DISSERTATION

TRIPLOID WALLEYE: A NEW FRONTIER FOR NONNATIVE PREDATOR MANAGEMENT IN THE WEST

Submitted by

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ABSTRACT

TRIPLOID WALLEYE: A NEW FRONTIER FOR NONNATIVE PREDATOR MANAGEMENT IN THE WEST

Walleye *Stizostedion vitreum* is a widely distributed and economically important freshwater fish species throughout the United States and Canada, but they are not native to Colorado. Walleye can be invasive and negatively impact native species in their introduced range, and stocking is prohibited in many parts of the western U.S. However, illegal introductions and natural dispersal are common, and managers need a means to limit the impact of introduced Walleye.

Stocking triploids has been proposed as an alternative stocking method for Walleye throughout the West, as a means to satisfy angler demand and limit dispersal while mitigating conflict with native species conservation goals. Triploid fish have three sets of chromosomes as compared to normal, or diploid, fish which have two sets of chromosomes. Triploid fish are sterile, which could prevent the establishment of new Walleye populations in sensitive areas. However, almost nothing is known about the performance of triploid Walleye in the wild. The overarching goal of my dissertation was to examine how triploid Walleye compare relative to diploid conspecifics.

In Chapter One, I tested how a novel technique for determining ploidy works for Walleye. Biologists, researchers, and managers working in field environments need practical methods to determine ploidy. Cytological methods (e.g., flow cytometry, Coulter counter) using erythrocytes are the most common for ploidy determination in fishes. However, collecting and

storing erythrocytes can be logistically challenging during field work, and donor fish need to be alive or freshly killed. With rapid advances in molecular genetics, biologists, researchers, and managers may be unaware of molecular approaches for ploidy determination that could alleviate the difficulties associated with cytological methods and allow ploidy determination from archived samples (e.g., fin clips, scales, otoliths). I analyzed the agreement between molecular-based (using fin tissue) and Coulter counter-based (using blood) ploidy determinations for Walleyes, the first assessment of concordance between molecular and cytological methods for determining ploidy in the family Percidae. I found that agreement between these two methods was > 98%. The high degree of agreement and greater ease of collecting and storing samples for molecular-based approaches relative to the traditional cytological ones support the utility of molecular methods for ploidy determination for Walleyes.

In Chapter Two, I assessed how the growth and survival of juvenile Walleye compared to diploids. There is a paucity of information regarding the performance of triploid Walleye in natural systems, which is essential to inform management decisions. I compared the relative growth and survival of diploid and triploid Walleye in two Colorado reservoirs for 3 years (2018, 2019, 2021). Walleyes were sampled with a combination of autumn shoreline electrofishing and gill netting. I found that triploid Walleyes were significantly smaller and survived at significantly lower rates than diploids in their first year of life, but no differences were observed in growth and survival between triploid and diploid Walleyes in ages 1 – 3. Further research should be conducted to identify alternative stocking strategies and to identify environmental conditions that may increase survival of age-0 triploid Walleyes.

In Chapter Three, I compared mercury bioaccumulation in adult triploid and diploid Walleye in Narraguinnep Reservoir, Colorado, USA, and made several hypotheses that sex- and

ploidy-specific differences in the allocation of energy towards reproductive development would affect mercury bioaccumulation. I tested my hypotheses with linear regression and a bioenergetics model informed by field data. I found diploid Walleye had 28%–31% higher mercury concentrations on average than triploids, but there were no differences between sexes of the same ploidy. Triploids of mature age exhibited minimal gonadal development when compared to diploids. After accounting for reproductive investment, the bioenergetics model accounted for most of the observed difference in average mercury concentration between ploidies for females. Conversely, the energetic cost of producing testes was low, and gonadal development could not explain observed patterns for males. Costs associated with elevated swimming activity and metabolism by diploid males relative to other groups could explain the difference but requires further investigation. The use of triploid fish in stocking programs could prove useful for reducing mercury in fish destined for human consumption.

In Chapter Four, I used triploid Walleye to probe a common, but untested, assumption that forms the basis of several theoretical models of life history theory and organismal growth. The Reproductive Drain Hypothesis is a long-held belief that the energetic cost of reproduction initiates and drives growth deceleration in organisms that grow indeterminately. This theoretical notion remains contentious and has been difficult to test given the inability to control reproduction in wild populations. I tested the Reproductive Drain Hypothesis by comparing the lifetime growth of sterile, triploid Walleye that invest negligible energy into reproductive development with normal, fertile conspecifics co-occurring in the same ecosystem. I found that sterile individuals reached similar maximum sizes as fertile ones (*e*-value = 0.92), and that models constructed with the Reproductive Drain Hypothesis as a key motivation for their development failed to describe the growth patterns of sterile fish (model weight = 0.049). This

evidence implies that reproduction does not limit energy available for growth in body size, challenging the validity of this long-held concept. Instead, I hypothesized that the energetic demand of reproduction is additive rather than compensatory and may motivate individuals to increase food intake to fuel elevated energetic demands post-maturation. My findings improve understanding of how organisms allocate energy and can help refine general mechanistic theories of ontogenetic growth.

