brazil)

	Candidate models	df	Loglik	AICc	Δ_i	w_i	acc w_i	ER	Mar. R^2
1	s(TM) + TL	6	-113.7	239.9	0.00	0.22	0.22	1.00	0.084
2	s(TM)	5	-115.0	240.4	0.52	0.17	0.39	1.29	0.059
3	s(TM) + s(TD)	7	-113.1	240.9	1.00	0.14	0.53	1.57	0.074
4	s(TM) + s(TR) + TL	7	-113.6	241.8	1.98	0.08	0.61	2.75	0.083
5	s(TM) + s(TD) + TL	8	-112.6	241.9	2.08	0.08	0.69	2.75	0.078
6	s(TM) + s(DY) + TL	8	-112.8	242.2	2.39	0.07	0.76	3.14	0.093
7	s(TM) + s(TR)	6	-115.0	242.5	2.64	0.06	0.82	3.67	0.059
8	s(TM) + s(TD) + s(TR)	8	-113.2	243.1	3.21	0.05	0.87	4.40	0.072
9	s(TM) + s(TD) + s(TR) + (s(TD) × s(TM))	11	-110.1	243.7	3.80	0.03	0.90	7.33	0.105
10	s(TM) + s(TD) + s(TR)	9	-112.6	244.1	4.21	0.03	0.93	7.33	0.072
11	s(TM) + s(TD) + s(TR) + TL	9	-112.8	244.4	4.57	0.02	0.95	11.00	0.078

where the covariates temperature (TM), total length (TL), time of day (TD), day of year (DY), and transparency (TR) are described using the model degrees of freedom df , the log likelihood *Loglik* fit by maximum likelihood estimation, the Akaike Information Criterion for small samples *AICc*, the AIC between model i and the top ranked model Δ_i , the model weight w_i , the cumulative weights of models *acc w_i* , the evidence ratio (ER) of w_j / w_i where w_j is the weight of the top model (1) and w_i the weights of the consecutive models (2 - 11), and the marginal R^2 values *Mar. R^2* . The smoothing function s is denoted when utilized for selected covariates.

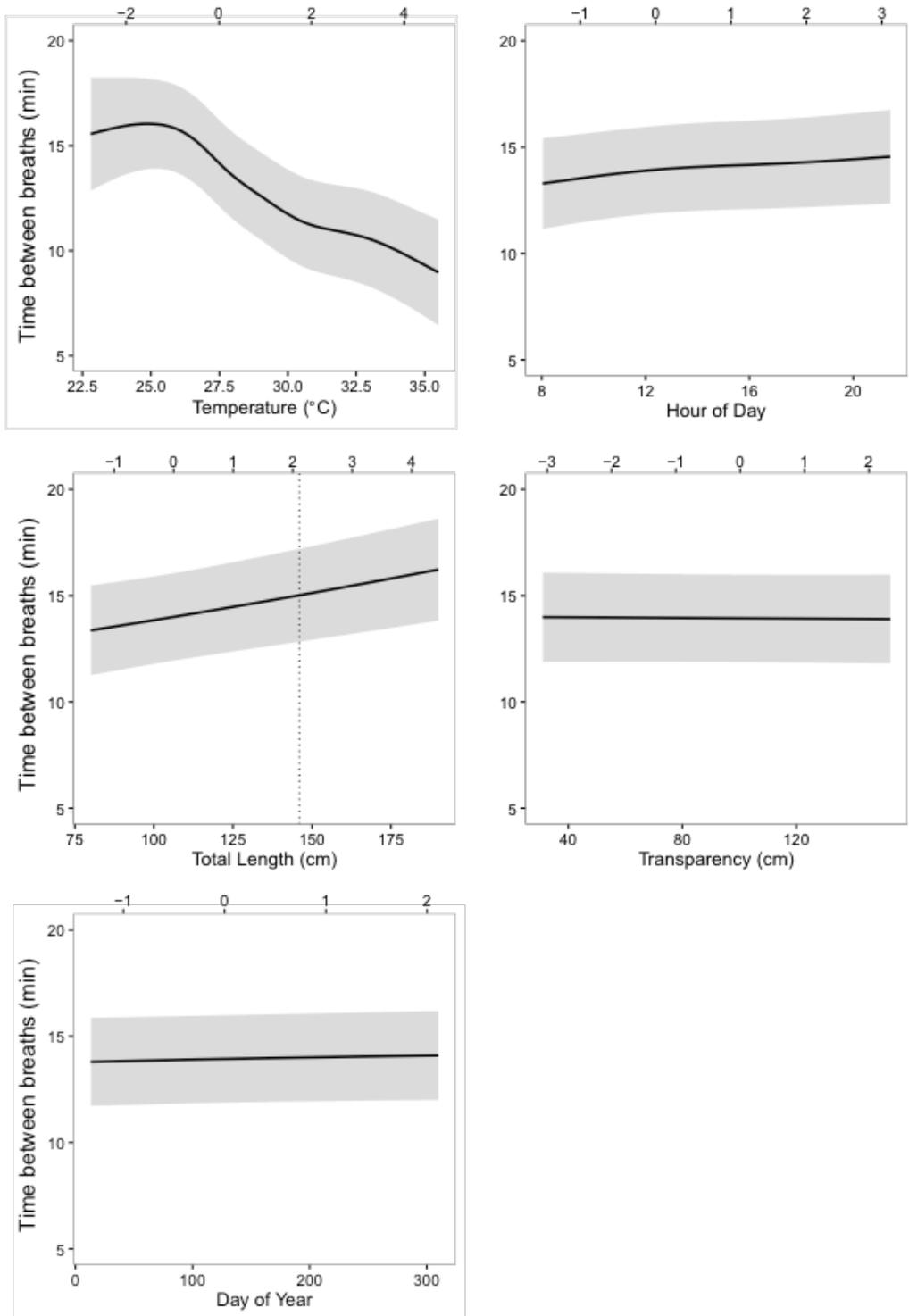


Figure 2.2. Model-averaged predictions of time between breaths (min) based on observations of each variable, with all other covariates at their mean values. Standardized values are shown on the top x-axis and non-transformed values on the bottom x-axis. Extrapolated predictions (i.e. beyond the observed range) appear to the right of the dotted line in the plot of total length. Shaded ribbon denotes 95% confidence interval.

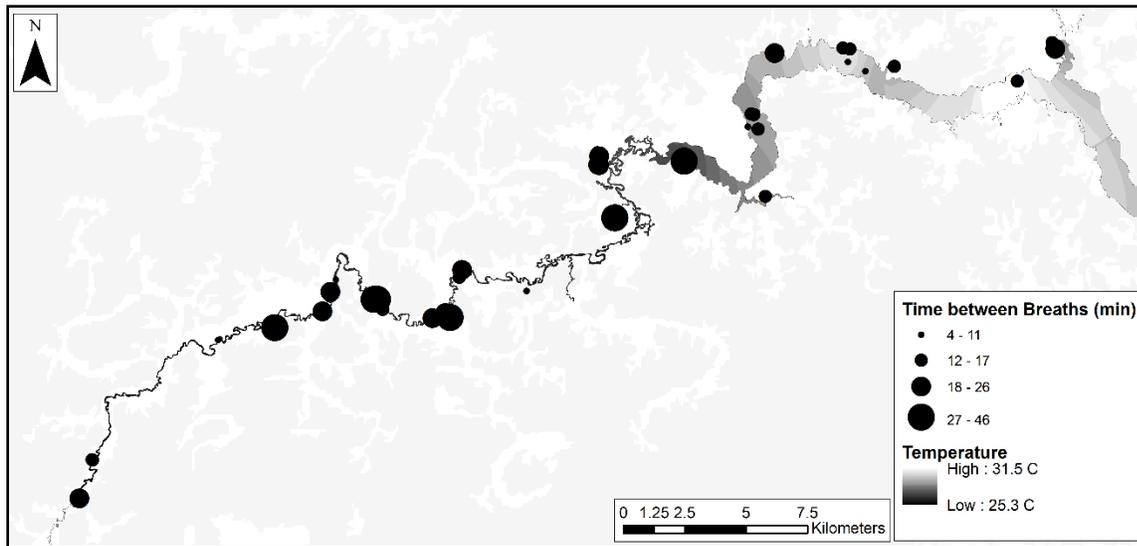


Figure 2.3. Lake Ayapuá study area with water temperature gradient (25 - 32°C) and breathing observations by time between breath, averaged for multiple fish in one location (proportional black circles) of *Arapaima* sp. (Purus River, Amazonas, Brazil). The temperature gradient was performed using an extrapolation of average water temperatures during the study.

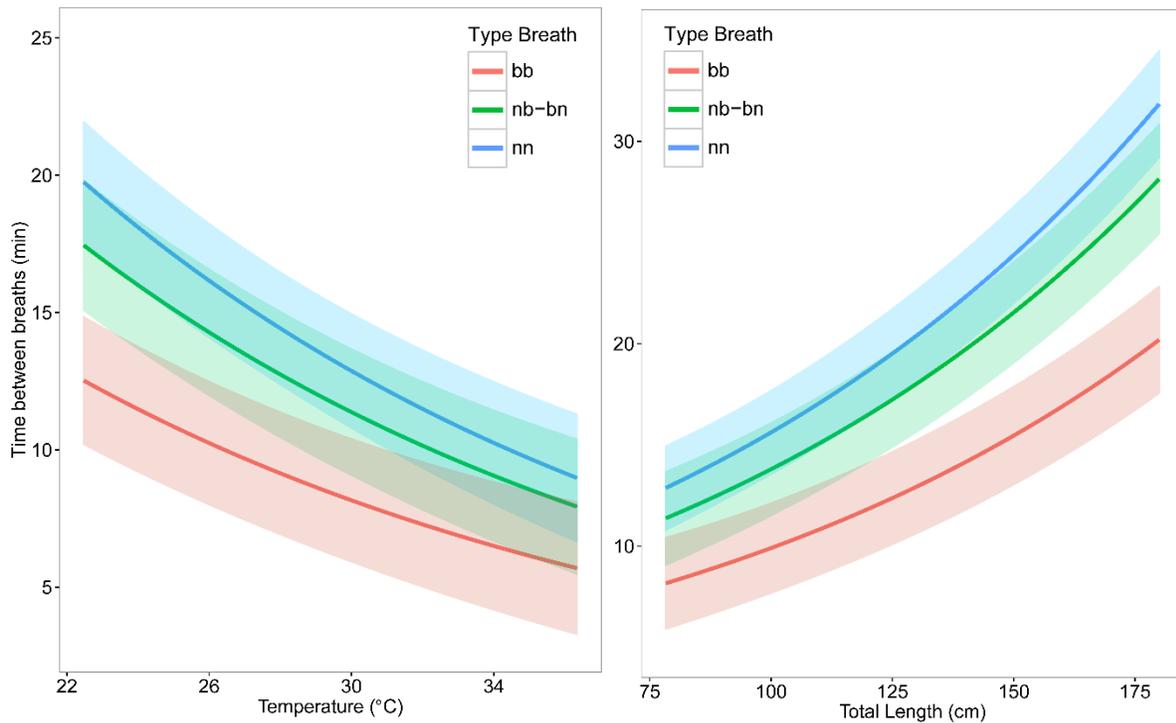


Figure 2.4. Predicted time between breaths (min), based on observed temperature and total length, by consecutive type of breath, where **aa** is *aggressive-aggressive*, **ac-ca** is *aggressive-calm* and *calm-aggressive* (combined due to extreme overlap) and **cc** is *calm-calm*.

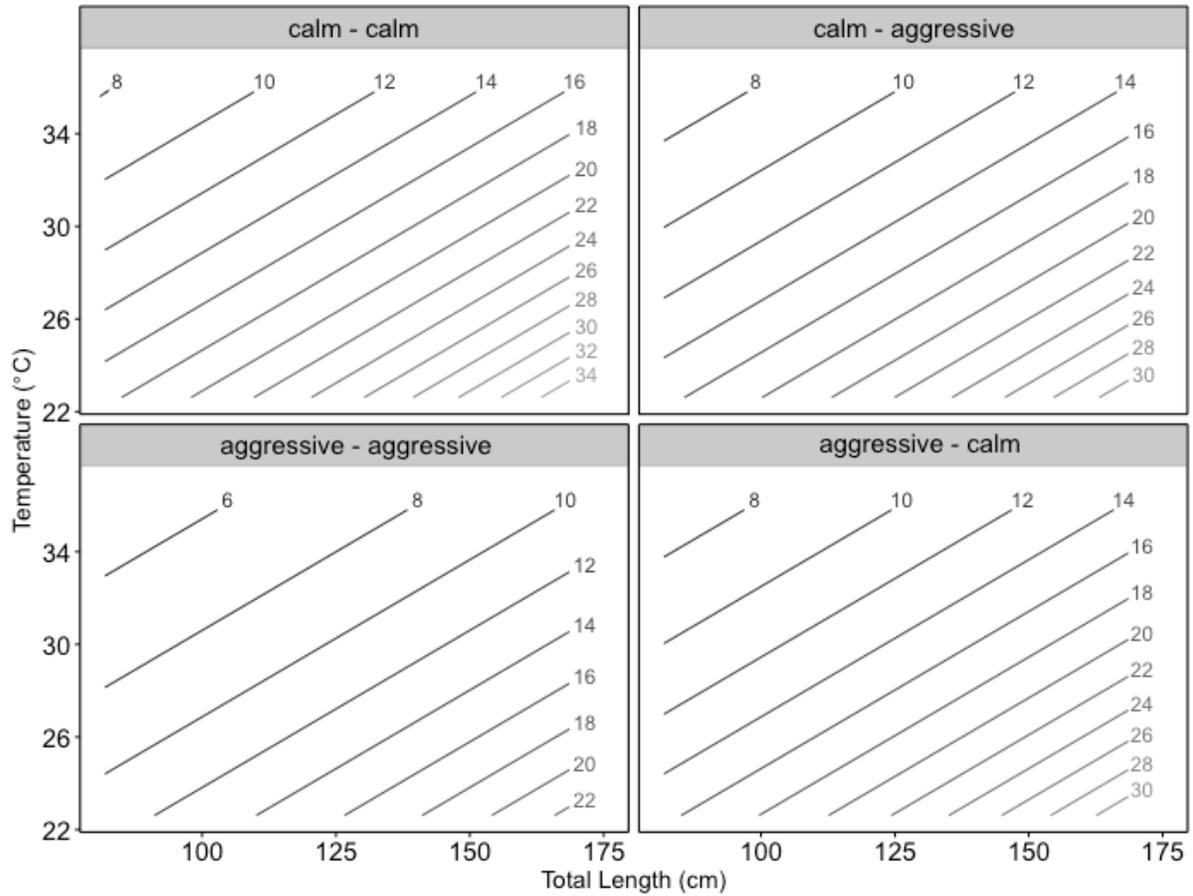


Figure 2.5. Predicted time between breaths (min, z = axis) based on observed values for temperature (°C) and total length (cm), faceted by breath type: **cc** is *calm-calm*, **ca** is *calm-aggressive*, **ac** is *aggressive-calm* and **aa** is *aggressive-aggressive*.