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DEDICATION

For Mom

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INTRODUCTION

I.1 Aquatic Biodiversity in Crisis

Freshwater ecosystems are among the most imperiled ecosystems in the world today and biodiversity declines are occurring at a much higher rate in freshwater ecosystems than in marine or terrestrial ecosystems (Ricciardi and Rasmussen 1999). A variety of stressors and drivers are implicated in the decline of freshwater ecosystem function and biodiversity: flow regulation and diversion; physical habitat alteration; degraded water quality; overharvest; and non-native fishes (Dudgeon et al. 2006). Predation, interspecific competition, disease transmission and alterations to food web dynamics caused by nonnative predators can drive declines of native species (Simberloff et al. 2013, Doherty et al. 2016). While interchanges of aquatic species between once isolated assemblages have occurred naturally (Vermeij 1991; Crisci et al 2003), human activities have greatly accelerated the rate and frequency in which these exchanges occur (Olden 2006). The vectors of movement of nonnative aquatic species into new environments include ballast-water discharge, legal and illegal intentional translocations of species for recreation purposes, unintentional releases from aquaculture operations and reservoirs stocked with nonnative sportfish, bait-bucket releases and the exotic pet trade (see Olden 2006). These human actions have resulted in biotic homogenization, the process by which native fish assemblages are gradually replaced by cosmopolitan nonnative assemblages (McKinney and Lockwood 1999; Olden 2006). The degree of biotic homogenization occurring on a global scale is alarming because of its role in decreasing biodiversity. Aquatic biodiversity provides a broad suite of valuable goods and services and is thought to increase resilience in the light of an uncertain future with regards to increasing demands placed on aquatic ecosystems by increasing human

appropriation of water resources, pollution, and climate change (Dudgeon et al. 2006; Schindler et al. 2010).

In the United States, the homogenization of fish assemblages has primarily been driven by intentional introductions of fish for food and/or sport (Rahel 2000). During the last century, the predominant introduction pattern was the introduction of sportfish native to the eastern United States to western states by state and federal agencies to develop recreational fisheries as European settlers considered western waters depauperate of desirable sportfish (Rahel 2000; Billington et al. 2011; Rahel 2016). Today, western United States native fish assemblages face a proportionally higher degree of endangerment than elsewhere in the nation because of significant anthropogenic alterations to natural flow regimes and habitat, coupled with range expansion of nonnative fishes via natural dispersal, as well as both authorized and unauthorized introductions (Warren and Burr 1994; Olden and Poff 2005; Rahel and Smith 2018).

The Colorado River Basin (Figure I.1) has been greatly affected by these patterns of introduction and ranks among the top five river basins in the United States most affected by introductions of nonnative fishes (Fuller et al. 1999). The native fishes of the Colorado River Basin are highly endemic, nearly 70% of the native fishes found in the Colorado River Basin are endemic to the region, a higher proportion than many other river basins in the United States (Burr and Mayden 1992). Historically, the highly endemic native fish assemblage of the Colorado River Basin was comprised of 36 species (Carlson and Muth 1989). Today, the Colorado River Basin is home to over 60 nonnative and less than 30 native fish species (Carlson and Muth 1989; Olden et al. 2006). The upper Colorado River Basin is now host to over 40 nonnative fishes and 14 native species, including six that are endemic to the basin (UCREFRP 1987; Johnson et al. 2008).

FIGURE I.1. Map of the Upper and Lower Colorado River Basin. Source: USGS

Consequently, Humpback Chub *Gila cypha*, Bonytail *G. elegans*, Colorado Pikeminnow *Ptychochelius lucius* and Razorback Sucker *Xyrauchen texanus* garnered endangered species status under the Endangered Species Act (U.S. Office of the Federal Register 1967, 1980, 1991). As a result of their listing, stocking of nonnative, non-salmonid sportfish in the upper Colorado River Basin is largely prohibited, with a notable exception that grants state fish and wildlife agencies the ability to stock sterile triploid sportfish (USFWS 1996). In addition, eradication and control of nonnative piscivores in the upper Colorado River Basin by mechanical removal, chemical control, dispersal barriers, and mandatory harvest began in the late 1980s, with an annual cost exceeding $1 million USD (Mueller 2005; Johnson et al. 2009; Martinez et al. 2014)

In the Upper Colorado River Basin, Channel Catfish *Ictalurus punctatus,* Northern Pike *Esox Lucius*, and Smallmouth Bass *Micropterus dolomieu* are the nonnative sportfish which have received the most attention for native fish recovery. Their increased abundance is of great concern to recovery efforts of native fishes (Hawkins and Nessler 1991; Johnson et al. 2008; Martinez et al. 2009). Efforts to remove Channel Catfish, Northern Pike and Smallmouth Bass are ongoing. Because density of Walleyes *Stizostedion vitreum* (Bruner 2021) is often inversely related to Northern Pike and Smallmouth Bass densities (Forney 1977; Johnson et al. 1977; Colby et al. 1987) suppression of Northern Pike and Smallmouth Bass could allow Walleye to increase their abundance, complicating native fish recovery efforts.

Concern about Walleyes invading critical habitat for native fishes in the rivers of the Upper Colorado River Basin has been growing in recent years. Between 1962 and 2006, Walleyes were seldomly encountered in the Colorado River and its tributaries (Michaud et al. 2018). Biologists conducting nonnative fish removal noticed a significant increase in Walleye encounters in the Green River starting in 2007, and in the upper Colorado River in 2010 (Figure

I.2; Michaud et al. 2018). It is thought that expanding range of Gizzard Shad *Dorosoma cepedianum* in the upper Colorado River and its tributaries has facilitated Walleye range expansion in these waters (Michaud et al. 2018). Furthermore, Walleyes in spawning condition and age-0 Walleyes were observed in the Green River (Monroe and Hendrick 2009; Skorupski and Breen 2012; Harding et al. 2013; Staffeldt et al. 2017; Michaud et al. 2018, Partlow et al 2018). Consequently, Walleyes were targeted for removal in the 2014 nonnative predator protocols of the Upper Colorado River Endangered Fish Recovery Program (Michaud et al. 2018). Even with increases in Walleye observations, they are still less abundant than Northern Pike and Smallmouth Bass in the Colorado River and its tributaries. Despite this, Walleyes have the potential to pose similar, if not greater, threats to native species recovery efforts in the upper Colorado River Basin should they establish a larger presence than currently observed.

I.2 Walleye: Biology and Invasion Potential

Walleye exhibit several characteristics common to successful invaders. These characteristics include Walleye's expansive native range, wide environmental tolerance, high fecundity, predatory behavior, migratory capabilities, and human affinity (Ricciardi and Rasmussen 1998; McKinney and Lockwood 1999). These characteristics suggest that Walleyes may continue to spread throughout the Upper Colorado River Basin and pose an increasing threat to native species there.

Walleyes are ecologically and economically important large-bodied piscivores found throughout North America (Figure I.3.; Scott and Crossman 1973; Collette and Bănărescu 1977; Becker 1983). Latitudinally, Walleyes have an expansive native range, extending from the Mackenzie River delta near the Arctic Coast in the Northwest Territories, southeastward through Quebec, and to as far south as the Gulf Coast drainages of Alabama and Mississippi (Scott and