Appendix A

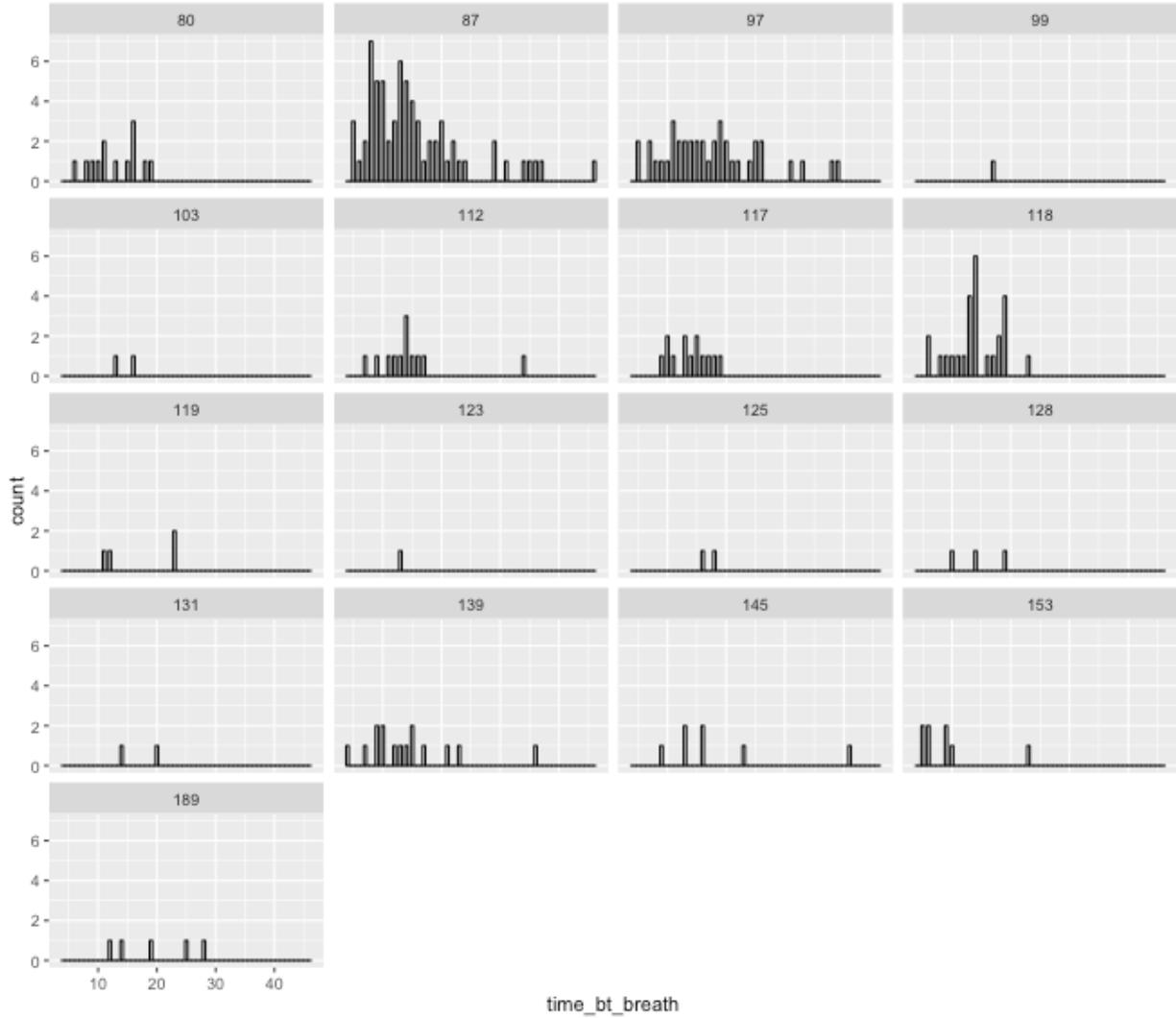


Figure A.1. Counts of breathing intervals by total length (cm) of arapaima for tagged fish in Lake Ayapuá (Purus River, Amazonas, Brazil).

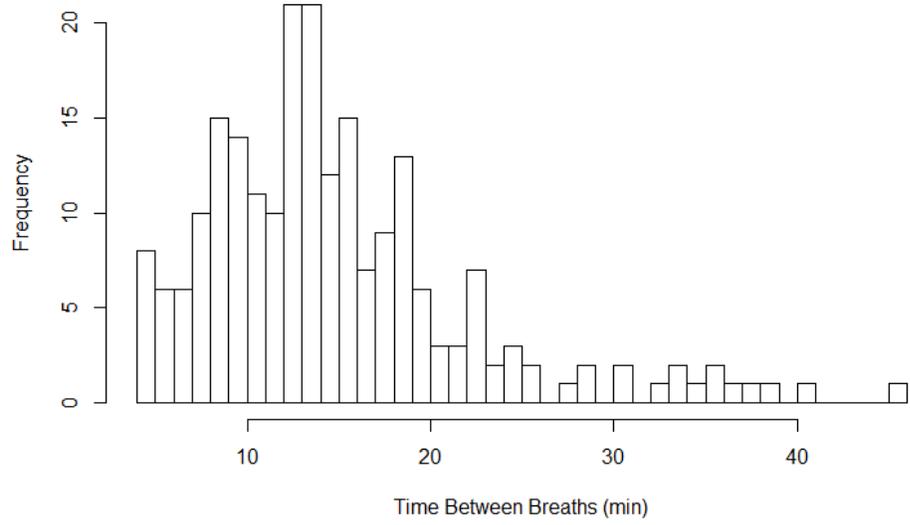


Figure A.2. Histogram of breathing intervals (n=281) for all radio-tagged fish in Lake Ayapuá (Purus River, Amazonas, Brazil).

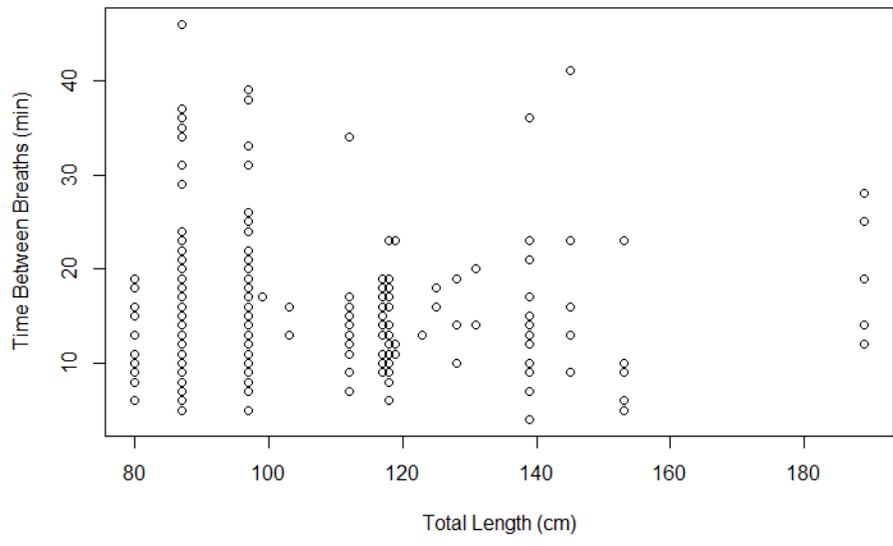


Figure A.3. Breathing intervals by fish length (cm) for all radio-tagged fish in Lake Ayapuá (Purus River, Amazonas, Brazil).

Chapter 3. Movement Ecology of *Arapaima* sp. in a Transitional River-Floodplain in the Amazon

Abstract

In dynamic river-floodplain ecosystems, fish move in response to changing environmental conditions, such as the annual rise and fall of water levels. In the Amazon, much remains unknown about fishes that move only short distances (i.e. sedentary fishes), typically between the river-floodplain and main channel. This study evaluated the movement ecology of the largest sedentary fish in the Amazon, *Arapaima* sp. This study examined relationships between movement patterns and environmental parameters (e.g. hydrology, depth) and body size for acoustic-tagged arapaima (n=15) in a transitional river-floodplain in the Amazon (Lake Ayapuá, Amazonas, Brazil). Generalized linear mixed models were used to evaluate the effect of environmental and size correlates on residency time, direction of movement, and movement rates. River flood stage was found to be the most important predictor of all movement responses. Total length, depth and habitat type correlates suggest that 1) larger fish spend more time in a given area and move faster than smaller fish, and 2) fish may spend longer in heterogeneous flooded forests than any other habitat type. Consecutive movements in the same direction were more likely to occur for upstream movements, whereas downstream movements were equally influenced by hydrology and previous movements. Arapaima were capable of using most of the available study area (up to 72%), swimming more than 300 km in a year, and using short burst movements (e.g. 4 km in 4 min). Such use of the full spatial and temporal heterogeneity of the river-floodplain adds to the framework of floodplain fish movement and provides useful information to inform management practices.

Keywords: arapaima, acoustic telemetry, fish movement, residency, river-floodplains

Introduction

Movement is one of the primary responses of fish to a heterogeneous availability of resources and the most common behavioral response to biotic and abiotic influences (Lucas & Baras 2001, Nathan et al. 2008). Because resources are not equally distributed or available across space and time, directed movements allow fish to minimize the cost of environmental changes while benefiting from richer resources and reduced competition elsewhere (Lucas & Baras 2001). The timing and probability of spatial and temporal movement patterns are influenced by physical, biological and environmental stimuli and individual factors (Rasmussen & Belk 2016). Factors such as time of day (i.e. light intensity) can influence movement to meet metabolic or refuge needs while other factors, such as hydrology, can influence movement patterns to increase reproductive success (Albanese et al. 2004).

Three descriptors of movement ecology are residency time (i.e. time spent in a given area), rate of movement (i.e. distance over time moved) and directional movement (i.e. probability of moving up or downstream given a previous direction). Some of the most important factors that influence freshwater fish movement are body size, time of day, habitat type and river flood stage. Body size influences movement primarily as a result of feeding ecology and reproduction (Beckman et al. 1998). For example, smaller fish may not have to move as far to find food, or larger, more mature fish may use directed movements to reach spawning grounds. Effects of the time of day can be evident in directional movements occurring differentially between day and night, and are associated with foraging behavior or predation avoidance (Nunn et al. 2010, Walsh et al. 2013). River flood stage can often provide cues to fish on optimal timing for initiation of movement, such as triggering fish movement at the start of water level rise (Lucas & Baras 2001). River flood stage and habitat type influence the timing, direction and rate

of fish movement as resource availability changes with seasonal water level fluctuations and fish move to maximize their fitness (Layman et al. 2009, Lyon et al. 2010).

These environmental and individual influences may result in fish spending more time in one location (residency events) to conserve energy, moving directionally to maximize resource use, or changing the rate at which they move. Residency time varies widely among freshwater fishes, with some fish spending only a few seconds in one location before moving (Greenberg 1991) while other species spend several months in a localized area without leaving (Hocutt et al. 1990). Similarly, body size and other environmental factors influence rate of movement, which can range from a several kilometers (Hocutt 1989) to more than 50 kilometers per day (Eiler 2000). Examining the influences on these metrics helps create a more complete understanding of fish movement patterns.

While freshwater fish movement has been studied in a wide variety of environments, fish movement ecology remains poorly understood in tropical river-floodplains. Previous studies provide a conceptual framework of migration patterns where fish closely followed water level fluctuations, (Winemiller & Jepsen 1998, Fernandes 1997, Fernandes et al. 2009, Castello 2008). The dynamic river-floodplain hydrological process that drives fish migrations in large rivers is best described as the “flood pulse” (Junk et al. 1989). Annual water level fluctuations of up to 10 m create a mosaic of seasonally flooded vegetation, temporary pools, channels, and meandering depressions that fish use as they become available (Junk et al. 1989). Fish optimize the heterogeneity of resources over space and time in river-floodplains through movement patterns called lateral migration (Castello 2008). Spawning occurs as floodwaters rise, and the newly inundated vegetation releases nutrients, increases production, and creates nursery habitat for larval fish (Bayley 1989). Young-of-the-year colonize the newly available floodplain and are

sustained by abundant food and lower densities of predators among the moving littoral zone (Bayley 1988). Fish move into increasingly available flooded forests and remain there during the highest water levels. As waters recede, fish move from the flooded forests to lower forest habitats or connected channels, and then become concentrated to fewer suitable habitats in lakes, pools or small channels during the lowest water levels (Welcomme 1979, Fernandes 1997, Castello 2008, Osorio et al. 2011). The factors that trigger fish movements and the metrics that describe such movements remain largely unstudied.

In order to fully understand fish movement ecology in tropical river-floodplains, it is necessary to examine fish movements in a river-floodplain with a natural hydrological cycle and native vegetation. One of the few remaining of such systems is the Amazon River basin. In the Amazon, there are three classes of migratory strategists: long-distance catfish migrants (i.e. catfishes that migrate as far as 8000 km from headwater rivers in the Andes to the mouth of the Amazon), floodplain migrants (i.e. characoid fishes that move several hundred kilometers between river-floodplains and river channels), and sedentary fishes (i.e. fishes that reside in floodplain lakes and migrate to adjacent floodplains) (Goulding 1980, Godinho & Kynard 2009, Duponchelle et al. 2016). According to existing studies, movements of sedentary fishes are typically restricted to movements from floodplain lakes to seasonally available floodplain habitats. Understanding and identifying the factors influencing sedentary fish movement is difficult, due to the small-scale and often subtle nature of such movement patterns, but important, in order to have a complete framework of fish migration in the Amazon.

One of the most economically and ecologically significant sedentary fishes in the Amazon is arapaima (*Arapaima* sp.). Despite their large size of up to 3 m and more than 200 kg, arapaima are considered a “sedentary”, or even “highly sedentary” species with no known long-

distance migrations and only short distance movements between habitats in close proximity (Nunez-Rodriguez et al. 2015, Hermann et al. 2016). Home ranges of arapaima were estimated to be areas of only 0.001 to 3.5 km in a floodplain lake (Nunez-Rodriguez et al. 2015). Unpublished reports describe directed movements of up to 50 - 80 km (Queiroz 2000, Crossa 2003, L. Castello, per. com.). Despite such observations, few studies examine the timing, influences and triggers of fish movement.

Arapaima spawning occurs at the beginning of the flood season, then fish begin to move to the flooded forest as it becomes flooded (Castello 2008). In high waters, males care for the young and may feed on the prey that inhabit flooded forests and floodplains (Queiroz 2000, Castello 2008). Juveniles with less than two months of age accompanied by paternal care, and young-of-the-year, use the aquatic macrophytes as an important nursery area, to feed and grow quickly, before water levels begin to fall (Castello 2008, Castello 2008b). During declining water levels, arapaima move to lakes and some connected channels where their populations become concentrated (Queiroz 2000, Castello 2008). Arapaima abundance and distribution in floodplain lakes have been positively related to lake depth and lake connectivity, possibly to increase survival, reproductive success and feeding potential during annual hydrological cycles (Arantes et al. 2013). Despite the widely variable river flood stage, arapaima have been found at nearly constant depths relative to the floodplain throughout the year, which suggests that they move to maintain a constant depth profile (Castello 2008).

The purpose of this study was to examine the ecology of arapaima movement and identify the factors that influence their year-round movements. To do this, we used three important metrics in movement ecology: 1) residency time, 2) probability of directional movements and 3) rate of movement. The objectives of this study were to identify the factors

that influence 1) residency events, 2) the probability of directed up or downstream movements and 3) the rate of movement of arapaima.

Methods

This study utilized acoustic telemetry to monitor fish movement in the transition zone between a river-floodplain and an upland lake in the Central Amazon region. Arapaima were tagged with electronic acoustic transmitters and fish movement was monitored using stationary acoustic receivers during a 12-month period. Environmental data, including water level and habitat type, were also collected and their influence was examined. Data were analyzed using generalized linear mixed models and candidate models were compared using the bias-corrected Akaike Information Criterion (AICc). Partial effect plots were generated to examine the influence of the strongest predictor variables.

Study area

This study was conducted in Lake Ayapuá, located within the Piagaçu-Purus Sustainable Development Reserve (State of Amazonas, Brazil) (Figure 3.1). Lake Ayapuá is a lake on the floodplain of the lower Purus River, which is a whitewater river characterized by high turbidity, high pH, high productivity, warm waters and abundant surface vegetation (Junk et al. 2012). The lake body is an oblong, shallow, turbid body bordered by seasonally flooded lowland vegetation (*várzea*). It is connected downstream to the Purus River and upstream to the lake's headwaters (Figure 3.1). When inundated in the flood stage, the lake's waters become more mixed with the contrastingly colder, less turbid and more acidic upstream headwaters. The lake headwaters, characterized by low biological production, are typical of blackwater ecosystems, (*igapó*), such

as that of the Rio Negro (Gourou 1950, Junk et al. 2012). The headwaters of Lake Ayapuá form a narrow valley (*ria*) lake with upland habitat (*terra firme*) on both sides of the main channel and many connective side channels (*igarapes*). All data in this study were collected in the lake headwaters (*cabeceira*), from the mouth of the headwaters to approximately 85 – 110 (dry – flood season) kilometers upstream between 10 November 2014 and 15 November 2015 (Figure 3.1).

Fish tagging

Eighteen arapaima [(Total length (TL) = 121 ± 22 cm) (Range = 83 - 170 cm)] were captured using a seine net (140 m x 6 m) in pools of the headwaters of Lake Ayapuá in November 2014 (Figure 3.2). Capture locations were selected based on high densities of arapaima, which increased the chance of a successful capture. Upon capture, arapaima were placed in a recovery canoe filled with lake water to rest and were monitored until air-breathing intervals normalized to approximately every 5 - 15 minutes. Release of each fish occurred at approximately the same location as where it was captured (Figure 3.2).

Two tags were placed on each fish: one acoustic tag was inserted surgically and one anchor tag was inserted externally. Surgical tag implantation was completed following standardized tagging procedures (Wagner et al. 2011). Two to four scales were removed over the peritoneal cavity anterior of the anal fin and a local anesthetic (topical benzocaine 20%) was applied. A uniquely coded acoustic transmitter (Vemco V13 - 69kHz) was inserted into the peritoneal cavity through a 2 - 3 cm incision. Incisions were closed using 2 - 3 interrupted sutures of absorbable monofilament synthetic surgical sutures. According to the discretion of the lead tagger (E. G. Martins) and based on the degree of incision exposure, some fish had 1 - 2

scales glued over the incision using Vetbond™ tissue adhesive. An external anchor tag with a unique fish identification number for each fish was inserted proximal to the base of the dorsal fin. After tagging, each fish was monitored for 5 - 10 minutes until it regained sufficient strength to swim away, at which point it was released. All capture and tagging protocols were approved by the International Animal Care and Use Committee (IACUC #14-085) at Virginia Polytechnic Institute and State University and the Sistema de Autorização e Informação em Biodiversidade (SISBIO #6289511) in Brazil.

Acoustic telemetry

Twenty acoustic telemetry receivers (model VR2W-69kHz, Vemco, Canada) were deployed in the headwaters of Lake Ayapuá in November 2014 (Figure 3.2). The single-channel, omni-directional receiver units recorded time, date and identity of animals fitted with acoustic transmitters that swam within range of the unit. Receivers were installed using a paired array (n = 5 pairs) when river width was greater than 100 m and a single array when less (n = 10). Each receiver was held in place by a 42 kg cement anchor and secured to a polypropylene mooring line approximately 1 m above the anchor with the hydrophone pointing up. Receivers were held vertically in place with 4 - 5 circular buoys attached to the mooring line 1.5 m above the receiver and a large surface buoy for retrieval and identification.

Receivers were fully submerged and continuously monitored the environment for the presence of uniquely coded transmitter signals within a radius of about 300 - 500 m, which we estimated based on range tests conducted in the area. Receivers were checked and maintained monthly for vegetative growth and sufficient depth. Data were manually downloaded from all receivers via Bluetooth transmission every other month and receivers were replaced in the same

location after a successful download. One receiver was relocated from a midstream point to a further point upstream in March and another was relocated near the mouth of the lake headwaters in July after it was run over by local boat traffic. All twenty receivers were retrieved in working condition at the conclusion of the study in November 2015.

Two sentinel tags (Vemco range tag 69kHz) were used to test whether receiver detection was constant for the duration of the study. The tags were configured with a fixed delay signal (10s) and deployed within 50 - 70 m of the most upstream (n tags = 1) and downstream (n tags = 1) receivers. Linear mixed effect models of the influences of flood stage and time of day on hourly receiver detections of the sentinel tags revealed that there was no significant difference between detection efficiency in dry and flood stages, nor between night and day (Figure 3.3). Such results indicated that any seasonal or daily variability in fish detections was therefore a reflection of fish activity patterns rather than changes in detection efficiency.

Data preparation

The raw detection data were prepared for analysis by removing false positive detections, aggregating receivers into regional zones, calculating distances between zones and rates of movement, and aggregating time spent at receivers for residency analysis. False-positive detections were removed from the dataset using the Vemco VUE False Detection Analysis (FDA) tool. Detections were considered valid if they were part of a sequence of at least two detections over a period of 60 minutes. The time series of detections for each fish were then visually checked and assessed for realistic spatiotemporal order of the movements (Beeman & Perry 2012).

Next, receivers were aggregated into zones, or regional areas where 1 or 2 receivers occurred. Any area with a paired receiver array was considered to be a single zone. All areas with only a single receiver were considered a separate zone. This resulted in sixteen unique zones in the study area (Figure 3.2). The distance between each zone was calculated as the shortest possible route that a fish could travel between zone midpoints given the available aquatic area. Distances were calculated for the flood and dry seasons using dual-season satellite imagery and geospatial measurement tools. The minimum distance between zones 1 and 16 in the dry season was 110.8 km and 85.3 km in the flood season. Daily distances were calculated as the sum of distances between locations at time t and time $t-1$. Movement rates for each fish were calculated as the total distance moved between zones (km) per day.

Fish detections were used to compute the amount of time (i.e. residency) spent at each zone. A residency event was initiated wherever a fish was detected at least two times at the same zone without being detected at a different zone within a period of 60 minutes. The duration of each residency event was calculated as the time (minutes) between arrival and the last time the fish was detected. Residency events were terminated whenever the time elapsed between consecutive detection was greater than 60 minutes or if the fish was detected in another zone. Residency events were classified by time of day (Night = 6p-6a, Day = 6a-6p) and flood stage (below). If the residency event fell within two or more levels of time of day or flood stage, the level that accounted for the most time spent at the zone was used.

Environmental data collection

Environmental data on depth, water level, river flood stage and habitat covariates were collected to examine their influence on movement. Depth measurements were collected in

November 2014 during receiver deployment and mean depth was calculated as the mean depth per zone. Depth was used as a relative measure between zones and not recalibrated to account for weekly water level changes. A data logger was used to record absolute pressure data for water level calculations (Onset HOBO Water Level Logger Series U20L-01). The logger was enclosed in a protective housing and deployed 100 m from shore in January 2015 (Figure 3.2). Due to the fact that the study began two months prior to data logger deployment, water level data for the first two months of the study was extrapolated from meteorological data for the study area (Agência Nacional de Águas 2016). Water level was calculated using pressure data from the Instituto Nacional de Meteorologia's weather station nearest to our study area (Beruri, Purus River, Amazonas 2016). Water level measurements from this location were used to calculate mean water level per week for the whole study area. A categorical variable for flood stage was calculated using water level data and visual observation of the hydrograph, where *Flood* = Apr-Jun, *Falling* = Jul-Sep, *Dry* = Oct-Dec, *Rising* = Jan-Mar.

Vegetative cover data from 100 m, dual-season satellite imagery were used to extract habitat class proportions per zone (Hess et al. 2015). The 100 m imagery data produced twenty habitat classes that reflected unique combinations of vegetative cover during the flood and dry season (Hess et al. 2015). For the purpose of this analysis, only classes that contained seasonally flooded potential fish habitat were included (green color, Figure 3.2). All other pixels (i.e. areas not available for fish during the flooded season) were excluded (tan color, Figure 3.2). Available habitat for each zone was considered to be all pixels within a 600 m radius around a receiver. In zones with two receivers, the total number of pixels in the radius around both receivers was considered. The total number of pixels of each habitat class were summed and used to calculate proportion of each habitat class per zone. Due to the high likelihood of correlated habitat

variables, a principal component analysis (PCA) was conducted using twelve vegetation classes to create orthogonal synthetic variables. Vegetation classes included open water, herbaceous, aquatic macrophytes, shrub, woodland and forest, with non-flooded and flooded categories of the latter three. Eigenvectors accounting for >50% of the observed variation were used as predictor variables in the analysis.

Data preparation

Data exploration included the examination of Cleveland and box-and-whisker plots to identify outliers and influential points (Zuur et al. 2010). Variance inflation factors were examined to check for collinearity, and variograms were used to assess spatial and temporal autocorrelation in model residuals; however, no collinearity or correlation issues were found and we proceeded with model selection.

Statistical modeling of residency events

To assess if there was a relationship between the time of residency events and environmental, temporal and habitat variables, generalized linear mixed models (GLMMs) were used. GLMMs allow for a linear model structure with the inclusion of random effects to account for correlation across repeated measures on the same subject (Bolker et al. 2009). Predictor variables corresponding to each zone (e.g. habitat, depth) were selected based on prioritization of the most likely influential characteristics on the zones. The following independent variables were selected for inclusion in the global model: flood stage (flood, falling, dry, rising), time of day (night, day), depth (m), habitat (PCA variables), and total length at the time of capture (cm),

based on *a priori* justification. All continuous variables were standardized prior to inclusion in the model to facilitate comparisons of the effect sizes of each variable (Schielzeth 2010).

All models included random effects for fish ID and zone to account for unexplained individual fish and zone effects. The model was fitted assuming a Gamma distribution and log link function (Zuur & Ieno 2016). The model has the following form:

$$\begin{aligned} \text{Residence}_{i,j,k} &= \text{Flood Stage}_{i,j} + \text{Total Length}_i + \text{Time of Day}_{i,j} + \\ &\quad \text{Habitat}_{i,j,k} + \text{Depth}_{i,j,k} + \gamma_i + \gamma_k \\ \gamma_i &\sim N(0, \sigma_i^2) \\ \gamma_k &\sim N(0, \sigma_k^2) \end{aligned} \tag{1}$$

where $\text{Residence}_{i,j,k}$ is the j th residency time (hours) per residency event for individual i at zone k , γ_i is the random deviation from the intercept for individual i , and γ_k is the random deviation from the intercept for zone. Both γ_i and γ_k were assumed to be normally distributed with mean 0 and variance σ_i^2 and σ_k^2 , respectively (Zuur & Ieno 2016). Models were validated by plotting residuals against fitted values and by inspecting semivariogram plots (Zuur et al. 2009).

Modeling directional movement

Upstream and downstream movements were examined to investigate the strongest predictors of the direction of movement, which was considered to be the state of the fish. Two binary time-series were created of weekly movement for each fish, one to show upstream movements and the other to show downstream movements. Movements were likely to be correlated over time; that is, a fish moving in one direction at week $t - 1$ was more likely to be detected moving in the same direction the next week at time t . Thus the binomial response variable was defined as the state (S) of an individual fish at week t denoted when a fish was detected moving upstream (1) or a fish was not detected moving upstream (i.e. residency in a zone, moving downstream, or not detected) (0). The time-series were analyzed by using a first-

order Markov process for fish movements and a binomial GLM with one of the predictors being the state at $t-1$ (Bestley et al. 2010).

The following covariates were selected for inclusion in the global model: state at $t - 1$ (0,1), flood stage (flood, falling, dry, rising), rate of water level change, and total length of each individual fish (cm). Rate of water change was calculated as the average rate of increase or decrease in water level, aggregated by week to normalize variation in daily fluctuations. All continuous variables were standardized prior to inclusion in the model to facilitate comparisons of the effect sizes of each variable (Schielzeth 2010). The model has the following form:

$$\begin{aligned} \text{State}_{i,t} &= \text{State}_{i,t-1} + \text{Flood Stage}_{i,t} + \text{Total Length}_i + \Delta \text{Water Level} + \gamma_i \\ \gamma_i &\sim N(0, \sigma_i^2) \end{aligned} \quad (2)$$

where $\text{State}_{i,t}$ is the state of individual i at week t , $\text{state}_{i,t-1}$ is the state of individual i at week $t - 1$, and γ_i is the random deviation from the intercept for individual i , assumed to be normally distributed with mean 0 and variance σ_i^2 (Zuur & Ieno 2016). Analyses were conducted separately for both a) upstream and b) downstream movements.

Modeling movement rates

Generalized linear mixed models (GLMMs) were used for analysis of movement rates while accounting for repeated observations of the same individuals (Zuur 2009). The following covariates were selected for inclusion in the global model: flood stage (flood, falling, dry, rising), time of day (night, day), rate of water level change, and total length of each individual fish (cm). The same procedures for the treatment of distribution, random effects, continuous variables and model visualization that were used in the analysis of residency events (model 1) were used here. The model has the following form:

$$\text{Rate}_{i,t} = \text{Flood Stage}_{i,t} + \text{Total Length}_i + \text{Time of Day}_{j,t} +$$

$$\begin{aligned} & \Delta \text{Water Level}_{j,t} + \text{Direction}_{j,t} + \gamma_i \\ \gamma_i & \sim N(0, \sigma_i^2) \end{aligned} \tag{3}$$

where $\text{Rate}_{i,t}$ is the movement rate for individual i at time t (km/day), γ_i is the random deviation from the intercept for individual i , which is assumed to be normally distributed with mean 0 and variance σ_i^2 (Zuur & Ieno 2016).

Model selection and averaging

All possible subsets of the global model were fit to the data and ranked by second-order AICc values using package “MuMIn” (Barton 2016). All models included in the 95% confidence set for the best model were used for generating model-averaged predictions and unconditional 95% confidence intervals (Burnham & Anderson 2002). The “zero method” was used for model averaging, which assumes the variable is included in every model but sets coefficients to 0 if not (Grueber et al 2011). Data for each prediction were generated using the minimum and maximum observed values for the predictor of interest while holding all other values at a constant mean. Relative variable importance (RVI) was calculated using the sum of model weights where each variable was found (Symonds & Moussalli 2011).

Results

Fifteen of the tagged fish (83%) were detected between 20 November 2014 and 15 November 2015 by a total of 5812 detections. The remaining tagged fish ($n = 3$) may not have been detected during the study due to use of areas outside the receiver range, mortality, or tag malfunction. Of those fish detected during the study, two-thirds were detected within the first month and all but one fish were detected within the first eight weeks. Individual fish were detected at 15 of the 16 receivers, with no fish ever being detected at the furthest upstream

receiver. Water level peaked at 17.9 m (flood season) on 20 June 2015 and dropped to its lowest (dry season) at 4.9 m on 27 October 2015 with an amplitude of 13 m. A total of 249 residency events in a given zone were identified from 13 individual fish, with a mean of 45 residency events per fish (Table 3.2). Median time of residency events at a given zone was 7.2 minutes; the mean was 23.4 minutes (Range = 2 min - 5 hrs). The longest single residency time of 5 hours occurred with the largest fish in the study (TL = 170).

Overall, the median rate of fish movement was 5.9 km/day and the mean was 14.7 km/day (range = 0.03 - 90.3 km/day) (Table 3.3). However, the amount of time elapsed between movements is important to consider, as the median was 6 minutes, and the mean was 21 minutes (range = 1 min - 5 hours). Mean rate of movement was 7.3 km/day faster for short bursts of movement (< 3 min) than longer time between movements (>15.6 min) (Table 3.3). Mean rate of movement that occurred over a timeframe greater than 1 hour was 12.7 km/day. All movements greater than 30 km/day (n = 32) occurred in the dry season between Sept – Nov 2015. The maximum movement rate for all fish occurred at the end of the dry season (Sept 2015, TL = 129). Fish were observed moving 21 km in 2.4 hours, 33 km in just under 1 hour, and even an estimated 25 km in 10 minutes, although it is possible that this fish took a shortcut in the area where distances were calculated. Total distance moved by fish during the study ranged from 8.18 km to 326 km (mean = 105 km \pm 103 km) (Table 3.3). The percent of total area in which fish were detected ranged from 11.5 to 72.3%, with a mean of 48% (Table 3.4). Based on the paired receiver design and estimated detection ranges, it can be assumed that that no fish crossed into the lake body, nor did they move farther upstream than the most upstream receiver. No tagged fish was detected back in the same location as where it was tagged one year prior. Of the seven

fish that were detected one year after tagging (± 2 weeks), they were found as far as 38 km downstream and 51.6 km upstream of where they were tagged (Table 3.5).

Time per residence event

The most influential variable in predicting residency time for arapaima was flood stage (Table 3.7). Total length, depth and habitat also showed support; however, relative variable importance (RVI) indicated that flood stage (RVI = 0.90) was almost three times as important as total length (RVI = 0.32) and depth (RVI = 0.30), and four times as important as habitat (RVI = 0.24). The corresponding PCA results for habitat was strongly positively correlated (threshold = 0.80) to flooded forest, and negatively correlated (threshold = - 0.80) to open water and herbaceous and aquatic macrophytes (Table 3.6).

Time per residence event was the longest during months of rising and falling waters and the shortest during months in the dry and flood season (Figure 3.4). Time per residence events had a positive relationship with total length, where larger fish (TL = 150 cm) were predicted to spend on average approximately 5 minutes longer in a zone than smaller fish (TL = 100 cm). Depth was also positively related to residence time, suggesting that fish spend longer in areas of deeper average depths (Figure 3.4). Time of day was not included in the 95% confidence set for the best models, indicating that it has little to no direct influence on the amount of time a fish spends in a given zone.