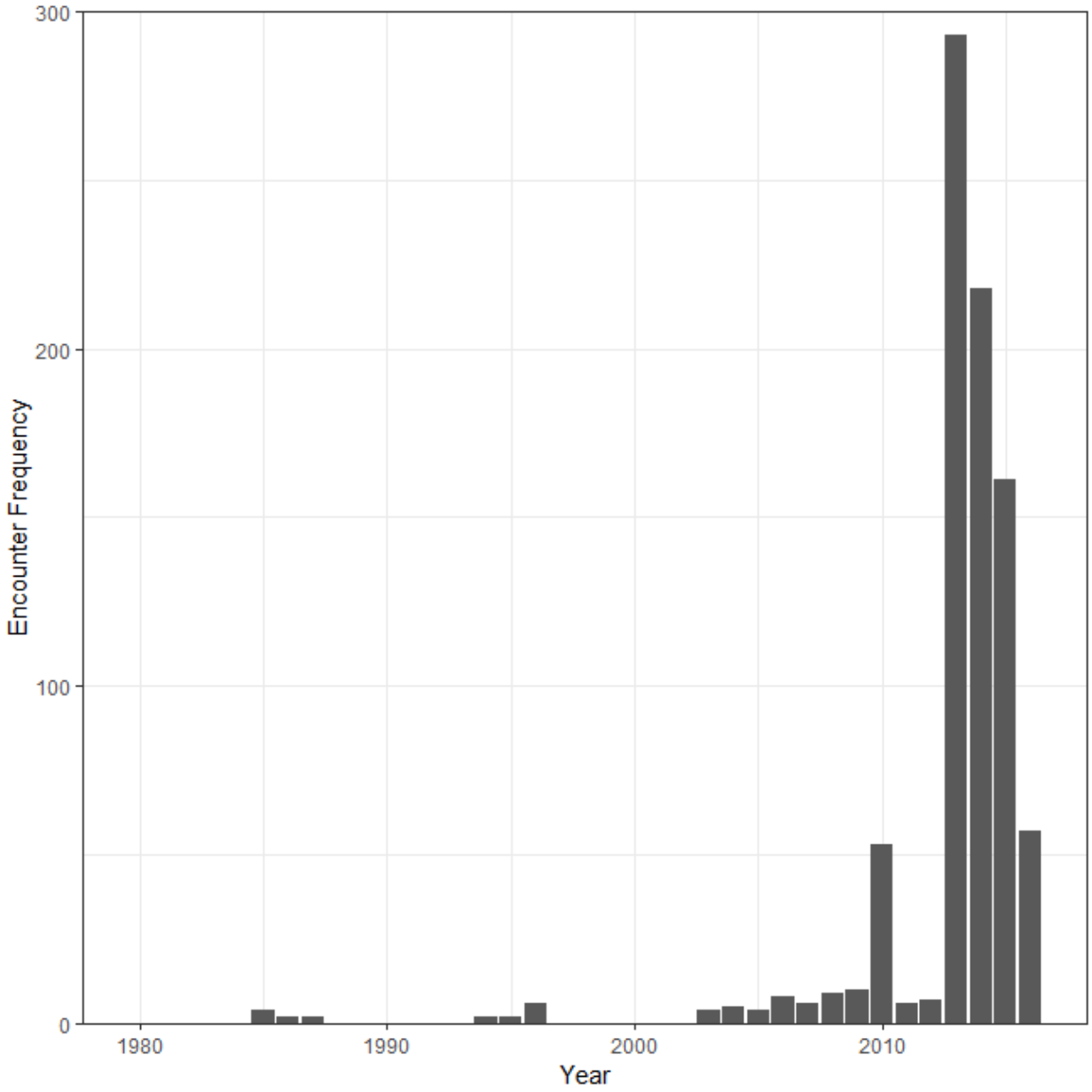

FIGURE I.2. Encounter frequencies for Walleyes during nonnative fish removals in the Upper Colorado River Subbasin, 1980-2018. Modified from Michaud et al. (2018).

Crossman 1973; Collette and Bănărescu 1977). The Walleye's southerly distribution is thought to be limited due to a physiological requirement for waters to cool to a minimum of 10°C during winter for successful gamete development (Bozek et al. 2011a). Walleye native range is bounded longitudinally east of the Rocky Mountains and west of the Appalachian Mountains (Bozek et al. 2011b). Historically, Walleyes were thought to be native to the Missouri River drainage downstream of the Yellowstone River confluence, which included the South Platte River drainage within Colorado (Propst and Carlson 1986). However, more recent studies concluded that Walleyes are not native to the Missouri River drainage, and thus not native to Colorado (Fausch and Bestgen 1997; Galat et al. 2005; Hoagstrom and Berry 2010).

Like many other popular sportfish, the Walleye's range has expanded considerably over the past two centuries, initially due to intentional introductions by federal and state fish and wildlife agencies to provide satisfactory sport fisheries to European settlers, and later, due to dispersal and unauthorized introductions. Native to 25 states, Walleyes now have populations in at least 44 states (Rahel 2000; Billington et al. 2011). Walleyes are present throughout the coastal drainages of the New England and Mid-Atlantic states, the Columbia and Colorado River Basins, and numerous other systems throughout the United States (Bozek et al. 2011a). Walleyes are now among the most widespread fishes in North America. In a geographic sense, Walleye could be considered the most successful piscivore on the continent with a latitudinal range which exceeds that of other top predators (Bozek et al. 2011a; Hoagstrom and Berry 2010). Being a coolwater species, Walleyes can tolerate a wider range of temperatures than coldwater or warmwater fishes (Bozek et al. 2011a). In addition, Walleye exhibit a high degree of life history plasticity, which contributes to their geographic success (Bozek et al. 2011a). Though they are typically thought of as a lacustrine species, Walleye evolved as a riverine species and thrive in

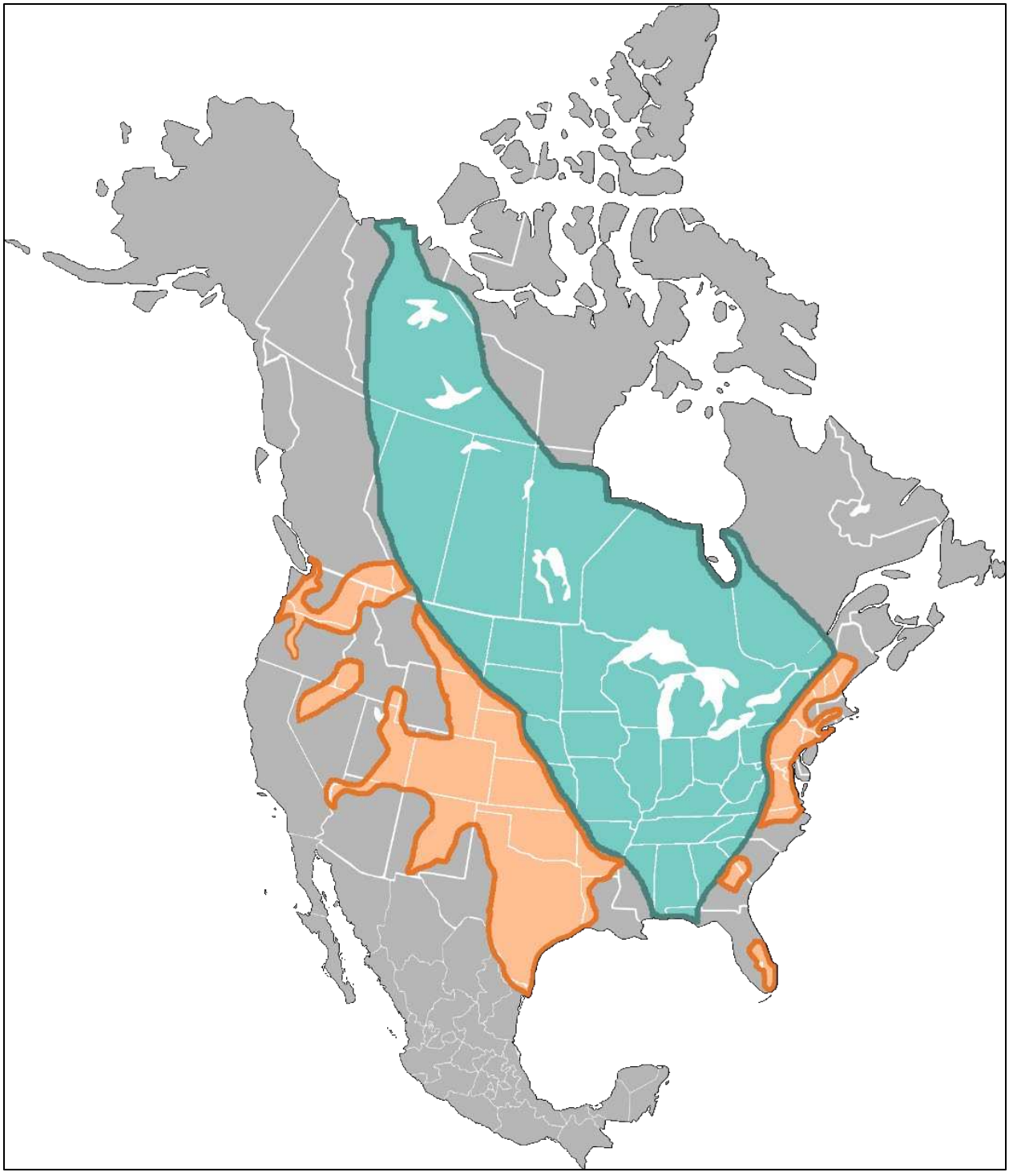

FIGURE I.3. Current distribution of Walleye, with its native range in blue and (putative) nonnative range in orange. Adapted from Scott and Crossman (1973) and Bozek et al. (2011a).

in lotic systems equally as well as lentic systems (Kitchell et al. 1977). This trait should allow Walleyes to move through and establish new populations in the networks of rivers and reservoirs common across the western United States.

Walleyes have reproductive traits that contribute to their potential invasiveness. Walleyes are annual iteroparous broadcast spawners typically preferring coarse substrate, though they can successfully spawn in a variety of habitat types (Preigel 1970; Minor 1980; Bozek et al. 2011a). Walleyes are more fecund than many other nonnative sport fishes (Table I.1), with an average relative fecundity of 60,000 eggs·kg^{-1} body weight (Nickum 1986; Barton and Barry 2011). Initiation of spawning in Walleyes is dependent on temperature and photoperiod; thus, timing varies latitudinally (Bozek et al. 2011a). Reproductive maturity in Walleyes is dependent on climate and sex. Male Walleyes typically reach 50% maturity after experiencing 6,900 cumulative growing degree-days greater than 5°C (GDD_c) and females after 10,000 GDD_c (Venturelli et al. 2010). Ovulation in Walleyes initiates when water temperatures increase to at least 5°C (Bozek et al. 2011a).

Walleyes are highly piscivorous and begin feeding on fish earlier in life than other nonnative predators (Table I.1). Following hatch, larval Walleyes have limited yolk reserves, and exogenous feeding begins at 8-9 mm, before yolk sacs are completely utilized (Engel et al. 2000; Chipps and Graeb 2011). At the onset of exogenous feeding, Walleyes begin feeding on zooplankton (primarily cladocerans and copepods; Mathias and Li 1982), but they will also consume larval fish. Walleyes generally switch to benthivory for a short period following planktivory (Chipps and Greab 2011), but they quickly become more piscivorous, at 30-40 mm (Mathias and Li 1982). Walleyes remain piscivorous for the remainder of their lives, though they

will consume invertebrate prey when availability of piscine prey is limited (Chipps and Graeb 2011). Walleyes are capable of consuming fish up to 50% of their total length (Campbell 1998).