Directional movement events

Models for examination of upstream and downstream movement suggested there was a difference in the factors that influenced directional movement. The previous state predictor

variable was selected for in all models for both directions of movement, supporting that if a fish was already moving in one direction, the probability that it would sequentially be detected moving in the same direction was high. Both downstream and upstream movements were highly influenced by flood stage, where a negative relationship was strongest in the flooded and falling stages (Table 3.8, Table 3.9). Models selected in downstream movement analysis indicated that flood stage and previous state were of highest importance (RVI equal to 0.98 in both cases), while change in water level (RVI = 0.29) and total length (RVI = 0.30) had slightly lower, but nearly equally important, support from the data (Table 3.8). However, upstream movement models showed the strongest support for the previous state (RVI = 0.95) and less support for flood stage (RVI = 0.53), with a small support for water level change (RVI = 0.33) and total length (RVI = 0.25) (Table 3.9). Such results suggested that upstream movements were best predicted only by the previous state while downstream movements were predicted equally as well by flood stage as using the previous state.

Rate of Movement

Flood stage, selected for in all models, was the most important predictor of movement rate (RVI = 0.96) (Table 3.10). Movement rate in the dry and flood seasons were predicted to be two to four times greater than that in the rising and falling stages (Figure 3.5). The difference between rate of movement during rising and falling seasons was estimated to be negligible. The models also show strong support for direction of movement (RVI = 0.75). Upstream movements were a slightly stronger predictor of rate of movement than downstream movements. Weekly rate of water level change (RVI = 0.62) showed a negative relationship with movement rates. Mean movement rates were predicted to be approximately 1.2 km/day faster with a water level change

of -10 cm/week than 10 cm/week (Figure 3.5). Movement rate was positively related to daylight hours (time of day) and total length. Time of day was selected for in half of the 95% confidence set (RVI = 0.47), which provides some evidence that fish move slightly faster at night than during the day. Total length had the weakest support from the data (RVI = 0.14) and predicted rates were only a small magnitude greater (< 0.1 km/hr) with a fish size increase of 70 cm.

Discussion

This study's findings on fish movement in river-floodplains contribute to understanding how fish maximize use of available resource within a spatially and temporally heterogeneous landscape. Previous studies have shown that as water levels rise, fish move to the newly inundated floodplains and use the of floodplains during the flooded season, then fish move back towards the main channel when waters recede (Saint-Paul 2000, Welcomme 1979, Fernandes 1997, Castello 2008). This study provides some of the first evidence that fish use the full extent of river-floodplains. The results of this study showed that: 1) river flood stage was the driving factor for predicting rate of fish movement and time spent in a given area (residency), 2) directed and nonrandom movements suggest a strategize to fully utilize the river-floodplain, 3) larger fish were more likely to spend more time in areas with deeper, heterogeneous habitats associated with flooded forests, and 4) arapaima were capable of using a large proportion of the available study area with short burst movements. To the knowledge of the author, no studies have used acoustic telemetry to assess residency, movement rates and directional fish movements in a river-floodplain with a natural hydrological regime and native vegetation. Such findings are essential to the understanding of fish ecology, as they provide insight of fish movement in natural systems, which can then be applied to modified systems.

Hydrological influence on fish movement in river-floodplains

Results of this study support the notion, as shown by previous studies, that hydrology is the dominant factor influencing fish movement in river-floodplains (Welcomme et al. 1979, Fernandes 1997, Castello 2008). This was determined from differences in the effect of each flood stage on residency, movement rate and directional movement and the importance of flood stage in all models. Residency times were longer in the rising and falling flood stages and shorter in flood and dry stages, while movement rates were higher in flood and dry stages and lower in rising and falling flood stages (Figure 3.4, Figure 3.5). In the dry season, when channels and available habitat are more restricted, fish could be expected to have to move farther and faster (i.e. faster movement rates) than in other seasons to reach any location. Movement rates were also high in the flood season (Figure 3.5). Inundation of the floodplain allows for increased access to habitats across the landscape. Possible explanations for faster movement rates in the flood season include that fish would be able to travel widely throughout the newly flooded landscape or that optimal habitats may be farther from the main channel during this time of year.

Longer residency times during the rising and flooded stages may be due to changes in resource availability. Rising water levels trigger biological production and increased primary production supports the growth of prey species (i.e. food resources) (Junk et al. 1989). Thus, it is likely that fish would not have to move as far or as fast to prey on food resources. Fish may take advantage of spending more time in the interterrestrial zones (i.e. edge of water and land) as waters rise and nutrients become easily accessible. Longer residencies in the falling stage may have been more related to a metabolic response to the changing environment. Water level change occurred faster in the falling stage than in the rising stage (Figure B.8). Energetically, it would be

less costly to remain in a location when the rest of the environment is rapidly changing from water levels falling 6-12 cm or more per day.

Non-Random Movements

This study also demonstrated that direction of movement patterns was not random. Upstream movements were best predicted by the previous state, whereas downstream movements were equally influenced by the previous state and flood stage (Table 3.8, Table 3.9). As waters rose, fish moved up and downstream, possibly to maximize the use of available resources during the flood, whereas as waters receded, they mostly migrated downstream because upstream habitats became too confined for suitable habitat. Fish move in order to maximize resource use (Lucas & Barras 2001); therefore, the difference in predictors for upstream versus downstream movements was likely due to changing resource availability. Based on the results, it is likely that once fish began searching for newly available habitat and new resources, they continued searching as habitats became available. Consecutive upstream movements in rising and flood stages support biological functions occurring this time of the year (e.g. reproduction, care for young) (Castello 2008). Consecutive downstream movements, predicted by both flood stage and previous state, were more likely to occur as waters recede (Table 3.8). These results are consistent with many other species known to use downstream movements in response to hydrology and decreasing water levels (Montgomery et al. 1983). Falling water levels restrict upstream and floodplain habitats, and in turn, fish begin to move towards the channel (Louca et al. 2009).

Influence of other predictors of movement in river-floodplains

Habitat, depth and body size were several of the other factors that predict fish movement patterns, though their influence was relatively small. Regardless of time of year, fish spent longer in areas with a greater proportion of flooded forest habitat. Fish are known to migrate to flooded forests as it becomes available in the high water season, and stay there for an extended period time, longer than any other habitat during the year (Castello 2008). Flooded forests support high productivity as the canopy trees release fruits and seeds and increased nutrients support higher levels of primary productivity (Goulding 1980). Arapaima were more likely to spend time in these habitats where food was abundant. In contrast, there was a negative association of residency to macrophytes and open water (**Error! Reference source not found.**). There also was a correlation between macrophytes and open water, which may be an indication that macrophytes were only found in the open water areas at the intersection of side channels and the main channel. Open water, due to a lack of structural and biological diversity, may not support optimal foraging, refuge or reproductive activities. Because the bulk of food sources for fish are near structural vegetation, fish are expected to be more often found in the flooded forests and structurally complex habitats. (Meleck & Forsberg 2001). As such, fish were never detected for long periods of time in zones with open water. Several studies provide evidence that arapaima use macrophytes for cover and feeding in floodplain lakes and that areas with greater proportions of macrophytes support more fish (Arantes et al. 2011). However, other studies note that adult arapaima are not typically found in macrophytes and instead seek prey species in the flooded forests (Querioz 2000, Richard in prep). All fish in this study were considered to be sub-adults or adults and as such, further supports the negative relationship to macrophytes.

Second, fish were more likely to spend more time in deeper areas (Table 3.7). Arapaima abundance has been positively associated with pools and deeper lakes as well as lakes with high connectivity to adjacent water bodies (Arantes et al. 2013). This may be to locate food resources, increase reproductive success, or seek refuge in deeper areas of the channel (Arantes et al. 2013).

Third, body size had a positive relationship with residency time and rate of movement (Figure 3.4, Figure 3.5). Larger fish displayed faster, directed movements and in turn, spent longer in the intermediate locations between movements. Another possible explanation is the inverse relationship of body mass and metabolism in teleost fish, which suggests that larger fish could spend more time in a given location between movements without having to search for forage (Clarke & Johnston 1999). Finally, it is important to note that while habitat, depth and body size all contribute to movement patterns, some unexplained variability is likely attributed to the complexity of the ecosystem and individual fish variation.

Movement ecology of sedentary fishes in the Amazon

Traditionally, many stream fishes were considered to be sedentary, according to the restricted movement paradigm (Gerking 1959). However, since then, this idea has largely been discarded by later studies. A similar concept has been applied to lakes in river-floodplains, assuming that fish that are found in lakes must be sedentary and do not move long distances. The concept that many fish in habitat floodplain lakes have restricted movement reinforces the notion of the restricted movement paradigm. In line with the notion that arapaima are considered to be sedentary, a previous study found arapaima to move only up to 30 km in 18 months (Nunez-Rodriguez et al. 2015). However, unlike the restricted movement paradigm's application in Amazonian lakes, this study found that arapaima move can more than 300 km in 12 months

(Table 3.3). This suggests that arapaima move during the whole year, as movements were detected year-round, not just between lakes or seasons. Such results are also a result of arapaima using nearly the full extent of the available habitat (72% of the study area). The results herein showed no evidence of homing or natal homing, as one year later, fish were found far from their tagging location (upstream 38 km to downstream > 50 km) (Table 3.5). Due to the fact that no fish was found to move past the farthest receiver or cross into the lake it is likely that arapaima home ranges would be confined to the areas where fish were detected and their connective floodplains. The disparity between our findings and previous estimates of arapaima home range (0.001 - 4 km²) is likely due to either a different species of arapaima or a very different habitat type (Nunez-Rodriguez et al. 2015).

Movements within our study area commonly occurred as short, burst movements. No sustained long distance movements were observed. Arapaima were capable of swimming quickly (4.1 km/4 min, up to 33 km in one hour; Table 3.3) but only over short time periods. This is a common attribute of elongated fishes with truncate caudal fins (Lighthill 1971). Burst movements were more commonly observed as fish moved upstream, perhaps a result of movements to seek out specific resources or as a result of swimming against the current. Such findings of arapaima movement patterns suggest that arapaima are capable of using the full extent of habitat to maximize resource use, and do so in a way that is most energetically efficient.

Conservation Implications

While home ranges were not explicitly determined for this study because movement in the side channels of the river-floodplains, movement within the channel of over 300 km in one

year provides support that these fish indeed use the full extent of available habitat. In this ecosystem, the home range of arapaima is much larger than previously thought and is important to consider in terms of use of total available habitat and hydrological connectivity. Fish never passed the most upstream receiver, suggesting that such upstream reaches may not be suitable habitat or that it may not be too energetically costly to move so far for available resources. Downstream, there is no evidence that fish cross into the lake. Therefore, the extreme headwaters and the mouth of the lake may be considered boundaries to the population of arapaima using the headwaters reaches.

Hydrology and topography define the units that comprise hydrological connectivity in river-floodplains. Understanding hydrological connectivity is a key factor in understanding population dynamics, home range, and movement ecology. In the area where this study took place, the entirety of the headwater area of Lake Ayapuá, plus connections to the main channels, are connected for six months of the year. During this time especially, when fish are not physically constrained, they use what is available. This study demonstrates the need to consider such expanses of space use in terms of hydrological connectivity. A previous study of hydrological connectivity in a whitewater floodplain ecosystem found an average diameter of hydrologically connected units for arapaima to be approximately 30 km (Richard in prep). In this study, median annual movement was 50 km, which, being close to that of a previous estimate for arapaima, suggests that arapaima are more mobile in highly connected areas and as such, must be managed at a larger scale than previously thought.

There are two main communities in the study area, plus one additional community on the northwestern side of the lake body and one indigenous community southwest of the upstream reaches of the headwaters (Figure 3.1). Since the start of management in the reserve in the early

2000s, the two communities in the headwaters, Uixi and Pinheiros, have counted and managed arapaima together as one. The data from this study support that these two communities manage at the best possible geographical scale. In terms of community based management, the large home ranges of arapaima in this study support the idea that collaborative stock assessment should occur with multiple communities. The other two communities are located in areas where arapaima in this study were not found, thus suggesting that they are managing independent arapaima populations at an appropriate scale.

Typically, conservation management areas are formed using political boundaries; however, if this were done in the area where this study was conducted, management would not be nearly so effective. Political boundaries rarely match ecological boundaries because ecosystems are contiguous and connected. Until we are able to fully understand the ecosystem and the ecology of the fishes within it, it is difficult to accurately delineate boundaries. It is recommended that the scale of community-based management be matched to the home ranges and space use of arapaima. Only then will best conservation practices be able to be most effectively implemented.

Figure and Tables

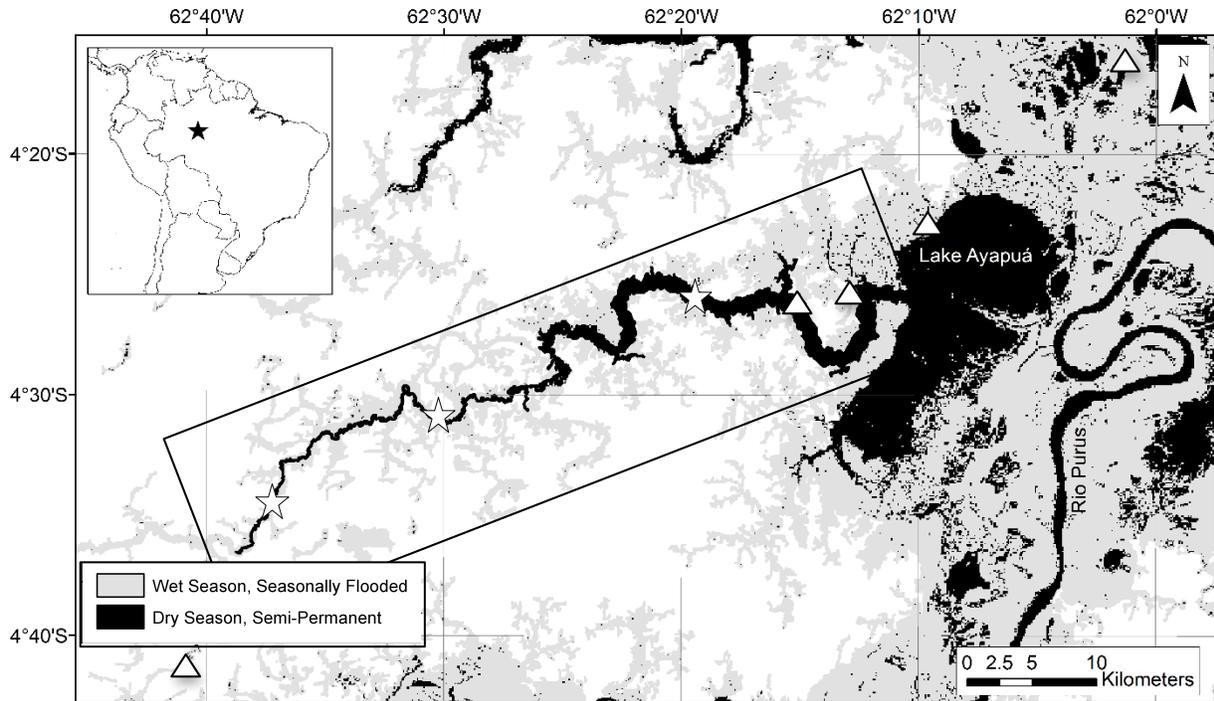


Figure 3.1. Lake Ayapua (Purus River, Amazonas, Brazil) in dry (black) and flood (gray) stages. Stars represent tagging sites, triangles represent communities and the black box highlights the study area in the headwaters of the lake.

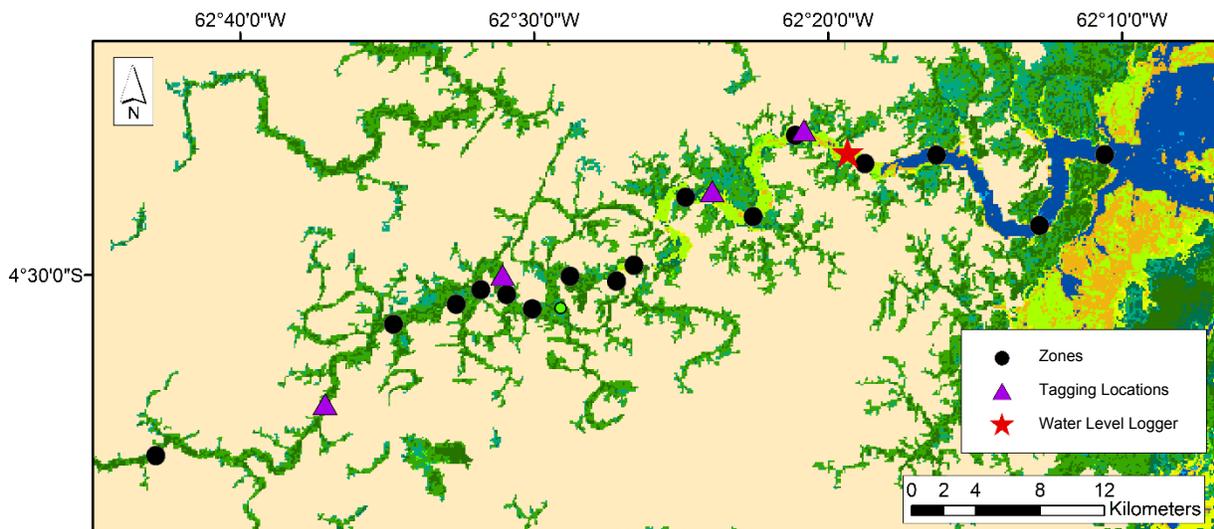


Figure 3.2. Flood stage habitat in Lake Ayapua study area (Purus River, Amazonas, Brazil) with zone locations, tagging location and water logger level location.

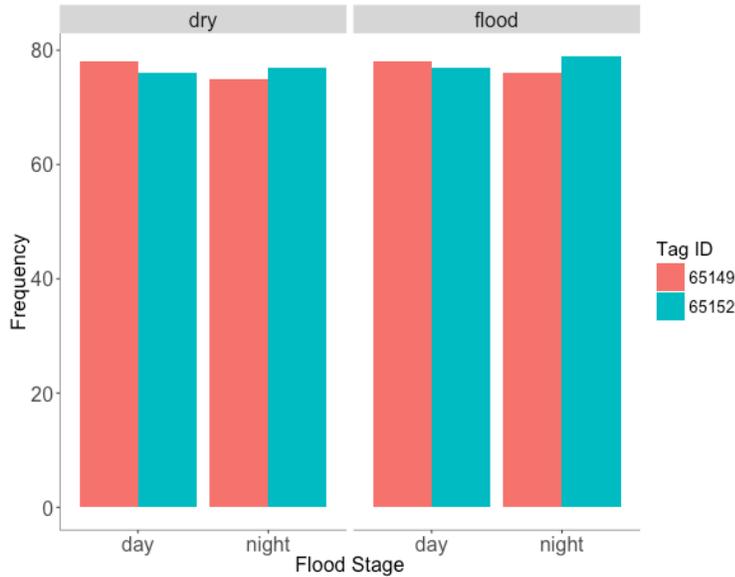


Figure 3.3. Acoustic test tag detections by time of day (night, day) and flood stage (dry, flood) showing very little variation in detection over time in Lake Ayapuá (Purus River, Amazonas, Brazil) from November 2014 – 2015.

Table 3.1. Summary and description of physical and temporal variables sampled from November 2014 - November 2015 in Lake Ayapuá (Purus River, Amazonas, Brazil) and that were selected for model inclusion for residency time, directional movement and movement rates.

Predictor	Description	Range	Mean \pm SD
Flood stage	4 categories: flood, falling, dry, rising	--	--
Depth	Mean depth at zone (m)	14.8 – 17.2	6.5 \pm 1.1 m
Habitat	PCA eigenvector synthetic values (1)	- 4.7 – 2.7	- 0.4 \pm 1.9
Total Length	Total standard length of tagged fish	83 – 170 cm	121 \pm 22 cm
Time of day	2 categories: night (6p-6a) & day (6a-6p)	--	--
Δ Water Level	Amount of water level change per week	-18.5 – 8.9 cm	-7.7 \pm 8.9 cm

Table 3.2. Summary of movement metrics of individual acoustic-tagged fish (n = 15) in Lake Ayapuá (Purus River, Amazonas, Brazil) from November 2014 – 2015. Metrics include total length of fish at tagging (fish length), the number of residency events (n resid events), the first and late date that a fish was detected (detections), the maximum and mean movement rates at which a fish swam (max rate, mean rate), and the total distance traveled by each fish (total dist).

Fish length (cm)	n resid events	Detections (first – last)	Max rate (km/day)	Mean rate (km/day)	Total dist (km)
83	9	12/18/14 – 04/04/15	0.48	0.24	18.98
88	8	12/03/14 – 08/21/15	26.16	3.36	32.55
89	40	12/02/14 – 11/14/15	46.56	3.12	161.40
106	34	11/24/14 – 10/21/15	20.64	2.4	210.97
110	2	01/04/15	0.48	0.48	22.66
115	19	11/29/14 – 03/01/15	58.8	6.48	105.52

117	121	11/25/14 – 05/20/15	8.4	0.24	44.27
126	68	11/25/14 – 10/18/15	48.72	0.96	111.12
129	92	11/28/14 – 10/16/15	90.24	12.72	326.10
129	16	11/20/14 – 10/27/15	26.64	3.6	8.18
132	59	11/29/14 – 11/09/15	44.16	6.96	302.92
135	7	12/27/14 – 02/24/15	0.48	2.4	43.03
139	110	12/12/14 – 11/15/15	13.44	0.24	111.93
139	48	11/25/14 – 10/18/15	59.04	10.08	20.35
170	50	10/08/15 – 11/15/15	43.2	1.68	49.58

Table 3.3. Summary metrics for rate of movement by the amount of time between movements for Arapaima sp. in Lake Ayapuá (Purus River, Amazonas, Brazil).

	<i>Time between movements</i>			
	Short < 3 min	Intermediate 3 - 6 min	Intermediate 6.1 - 15.6 min	Long > 15.6 min
n	32	60	45	48
Median rate	9.37	8.57	6.33	3.54
Mean rate	19.13 (\pm 16.5)	18.9 (\pm 21.2)	13.29 (\pm 13.87)	11.87 (\pm 16.17)
% up	53.1%	53.3%	35.6%	41.7%
% down	46.9%	46.7%	64.4%	58.3%

Table 3.4. Distance between most upstream detection and most downstream detection for each fish and percent of total available dry stage channel area used by total length of arapaima in Lake Ayapuá (Purus River, Amazonas, Brazil).

Dist (km)	% area used	TL (cm)
9.6	11.5	83
25.0	29.9	88
43.0	51.4	135
42.1	50.3	139
55.8	66.7	132
19.2	22.9	117
58.9	70.4	129
21.1	25.2	110
57.0	68.1	139
46.4	55.5	115
40.2	48.1	126
60.5	72.3	129
55.8	66.7	170
24.8	29.6	89

Table 3.5. Distance and direction where arapaima were found at the end of the study in relation to where they were tagged by total length of arapaima in Lake Ayapuá (Purus River, Amazonas, Brazil).

Dist (km) from tag site	Direction from tag site	TL
38.0	downstream	139
9.6	downstream	132
38.0	downstream	139
25.5	upstream	126
35.1	upstream	129
22.0	downstream	106
51.6	upstream	89

Table 3.6. PCA Habitat eigenvector values for each of the habitat classes by flood stage in the study area in Lake Ayapuá (Purus River, Amazonas, Brazil). Bolded values indicate habitat variables that were greater or less than the designated thresholds (0.80, -0.80 respectively).

Dry Stage	Flood Stage	PCA Value
	Open water	-0.80
Open water	Aquatic macrophyte	-0.68
Herbaceous	Open water	-0.56
Herbaceous	Aquatic macrophyte	-0.80
	Aquatic macrophyte	-0.53
Non-flooded shrub	Open water	-0.31
Non-flooded shrub	Flooded shrub	0.04
Flooded shrub	Open water	-0.22
	Flooded woodland	0.06
	Non-flooded forest	0.76
Non-flooded forest	Flooded forest	0.86
	Flooded forest	0.83

Table 3.7. 95% confidence set of models examining the effect of total length and environmental covariates on residency time (min) of *Arapaima* sp. in Lake Ayapuá (Purus River, Amazonas, Brazil).

Candidate models	df	Loglik	AICc	Δ_i	w_i	acc	ER
1 FS	7	-222.084	458.3	0.00	0.28	0.22	1.00
2 FS + TL	8	-221.578	459.4	1.03	0.17	0.45	1.68
3 FS + Depth	8	-221.804	459.8	1.49	0.13	0.58	2.10
4 FS + Habitat	8	-221.973	460.2	1.83	0.11	0.71	2.49
5 FS + Depth + TL	9	-221.327	460.9	2.59	0.08	0.79	3.62
6 FS + Habitat + TL	9	-221.368	461	2.67	0.07	0.86	3.82
7 FS + Depth + Habitat	9	-221.666	461.6	3.27	0.06	0.92	5.07
8 Depth	5	-226.105	462.3	3.96	0.04	0.95	7.34

where the covariates for flood stage (FS), PCA habitat synthetic variables (Habitat), depth, and total length (TL) are described using the model degrees of freedom df , the log likelihood $Loglik$ fit by maximum likelihood estimation, the Akaike Information Criterion for small samples $AICc$, the AIC between model i and the top ranked model Δ_i , the model weight w_i , the cumulative weights of models acc w_i , the evidence ratio (ER) of w_j / w_i where w_j is the weight of the top model (1) and w_i the weights of the consecutive models (2 - 8). Time of day (TOD) was not selected in the 95% confidence set.

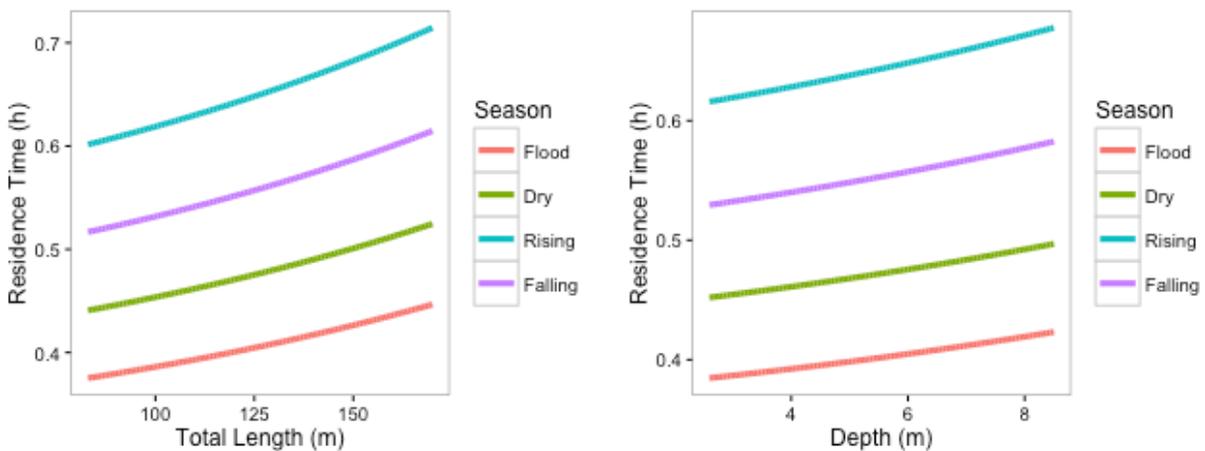


Figure 3.4. Partial effects plot of the effect of total length and depth by flood season on residency time using model averaged results from generalized linear mixed models for *Arapaima* sp. in Lake Ayapuá (Purus River, Amazonas, Brazil).

Table 3.8. 95% confidence set of models examining the effect of environmental and temporal covariates on the probability of *downstream* movement of Arapaima sp. in Lake Ayapuá (Purus River, Amazonas, Brazil).

	Candidate models	df	Loglik	AICc	Δ_i	w_i	acc w_i	ER
1	State + FS	6	-215.0	442.0	0.00	0.48	0.48	1.00
2	State + FS + TL	7	-214.8	443.7	1.63	0.21	0.69	2.28
3	State + FS + Δ WL	7	-214.8	443.8	1.75	0.20	0.89	2.40
4	State + FS + TL + Δ WL	8	-214.6	445.4	3.38	0.09	0.98	5.33

where the covariates for state, flood stage (FS), mean weekly water level change (Δ WL) and total length (TL) are described using the model degrees of freedom df , the log likelihood *Loglik* fit by maximum likelihood estimation, the Akaike Information Criterion for small samples *AICc*, the AIC between model i and the top ranked model Δ_i , the model weight w_i , the cumulative weights of models *acc w_i* , the evidence ratio (ER) of w_j / w_i where w_j is the weight of the top model (1) and w_i the weights of the consecutive models (2 - 4).

Table 3.9. 95% confidence set of models examining the effect of environmental and temporal covariates on the probability of *upstream* movement of Arapaima sp. in Lake Ayapuá (Purus River, Amazonas, Brazil).

	Candidate models	df	Loglik	AICc	Δ_i	w_i	acc w_i	ER
1	State + FS	6	-180.2	372.4	0.00	0.28	0.28	1.00
2	State	3	-183.9	373.7	1.33	0.14	0.43	1.95
3	State + Δ WL	4	-182.9	373.8	1.39	0.14	0.57	2.00
4	State + FS + Δ WL	7	-179.9	374.0	1.58	0.13	0.69	2.20
5	State + FS + TL	7	-180.0	374.1	1.66	0.12	0.82	2.30
6	State + TL	4	-183.6	375.2	2.81	0.07	0.88	4.07
7	State + TL + Δ WL	5	-182.7	375.5	3.08	0.06	0.95	4.68

where the covariates for state, flood stage (FS), mean weekly water level change (Δ WL) and total length (TL) are described using the model degrees of freedom df , the log likelihood *Loglik* fit by maximum likelihood estimation, the Akaike Information Criterion for small samples *AICc*, the AIC between model i and the top ranked model Δ_i , the model weight w_i , the cumulative weights of models *acc w_i* , the evidence ratio (ER) of w_j / w_i where w_j is the weight of the top model (1) and w_i the weights of the consecutive models (2 - 7).

Table 3.10. 95% confidence set of models examining the effect of environmental and temporal covariates on rate of movement of *Arapaima* sp. in Lake Ayapuá (Purus River, Amazonas, Brazil).

	Candidate models	df	Loglik	AICc	Δ_i	w_i	acc w_i	ER
1	FS + D + Δ WL	8	-90.3	196.9	0.00	0.20	0.20	1.00
2	FS + D + Δ WL + TD	9	-89.3	197.1	0.22	0.18	0.38	1.11
3	FS + D + TD	8	-90.7	197.8	0.93	0.13	0.51	1.53
4	FS + D	7	-92.0	198.4	1.45	0.10	0.61	2.00
5	FS + D + Δ WL + TL	9	-90.3	199.0	2.10	0.07	0.68	2.86
6	FS + D + Δ WL + TL + TD	7	-92.3	199.1	2.18	0.07	0.75	2.86
7	FS + Δ WL	10	-89.3	199.2	2.33	0.06	0.81	3.33
8	FS	6	-93.5	199.3	2.41	0.06	0.87	3.33
9	FS + TD	7	-92.6	199.6	2.68	0.05	0.92	4.00
10	FS + Δ WL + TD	8	-91.8	199.9	3.01	0.04	0.96	5.00

where the covariates for flood stage (FS), mean weekly water level change (Δ WL), time of day (TD), direction (D) and total length (TL) are described using the model degrees of freedom df , the Akaike Information Criterion for small samples $AICc$, the AIC between model i and the top ranked model Δ_i , the model weight w_i , the cumulative weights of models $acc w_i$, the ER evidence ratio of w_j / w_i where w_j is top model (1) and w_i is the consecutive model (2 - 10).