Walleyes have a unique sensory trait which may increase their ability to negatively impact species in systems they invade. Walleyes develop a tapetum lucidum in their retinas during their first year of life (Ali and Anctil 1977). This trait enhances their ability to see in low light conditions, which enables effective foraging during nighttime and in turbid waters (Bozek et al. 2011a). This ability is not common among North American piscivores and gives Walleyes a temporal niche that may separate them from other top predators (Bozek et al. 2011a). In addition, this scotopic vision often represents a novel feeding behavior in systems where Walleyes are nonnative, increasing their potential success as an invader. Walleyes can migrate long distances to spawn in lakes and rivers. This gives Walleyes the potential to rapidly expand their range following introduction to new waters. In Lake Huron, Walleye migrations of up 350 km were observed via acoustic telemetry (Hayden et al. 2014). In the middle Missouri River, 24 radio tagged Walleye migrated a mean distance of 130 km, with one individual migrating 264 km (Bellgraph et al. 2008).

In addition to natural dispersal, illegal stocking of nonnative sportfish poses a serious threat to native fish. Over 46 known incidents of illegal stocking have led to the establishment of populations of nonnative sportfish in Colorado (Johnson et al. 2009). Montana has documented more than 200 illegal fish introductions (Vashro 1995). Despite the fact that government agencies discontinued introductions of nonnative sportfish throughout the western United States, illegal introductions of Walleye continue (Bourret and Clancy 2018) because of their high popularity with anglers. Walleyes are prized among anglers throughout their native and introduced range in North America, and their popularity increases their potential to invade new

TABLE I.1. Life history traits of some non-native piscivores found in the western United States.

Species	Fecundity (eggs/kg)	Age at maturity (y)	Maximum size (kg)	Lifespan (y)	Onset of piscivory (mm)
Brown Trout	3,600[14]	2-4[14]	15[1]	11[15]	130-160[7]
Burbot	49,000[10]	4-6[15]	30[18]	20[18]	103[7]
Lake Trout	1,506[3]	5-19[19]	46[1]	62[5]	150-400[7]
Largemouth Bass	45,000[9]	<1-5[9]	10[1]	24[13]	50-100[7]
Northern Pike	22,700[6]	2-5[17]	25[1]	29[16]	45-100[7]
Smallmouth Bass	15,400[1]	3-4[10]	5.5[1]	15[1]	40-100[9]
Walleye	60,000[2]	2-4[4]	11[1]	32[4]	35-80[11]

Sources: (1) Scott and Crossman 1973; (2) Barton and Barry 2011; (3) Shuter et al. 1998; (4) Bozek et al. 2011a; (5) Behnke 2002; (6) Priegel 1975; (7) Mittelbach and Persson 1998; (8) Muus and Dahlstrom 1972; (9) Carlander 1977; (10) Becker 1983; (11) Mathias and Li 1982; (12) van Densen 1994; (13) Green and Heidinger 1994; (14) Hoitsy et al. 2012; (14) Jonsson et al. 1991; (15) Bailey 1972; (16) Casselman 1996; (17) Billard 1996; (18) Muus and Dahlstrom 1971; (19) Healy 1978.

systems (Quinn 1992). Walleyes were ranked as the fifth most sought-after species in a national survey of U.S. freshwater anglers (excluding Great Lakes anglers) in terms of angler effort in 2016 (USFWS and U.S. Census Bureau 2016). Likewise, Walleyes are popular among Colorado anglers, as more Walleyes are stocked (numerically) in Colorado than any other species of fish (Fetherman et al. 2015). The Upper Colorado River Basin is a complex socio-ecological system, and as a result, conflict has arisen as how to simultaneously manage for endangered native fishes while providing high value nonnative sport fisheries (McMahon and Bennett 1996; Clarkson et al. 2005). The stocking of triploid nonnative sportfish may ameliorate some of these conflicts by giving anglers the opportunity to have access to robust nonnative sport fisheries, while minimizing the potential for nonnative sportfish to establish populations in critical habitat and cause long-term harm to native fishes.

I.3 Induced Triploidy: Theory and Management Implications

Induced triploidy has great potential as a tool to manage nonnative sportfish. Somatic cells of triploid Walleyes contain three sets of chromosomes, rather than two sets found in normal diploid Walleyes (Legatt and Iwama 2003). Triploidy is caused by blocking the extrusion of the second polar body following fertilization (Flajšhans et al. 1993). Induced triploidy usually causes sterility because the odd number of chromosome sets disrupts meiosis and leads to a failure of gonadal development or the production of aneuploid gametes (Thorgaard 1983).