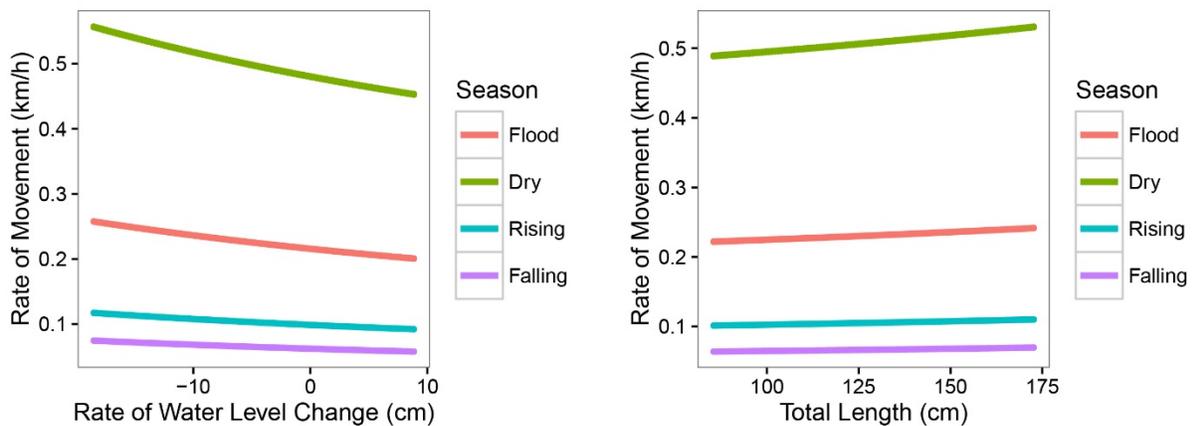


Figure 3.5. Partial effects plot of the effect of rate of water level change, total length and flood season on rate of arapaima movement using model-averaged results.

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References

- Agência Nacional de Águas. 2016. Serviços de Informações Hidrológicas. Setor Político. Area 5, Quadra 3, Brasília, Brazil.
- Albanese, B., Angermeier, P. L., & Dorai-Raj, S. 2004. Ecological correlates of fish movement in a network of Virginia streams. *Canadian Journal of Fisheries and Aquatic Sciences*, 61(6), 857-869.
- Arantes, C. C., Castello, L., Stewart, D. J., Cetra, M., & Queiroz, H. L. 2010. Population density, growth and reproduction of arapaima in an Amazonian river-floodplain. *Ecology of Freshwater Fish*, 19(3), 455-465.
- Arantes, C. C., Castello, L., Cetra, M., & Schilling, A. 2013. Environmental influences on the distribution of arapaima in Amazon floodplains. *Environmental Biology of Fishes*, 96(10), 1257-1267.
- Araripe, J., do Rêgo, P. S., Queiroz, H., Sampaio, I., & Schneider, H. 2013. Dispersal capacity and genetic structure of *Arapaima gigas* on different geographic scales using microsatellite markers: E54470. *PLoS One*, 8(1).
- Barton, K. 2016. MuMIn: Multi-Model Inference. Retrieved from <https://cran.r-project.org/package=MuMIn>.
- Bayley, P.B. 1988. Factors affecting growth rates of young tropical floodplain fishes: seasonality and density-dependence. *Environmental Biology of Fishes*, 21, 127-142.
- Bayley, P. B., & Petrere, M. Jr. 1989. Amazon fisheries: assessment methods, current status and management options. *Canadian Special Publication of Fisheries and Aquatic Sciences*, 106, 385-398.

- Beckman, B. R., Larsen, D. A., Lee-Pawlak, B., & Dickhoff, W. W. (1998). Relation of fish size and growth rate to migration of spring chinook salmon smolts. *North American Journal of Fisheries Management*, 18(3), 537-546.
- Beeman, J. W., & R. W. Perry. 2012. Bias from false-positive detections and strategies for their removal in studies using telemetry (pp 505-518). In N. S. Adams, J. W. Beeman, & J. H. Eiler (Eds.). *Telemetry Techniques: A user Guide for Fisheries Research*. Bethesda, Maryland: American Fisheries Society.
- Bestley, S., Patterson, T. A., Hindell, M. A., & Gunn, J. S. 2010. Predicting feeding success in a migratory predator: Integrating telemetry, environment, and modeling techniques. *Ecology*, 91(8), 2373-2384.
- Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J. S. 2009. Generalized linear mixed models: A practical guide for ecology and evolution. *Trends in Ecology & Evolution*, 24(3), 127-135.
- Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: A practical information-theoretic approach* (2nd ed.). New York: Springer.
- Castello, L. 2004. A method to count pirarucu *Arapaima gigas*: fishers, assessment, and management. *North American Journal of Fisheries Management*, 24, 379-389.
- Castello, L. 2008. Lateral migration of *Arapaima gigas* in floodplains of the Amazon. *Ecology of Freshwater Fish*, 17(1), 38-46.
- Castello, L. 2008. Nesting habitat of *Arapaima gigas* (Schinz) in Amazonian floodplains. *Journal of Fish Biology*, 72(6), 1520-1528.

- Castello, L., Arantes, C. C., Mcgrath, D. G., Stewart, D. J., & Sousa, F. S. D. 2015. Understanding fishing-induced extinctions in the Amazon. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 25(5), 447-458.
- Castello, L. & Macedo, M. N. 2016. Large-scale degradation of Amazonian freshwater ecosystems. *Glob Change Biol*, 22: 990-1007.
- Clarke, A., & Johnston, N. M. 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology*, 68(5), 893-905.
- Crossa, M. 2003. Una experiencia promisoriosa para el subsidio de programas de manejo del pirarucu (*Arapaima gigas Cuvier*) en el Bajo Amazonas. In *IIAP - Taller Internacional de Manejo de Paiche o Pirarucu*. Iquitos, Perú: Resumos.
- Duponchelle, F., Pouilly, M., Pécheyrán, C., Hauser, M., Renno, J., Panfili, J., Heino, J. 2016. Trans-Amazonian natal homing in giant catfish. *Journal of Applied Ecology*, 53(5), 1511-1520.
- Eiler, J.H. 1995. A remote satellite-linked tracking system for studying Pacific salmon with radio telemetry. *Transactions of the American Fisheries Society* 124, 184-193.
- Fernandes, C. C. 1997. Lateral migration of fishes in Amazon floodplains. *Ecology of Freshwater Fish*, 6(1), 36-44.
- Fernandes, R., Gomes, L. C., Pelicice, F. M., & Agostinho, A. A. 2009. Temporal organization of fish assemblages in floodplain lagoons: The role of hydrological connectivity. *Environmental Biology of Fishes*, 85(2), 99-108.
- Gerking, S. D. 1959. The restricted movement of fish populations. *Biological Reviews*, 34(2), 221-242.

- Godinho, A. L., & Kynard, B. 2009. Migratory fishes of Brazil: Life history and fish passage needs. *River Research and Applications*, 25(6), 702-712.
- Goulding, M. 1980. *The Fishes and the Forest*. University of California Press, Los Angeles.
- Gourou, P. 1950. Observações geográficas na Amazônia. *Revista Brasileira De Geografia*, 11, 355-408.
- Greenberg, L. 1991. Habitat use and feeding behavior of thirteen species of benthic stream fishes. *Environmental Biology of Fishes*, 31(4), 389-401.
- Grueber, C. E., Nakagawa, S., Laws, R. J., & Jamieson, I. G. 2011. Multimodel inference in ecology and evolution: Challenges and solutions. *Journal of Evolutionary Biology*, 24(4), 699-711.
- Hermann, T. W., Stewart, D. J., Limburg, K. E., & Castello, L. 2016. Unravelling the life history of Amazonian fishes through otolith microchemistry. *Royal Society Open Science*, 3(6), 160206.
- Hess, L.L., Melack, J.M., Affonso, A.G., Barbosa, C.C.F., Gastil-Buhl, M., & Novo, E.M.L.M. 2015. LBA-ECO LC-07 Wetland Extent, Vegetation, and Inundation: Lowland Amazon Basin. Oak Ridge, Tennessee: ORNL DAAC.
- Hocutt, C. 1989. Seasonal and diel behaviour of radio-tagged *Clarias gariepinus* in Lake Ngezi, Zimbabwe (Pisces: Clariidae). *Journal of Zoology*, 219(2), 181-199.
- Hocutt, C. H., Seibold, S. E., Harrell, R. M., Jesien, R. V., & Bason, W. H. 1990. Behavioral observations of striped bass (*Morone saxatilis*) on the spawning grounds of the Choptank and Nanticoke rivers, Maryland, USA. *Journal of Applied Ichthyology*, 6(4), 211-222.

- Junk, W.J., Bayley, P.B., & Sparks, R.E. 1989. The flood pulse concept in river floodplain systems. In: Proceedings of the international large river symposium. *Canadian Journal of Fisheries and Aquatic Sciences*, 110-127.
- Junk, W. J., Piedade, M. T. F., Schöngart, J., & Wittmann, F. 2012. A classification of major natural habitats of Amazonian white-water river floodplains (várzeas). *Wetlands Ecology and Management*, 20(6), 461-475.
- Layman, C. A., Montaña, C. G., & Allgeier, J. E. 2010. Linking fish colonization rates and water level change in littoral habitats of a Venezuelan floodplain river. *Aquatic Ecology*, 44(1), 269-273.
- Lighthill, M. J. 1971. Large-amplitude elongated-body theory of fish locomotion. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, 179(1055), 125-138.
- Lucas, M. C., & Baras, E. 2001. Migration and spatial behaviour. In *Migration of freshwater fishes*. M. C. Lucas, E. Baras, T. J. Thom, A. Duncan & O. Slavík (Eds). Malden, Massachusetts: Blackwell Science Ltd.
- Lyons, J. A. 2010. The effect of water level on lateral movements of fish between river and off-channel habitats and implications for management. *Marine and Freshwater Research* 61(3).
- Montgomery, W. L., Stephen, D., McCormick, R.J., Naiman, F.G., Whoriskey Jr., F. G., & Spring, A.B. 1983. Spring migratory synchrony of salmonid, catostomid, and cyprinid fishes in Rivière à la Truite, Québec. *Canadian Journal of Zoology*, 61(11): 2495-2502.

- Nathan, R., Getz, W. M., Revilla, E., Holyoak, M., Kadmon, R., Saltz, D., & Smouse, P. E. 2008. A movement ecology paradigm for unifying organismal movement research. *Proceedings of the National Academy of Sciences of the United States of America*, 105(49), 19052-19059.
- Núñez-Rodríguez, J., Duponchelle, F., Cotrina-Doria, M., Renno, J., Chavez-Veintimilla, C., Rebaza, C., Baras, E. 2015. Movement patterns and home range of wild and re-stocked *Arapaima gigas* (Schinz, 1822) monitored by radio-telemetry in Lake Imiria, Peru. *Journal of Applied Ichthyology*, 31(S4), 10-18.
- Nunn, A., Copp, G., Vilizzi, L., & Carter, M. 2010. Seasonal and diel patterns in the migrations of fishes between a river and a floodplain tributary. *Ecology of Freshwater Fish*, 19(1), 153-162.
- Osorio, D., Terborgh, J., Alvarez, A., Ortega, H., Quispe, R., Chipollini, V., & Davenport, L. C. 2011. Lateral migration of fish between an oxbow lake and an Amazonian headwater river. *Ecology of Freshwater Fish*, 20(4), 619–627.
- Queiroz, H.L. 2000. Natural history and conservation of pirarucu, *Arapaima gigas*, at the Amazonian varzea: Red giants in muddy waters. PhD thesis, University of St. Andrews, St. Andrews, Scotland.
- Rasmussen, J. E., & Belk, M. C. 2016. Individual movement of stream fishes: Linking ecological drivers with evolutionary processes. *Reviews in Fisheries Science & Aquaculture*, 25(1), 70-83.
- Saint-Paul, U., Zuanon, J., Correa, M.A.V., García, M., Fabr e, N.N., Berger, U., & Junk, W.J. 2000. Fish communities in central Amazonian white- and blackwater floodplains. *Environmental Biology of Fish*, 57, 235–250.

- Schielzeth, H. 2010. Simple means to improve the interpretability of regression coefficients. *Methods in Ecology and Evolution*, 1(2), 103-113.
- Sommer, T. R., Harrell, W. C., & Feyrer, F. 2014. Large-bodied fish migration and residency in a flood basin of the Sacramento river, California, USA. *Ecology of Freshwater Fish*, 23(3), 414-423.
- Symonds, M. R. E., & Moussalli, A. 2011. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using akaike's information criterion. *Behavioral Ecology and Sociobiology*, 65(1), 13-21.
- Wagner, G. N., Cooke, S. J., Brown, R. S., & Deters, K. A. 2011. Surgical implantation techniques for electronic tags in fish. *Reviews in Fish Biology and Fisheries*, 21(1), 71-81.
- Walsh, C., Reinfelds, I., Ives, M., Gray, C., West, R., & van der Meulen, D. 2013. Environmental influences on the spatial ecology and spawning behaviour of an estuarine-resident fish, *Macquaria colonorum*. *Estuarine, Coastal and Shelf Science*, 118, 60-71.
- Welcomme, R.L. 1979. *Fisheries Ecology of Floodplain Rivers*. Longman Press, London.
- Winemiller, K.O. & Jepsen, D.B. 1998. Effects of seasonality and fish movement on tropical river food webs. *Journal of Fish Biology* 53(Suppl A), 267-296.
- Zuur, A. F. 2009. *Mixed effects models and extensions in ecology with R* (1st ed.). New York, NY: Springer.
- Zuur, A., Ieno, E. N., & Walker, N. 2009. *Statistics for biology and health: Mixed effects models and extensions in ecology with R*. New York, NY: Springer.

Zuur, A. F., Ieno, E. N., & Elphick, C. S. 2010. A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, 1(1), 3-14.

Zuur, A. F., Ieno, E. N., & Freckleton, R. 2016. A protocol for conducting and presenting results of regression-type analyses. *Methods in Ecology and Evolution*, 7(6), 636-645.

Appendix B

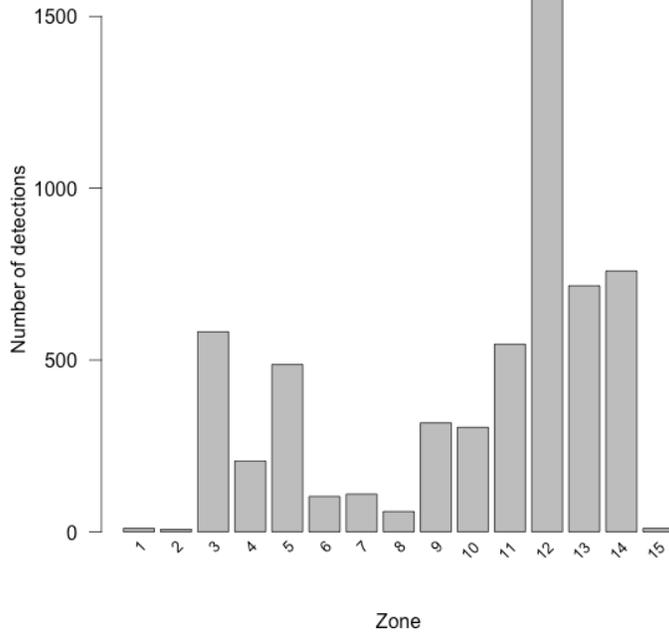


Figure B.1. Number of detections per zone (zone 1 = mouth of Lake Ayapuá).

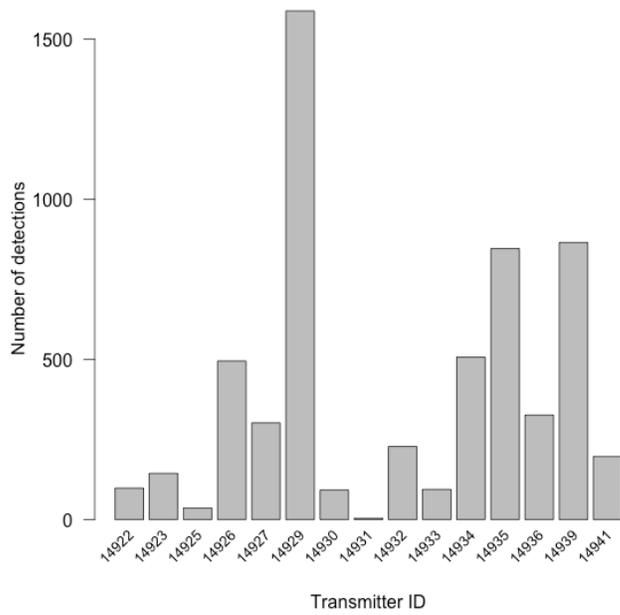


Figure B.2. Number of detections by fish ID (n fish = 15).

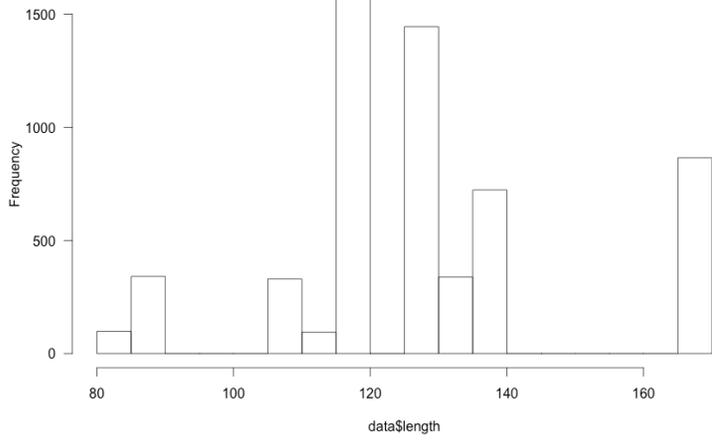


Figure B.3. Number of detections by fish length (lengths = 83 – 170 cm).

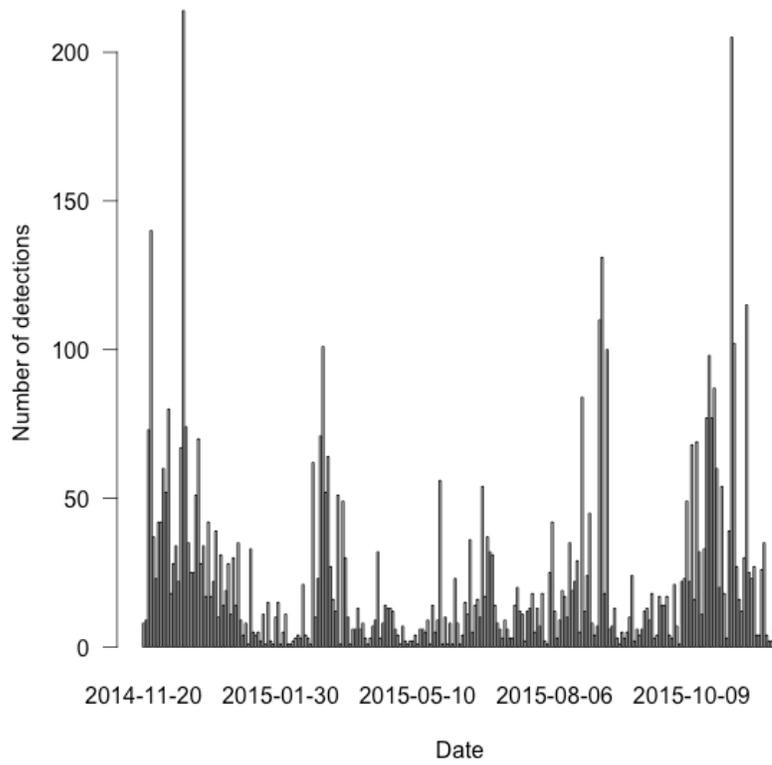


Figure B.4. Number of detections over study duration (11/10/14 – 11/15/15).

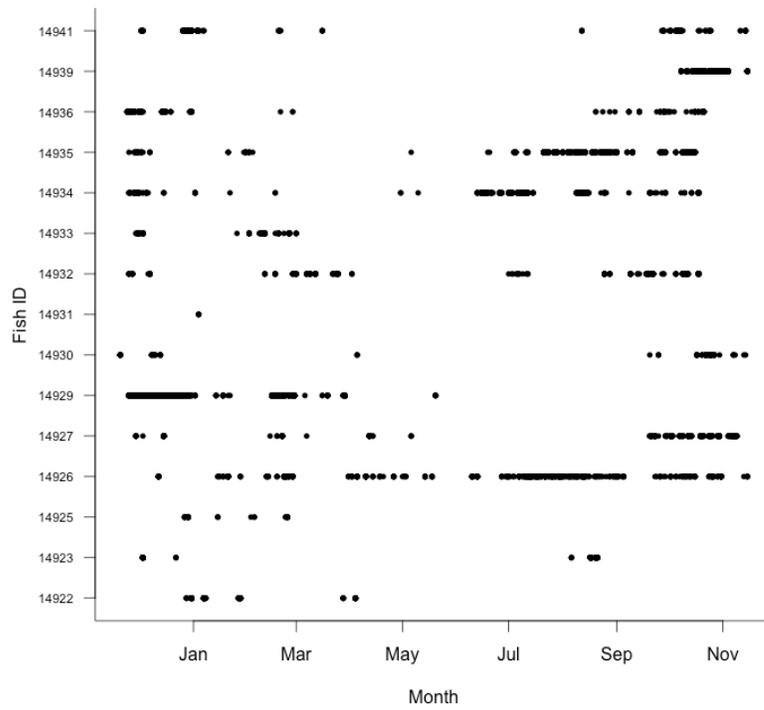


Figure B.5. Detections per fish by month of the year (Nov 2014 – Nov 2015).

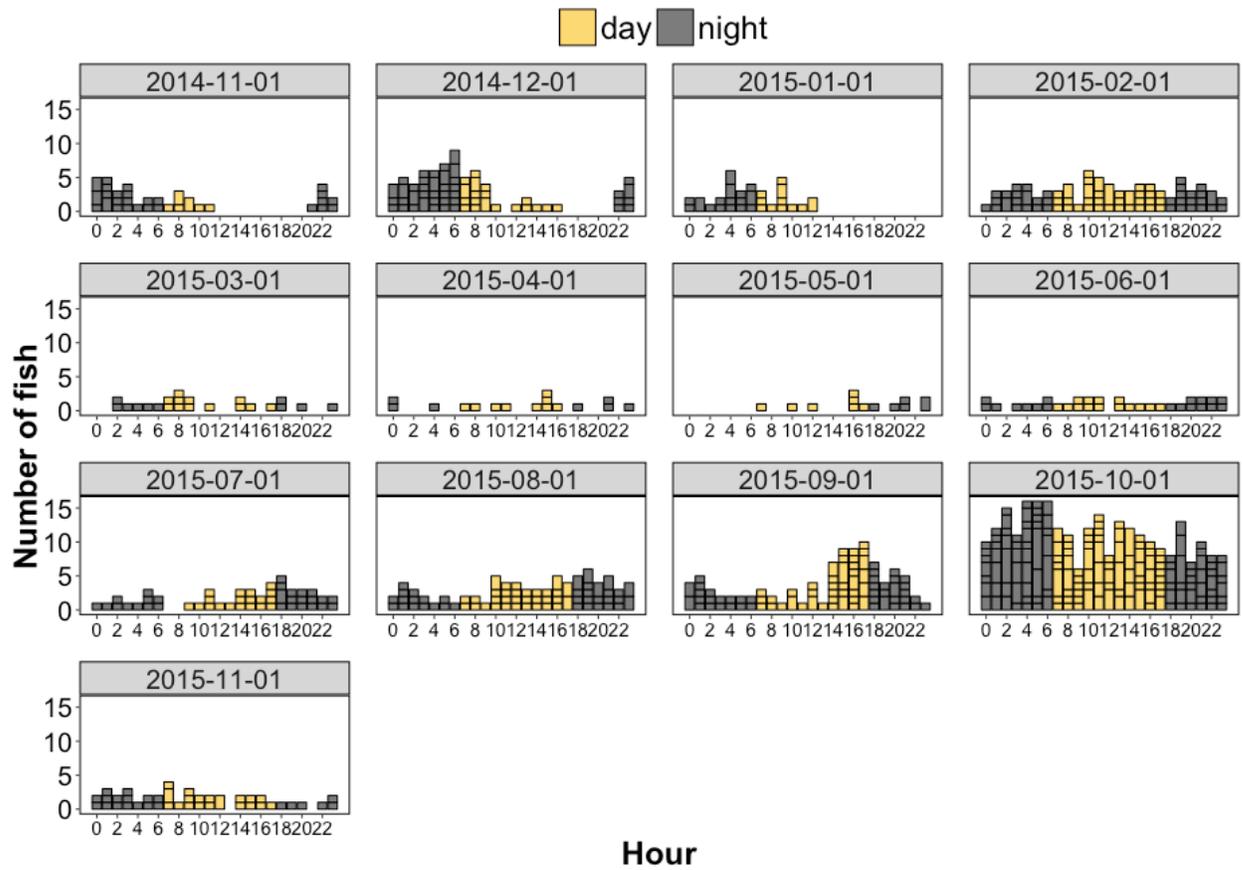


Figure B.6. Detections each hour of the day by month of the year (Nov 2014 - 2015).

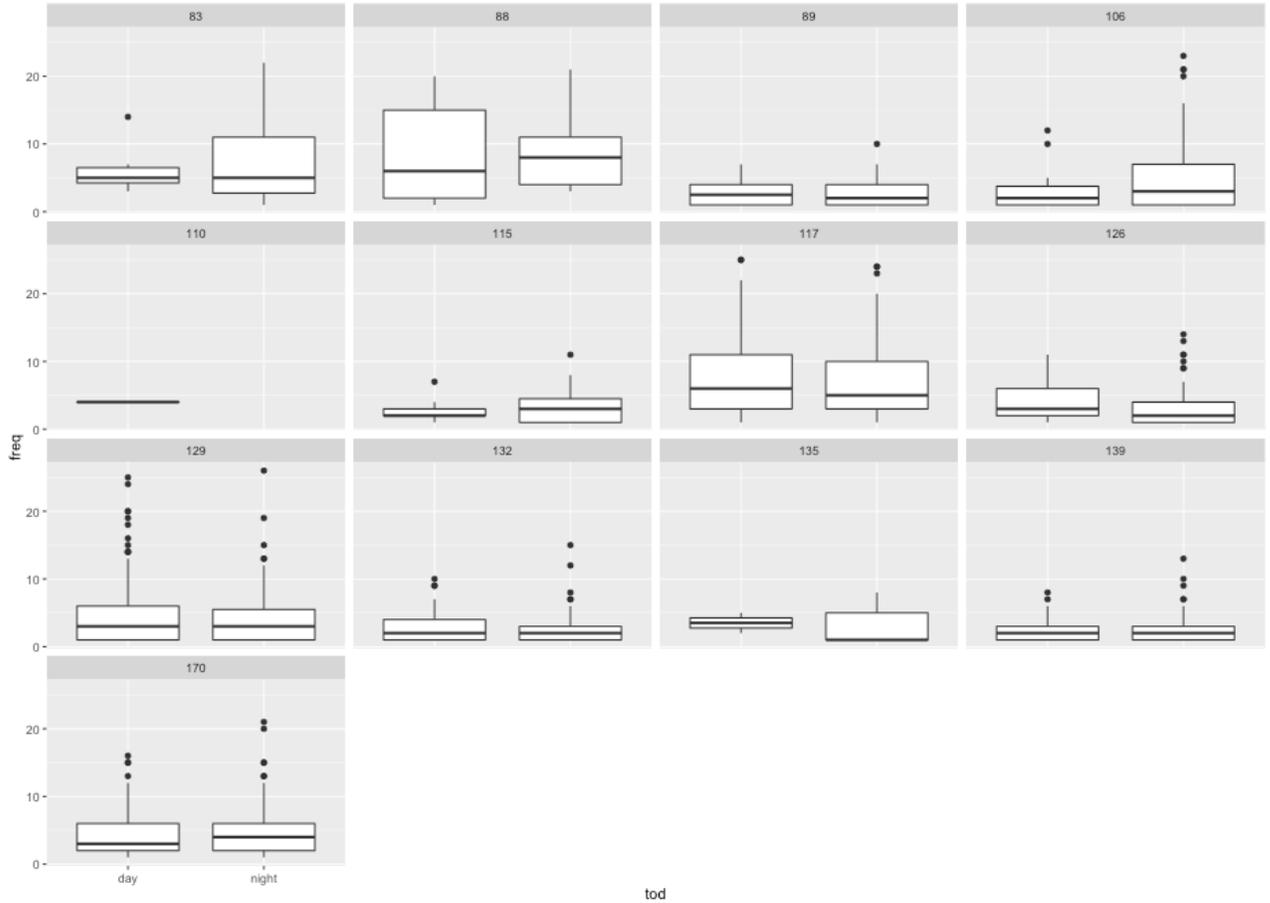


Figure B.7. Detections by time of day, faceted by fish length (83 – 170 cm).

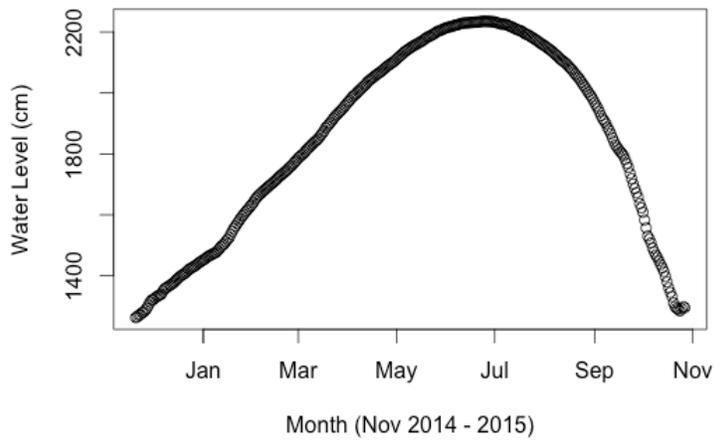


Figure B.8. Water level hydrograph for study duration (Nov 2014 - 2015).

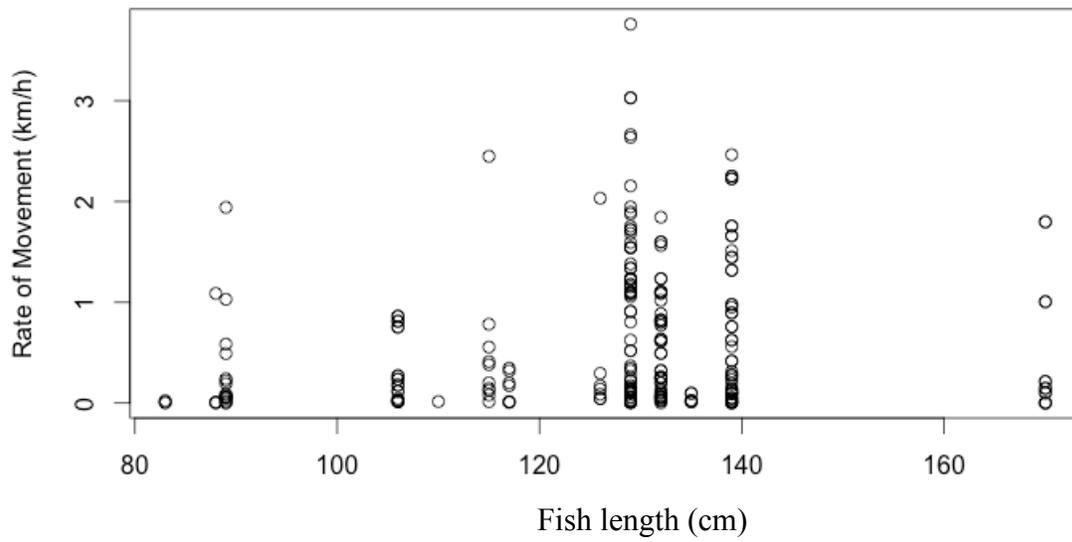


Figure B.9. Rate of movement (km/h) traveled by fish size (cm).