Swarup (1959a, 1959b) was the first to successfully raise induced triploid fish to adulthood and compare their growth and gonadal development to diploids. Baumgartner et al. (1986) was the first to successfully induce triploidy in Walleyes. Triploidy can be induced by a variety of methods including heat shocks, cold shocks and chemical shocks, but the preferred method to induce triploidy in Walleyes is by hydrostatic pressure shocks and is the method

Colorado Parks and Wildlife (CPW) uses to produce triploid Walleyes (Malison et al. 2001; Fetherman et al. 2015).

Triploid males often appear to show similar rates of gonadal development to that of diploid males, likely because triploidy does not limit the mitotic divisions associated with testes development; however, triploid spermatozoa are aneuploid and cannot fertilize diploid ova (Thorgaard 1983; Malison and Garcia-Abiado 1996; Benfey 1999). Triploid females typically do not show much gonadal development relative to diploid females, because meiosis is much more involved in the development of ovaries than it is for the development of testes (Benfey 1999). However, Malison and Garcia-Abiado (1996) found gonadal development in juvenile triploid Walleyes to be less than that of diploid controls for both males and females.

In diploid Walleyes males and females follow similar growth trajectories until they reach sexual maturity (Bozek et al. 2011a). Males typically reach sexual maturity prior to females, at about 80% of the length at which females reach sexual maturity, at which point sexually dimorphic growth begins (Bozek et al. 2011a). Females ultimately reach larger sizes than males, where male maximum length is approximately 80% of female maximum length (Bozek et al. 2011a). Theoretically, due to reduced energetic requirements for gonadal development in triploid fishes relative to diploids, it is hypothesized that triploids will grow faster and reach larger sizes relative to their diploid counterparts (Leary et al. 1985; Tiwary et al. 2004; Maxime 2008). In fact, this expectation has been a primary rationale for producing triploids for aquaculture (Thorgaard 1983; Tiwary et al. 2004). However, the literature on triploid growth performance is inconclusive; triploids have been observed to grow poorer than, equal to, and greater than diploid controls both among and within species (Galbreath et al. 1994; McGeachy et al. 1995; Tiwary et al. 2004). Several studies have found triploids to be less aggressive than diploid controls, which

could help explain the variable growth observed in the literature, as less aggressive individuals may be at an energetic disadvantage (Benfey 1999).

The effects induced triploidy has on survival are complex, affecting life stages and species differently. In early life stages, triploids often exhibit lower survival than diploids. The induction process is likely stressful to the organisms and may lead to decreased survival rates among triploids relative to diploids. For example, during the experimental production of triploid Rainbow Trout *Oncorhynchus mykiss,* Lincoln and Scott (1983) found that hatching rates of triploids were 30-59% to that of diploids. During CPW's production of triploid and diploid Walleye fry in 2018, hatching rates of triploids were 73% to that of diploids (A.G. Hansen, CPW, unpublished data). After hatching, triploid fish may have increased rates of skeletal and anatomical malformations relative to diploids, which may increase early mortality following hatch (Maxime 2008; Koch et al. 2018). However, once triploids reach sexual maturity, they may have increased survival relative to diploids, as they may experience less of the energetically taxing and stressful effects of spawning that diploids face (Thorgaard 1983).

The application of triploid stocking programs offers many potential advantages to fisheries managers. Sterility of triploids gives managers a greater degree of control over population characteristics, limits the potential for introgression between cultured and wild stocks, and limits the potential for nonnative sportfish to establish populations in novel environments (Piferrer et al. 2009). In addition, the potential for triploids to grow faster and to greater maximum sizes may give managers the opportunity to produce trophy sport fisheries (Thorgaard 1983).

For these reasons, state fish and wildlife agencies throughout the West are excited about the prospect of stocking triploid Walleye. Colorado was the first state to stock triploid Walleyes,

beginning their triploid Walleye stocking program in 2008. To date, triploid Walleyes have been stocked in at least nine waters in Colorado. Montana Fish, Wildlife & Parks stocked triploid Walleyes from 2009 to 2014 at Bighorn Lake to minimize introgression with genetically pure Sauger *Sander canadensis* populations (Dalbey et al. 2016). Utah Division of Wildlife Resources began stocking triploid Walleye in 2017. Despite the great interest in stocking triploid Walleyes by state fish and wildlife agencies in the West, very little is known about how these fish perform in the wild.