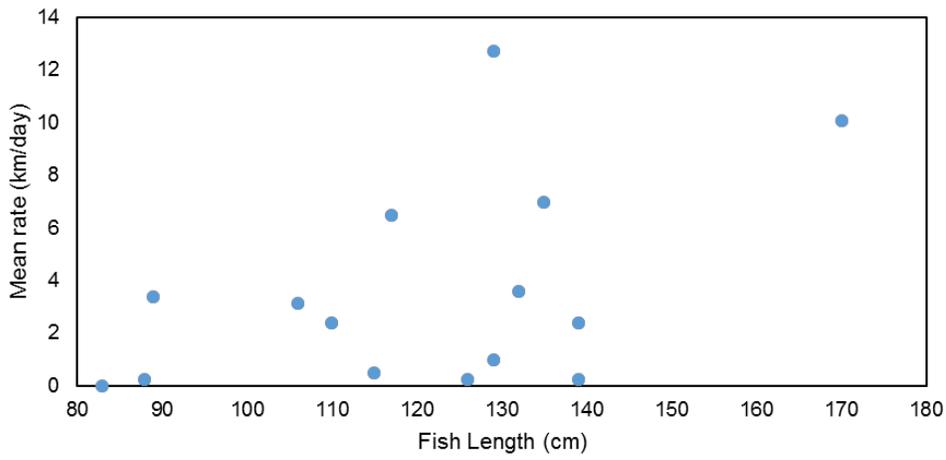


Figure B.10. Mean daily distance traveled by size of body (n=15).

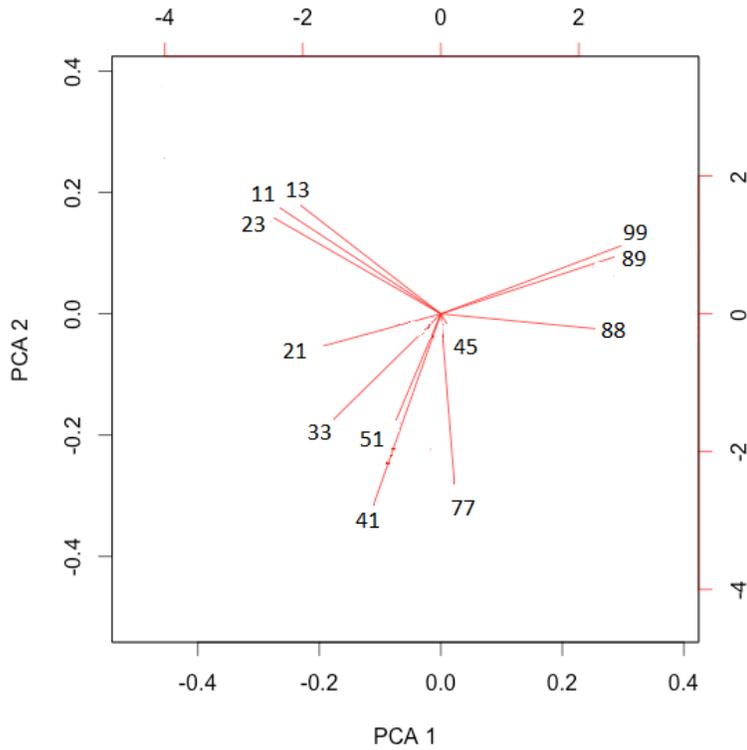


Figure B.11. PCA Analysis results of habitat variables where the x and y axes show the strongest two sets of eigenvalues. Habitat types correspond as follows for dry (wet) stages: 11=open water, 13=open water (aquatic macrophyte), 23=herbaceous (aquatic macrophyte), 21=herbaceous (open water), 33=aquatic macrophyte, 51=flooded shrub (open water), 41 = non-flooded shrub (open water), 77=flooded woodland, 88=non-flooded forest, 89=non-flooded forest (flooded forest), 99=flooded forest.

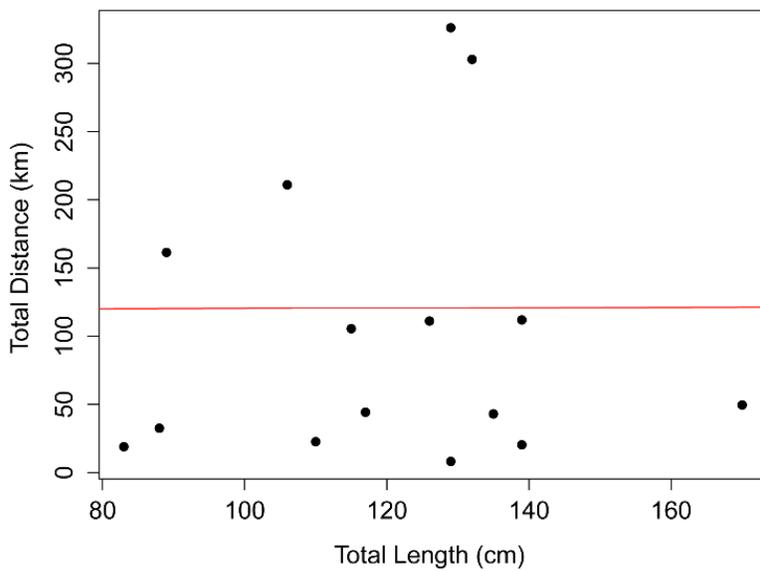


Figure B.12. Scatterplot with best-fit line (red) of total distance moved by total length.

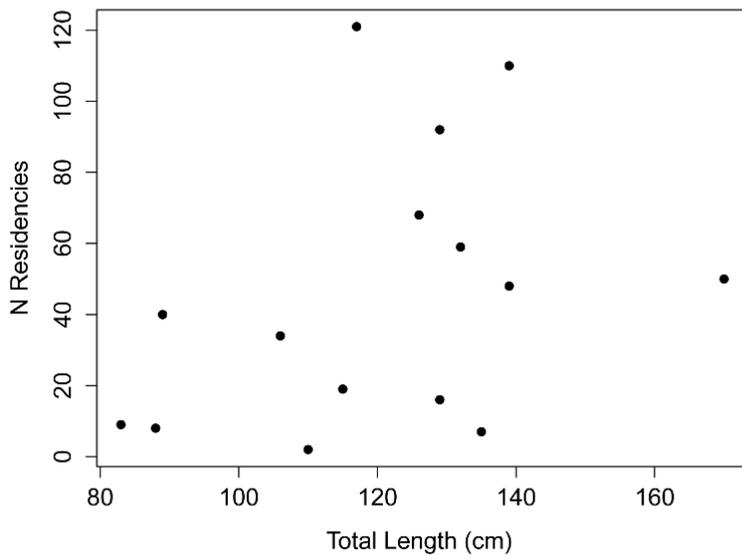


Figure B.13. Scatterplot of number of residency events by total length.

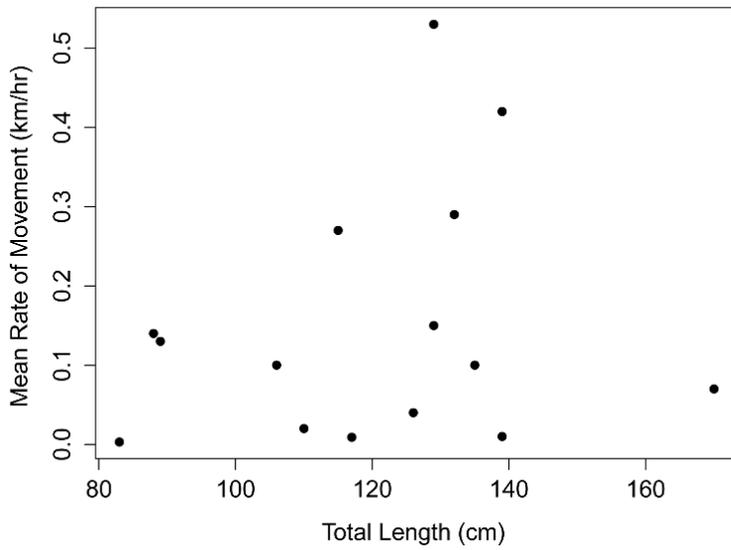


Figure B.14. Scatterplot of mean rate of movement by total length.

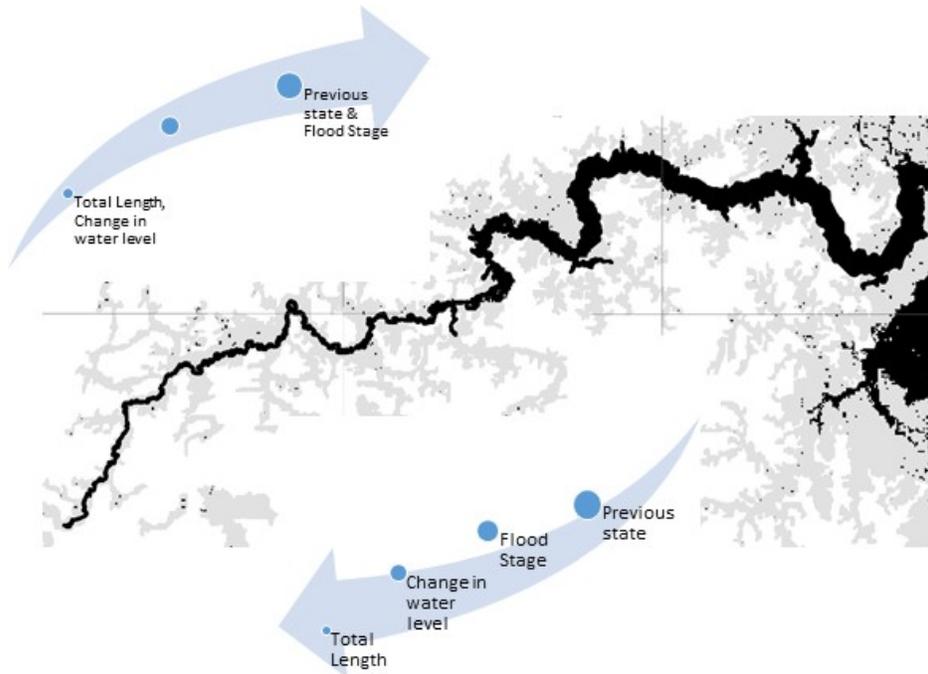


Figure B.15. Visual representation of the influences on directional movement of arapaima.

Chapter 4. Conclusion

Summary

At the interface of terrestrial and aquatic resources lie complex and dynamic river-floodplain ecosystems. A complete understanding of fish ecology will come only from examining natural river-floodplain systems where fish have the capacity to move to their full extent and behave in response to the annual flood pulse. The preceding two chapters strived to do that using a sedentary, air-breathing fish in a near-pristine area of the Amazon. Results of the studies yield several important findings: 1) air-breathing intervals were influenced most by temperature and body size; but were likely to be variable between and among individuals, 2) Two-thirds of fish (15% of breaths) took longer than 20 minutes to breathe, 3) surfacing characteristics categorized as “aggressive” were more likely to occur more rapidly than those categorized as “calm,” 4) hydrology was the most important predictor of movement patterns, followed by body size, direction of movement, and habitat, and 5) fish were capable of using the full extent of available habitat, moving throughout the entire year and utilizing short burst movements. Such findings are relevant across a wide scale, including to other sedentary fishes in the Amazon, fishes in transitional ecosystems, fishes in other river-floodplains and fishes with similar traits found in modified systems.

Study Implications

Studies, including this one, on the mobility and seasonality of fish movements are important to the overall understanding of production and biomass of floodplain fisheries

(Castello et al. 2013, Borie et al. 2014). It would be expected that other sedentary Amazon fishes of ecological and economic importance, such as *Plagioscion squamosissimus* (silver croaker) or *Cichla* sp. (peacock bass, *tucunaré*), may follow similar movement patterns. Findings of this study are also relevant across similar landscapes in the Amazon, particularly the Negro and Tapajós Rivers. These two river systems have some of the largest transitional *ria* lake ecosystems in the Amazon, many of which extend well over 100 km in length (Junk 2010).

This study was also one of the first to describe the surfacing characteristics of an air-breathing fish in the wild. Such characterization should be explored in the 21 other species of continuous, obligate air-breathing fishes in the world, including the family *Lepidosirenidae* (South American lungfish), family *Protopteridae* (African lungfish), genus *Megalops* (tarpon), genus *Channa* (snakehead), and genus *Electrophorus* (electric eel). Further, none of the above groups of fishes have a known management program based on counts from breathing intervals, but results of this study suggest that it would be possible to implement a similar program for other obligate air-breathers.

These findings are relevant not only to other similar species in the Amazon, but also to modified rivers where no such study is possible. The fact that arapaima move throughout the entire extent of the river-floodplain during the year and that they move continuously rather than in discrete seasonal movements may have implications to other species in rivers thought to be very sedentary. It is recommended that additional, robust movement studies be conducted to understand the full extent to which such species move in order to devise conservation management plans suitable for the ecological patterns of each species.

Management Implications

The conservation of fishes relies on robust biological assessments, which provide managers with an improved understanding of where, when and how fish move (Cooke et al. 2016). Locally, these results indicated that arapaima are not using the farthest reaches of the channel upstream nor was there evidence to suggest that fish crossed the lake, likely due to poor habitat in the warmer, shallower lake body. Therefore, managers should be able to consider the upstream *ria* lake a separate management area. However, the findings of the movement study suggest that arapaima are capable of using a relatively large area and move throughout it during the year. Rather than managing discrete populations, it is recommended that emphasis be put on managing ecological units, with consideration of the extent to which arapaima are capable of moving, as it may involve a number of different communities (Richard in prep).

Breathing interval results yield two suggestions: 1) managers should consider the fact that breathing is so variable and can occur as rapidly as every 4 minutes or longer than 45 minutes, and 2) adaptive management should be considered, particularly in colder areas, based on the fact that two-thirds of fish (15% of breaths) were found to take longer than 20 minutes to breathe.

Recommendations

Like any biotelemetry study in a complex environment, such as tropical river-floodplains, this study has its limitations. Future study designs should include the use of the best available technology. Evolving technologies in satellite telemetry are becoming

more useful and practical for the study of fish movement (Cooke et al. 2012). Arapaima offer a unique opportunity for studying floodplain fish movement strategies due to the fact that they are a highly obligate air-breathing species, requiring surface respirations every 10 - 15 minutes (Castello 2008). As arapaima surface, a signal would likely be able to transmit to satellites using the newly designed fast-acquisition global positioning system tags (Dujon et al. 2014, Sims et al. 2009). Paired with external sensors, this system would offer new insights on the spatiotemporal movement ecology of arapaima particularly in the gaps that still exist and in areas where telemetry tracking was not possible. Studies using accelerometers or cameras attached to fish would further enhance findings in behavior and movement by the use of real-time, high resolution data on movements and breathing.

The future of arapaima conservation relies on these technological tools and biological findings, but ultimately will be defined by local community involvement. It is recommended that local ecological knowledge be utilized when possible, to enhance scientific findings and provide insight from a local perspective. Community involvement and conservation investment from local fishers is key to protecting fisheries resources and cannot be overlooked. It is imperative to use these scientific findings, as well as those to come, in collaboration with local communities, in order to ultimately sustain, protect and conserve fisheries populations in the Amazon.

References

- Borie, A., Mok, H., Chao, N. L., & Fine, M. L. 2014. Spatiotemporal variability and sound characterization in silver croaker *Plagioscion squamosissimus* (Sciaenidae) in the central Amazon: E99326. *PLoS One*, 9(8).
- Castello, L. 2008. Lateral migration of *Arapaima gigas* in floodplains of the Amazon. *Ecology of Freshwater Fish*, 17(1), 38-46.
- Castello, L., McGrath, D. G., Hess, L. L., Coe, M. T., Lefebvre, P. A., Petry, P., Arantes, C. C. 2013. The vulnerability of Amazon freshwater ecosystems. *Conservation Letters*, 6(4), 217-229.
- Cooke, S., Paukert, C., & Hogan, Z. 2012. Endangered river fish: Factors hindering conservation and restoration. *Endangered Species Research*, 17(2), 179-191.
- Cooke, S. J., Martins, E. G., Struthers, D. P., Gutowsky, L. F. G., Power, M., Doka, S. E., Krueger, C. C. 2016. A moving target-incorporating knowledge of the spatial ecology of fish into the assessment and management of freshwater fish populations. *Environmental Monitoring and Assessment*, 188(4), 1-18.
- Dujon, A. M., Lindstrom, R. T., Hays, G. C., & Backwell, P. 2014. The accuracy of Fastloc-GPS locations and implications for animal tracking. *Methods in Ecology and Evolution*, 5(11), 1162-1169.
- Junk, W. J. 2010. Amazonian floodplain forests: Ecophysiology, biodiversity and sustainable management. New York: Springer.

Sims, D. W., Queiroz, N., Humphries, N. E., Lima, F. P., Hays, G. C., & Ropert-Coudert, Y. 2009. Long-term GPS tracking of ocean sunfish *mola mola* offers a new direction in fish monitoring. *Plos One*, 4(10), e7351-e7351.