The literature on triploid Walleyes is relatively sparse; a recent search of Web of Science conducted on April 28th, 2019, using the search terms "*ploid*" AND ("Sander vitreus" or "Stizostedion vitreum*")[1] found 12 scientific articles. Of these 12, only four were focused solely on Walleyes. Of these four publications, the topic of investigation was limited to the production and identification of triploid Walleyes. Prior to 2022, there were no peer-reviewed publications evaluating performance of triploid Walleyes in a field or laboratory setting. However, a few experimental and observational studies comparing triploid and diploid saugeyes *Stizostedion vitreum x S. canadense* have been published.

Garcia-Abiado et al. (2002) examined mean length, weight, and survival of heat-shocked triploids relative to heat-shocked diploid controls after periods of 40 and 193 days. After 40 days, triploids were significantly larger ($P < 0.001$) than diploids, but triploids experienced much lower survival (22.4%) than diploids (94.7%). After this 40-day period in hatchery ponds, 25,851 heat-shocked saugeyes were stocked into O'Shaughnessy Reservoir, Ohio. Twenty-three saugeyes were sampled from O'Shaughnessy Reservoir 153 days later, and triploids ($n = 12$) had

[1] The long recognized binomial name for Walleye, *Stizostedion vitreum,* was changed to *Sander vitreus* in 2003 (Nelson et al. 2003), though there is debate as to which name is correct (Bruner 2021; Scharpf and Fricke 2022). Thus, I included both names in the search to capture all papers published on triploid Walleye.

a significantly smaller ($P < 0.01$) mean length (211.9 ± 16.3 mm; mean ± SD) and weight (70.8 ± 20.9 g) relative to heat-shocked diploids (n = 11; 245.5 ± 18.9 mm; 111.3 ± 33.4 g).

Czesny et al. (2002) conducted three feeding experiments with juvenile diploid and triploid saugeyes to investigate potential differences in foraging behavior by comparing prey selection, ingestion rates, and handling time between the two ploidies. They found that diploids selected larger and more energy dense prey, had greater foraging efficiency, and required less handling time than triploids. The authors concluded that the advantage diploids showed over triploids would likely translate to faster growth rates and higher survival for diploids in a natural environment, but this prediction has not been tested.

There is one other paper evaluating diploid and triploid saugeye recruitment, growth and condition (Koch et al. 2018). Paired lots of diploid and triploid saugeye fry (2,500 fry ha^{-1}) were stocked into four Kansas reservoirs in 2014, 2015, and 2016. These fish were sampled annually in autumn via nighttime electrofishing. Averaged across age-class, catches were dominated by diploids, which represented 75-87% of the catch in the four reservoirs, suggesting that diploids had higher survival than triploids. Diploid saugeyes were generally larger than triploids at age-0, but age-1 and age-2 diploids and triploids were similarly sized. However, almost nothing is known about the performance of triploid saugeye adults, and there have been no studies of adult Walleye triploids. Clearly, there is a need to conduct field evaluations of the growth and survival of triploid Walleyes, and its long history of stocking triploid Walleyes makes Colorado an ideal location to perform this research.

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CHAPTER ONE

Cytological and molecular approaches for ploidy determination: results from a wild Walleye population[2]

1.1 Introduction

Artificially induced triploidy is used in aquaculture worldwide to increase growth efficiency, improve flesh quality, and prevent unwanted reproductive development of cultured organisms (Piferrer et al. 2009; Zhou and Gui 2017). Interest in stocking sterile fish is rising among fisheries managers for situations where natural reproduction is undesirable (Budy et al. 2012; Farrell et al. 2022) and may even be required by state regulation (California Code of Regulations 2021). While there are emerging techniques for producing sterile fish that show great promise (Zohar 2021), inducing triploidy is one of the most common and practical methods for large-scale production of sterile fish (Maxime 2008), especially in cases where interspecific hybrids are capable of successful reproduction (Bartley et al. 2000). For example, triploid Grass Carp *Ctenopharyngodon idella* are stocked outside their native range for vegetation control and triploid sport fish like Walleyes *Stizostedion vitreum* (Farrell et al. 2022), saugeyes (Walleye × Sauger *S. canadense*; Koch et al. 2018), and Cutthroat Trout *Oncorhynchus clarkii* (Cassinelli et al. 2019) are stocked to provide angling opportunities where reproductive and genetic containment are necessary to protect native fish stocks. As perfect induction of triploidy is rarely achieved (Fetherman et al. 2015), prestocking ploidy determinations are necessary to ensure that

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batches of fish with low induction rates are not released onto the landscape. Likewise, since ploidy is rarely visually identifiable (Maxime 2008), those interested in studying the effects of induced triploidy on life-history traits and population dynamics must determine the ploidy of sampled individuals.

Flow cytometry is a cytological method for determining ploidy and is considered the "gold standard" among practitioners (Fiske et al. 2019; Hubalek and Flajshans 2021), as it has been deemed the most accurate technique for determining ploidy in fish (Maxime 2008). Flow cytometry quantifies the relative nuclear DNA content of individual cells, typically erythrocytes in fishes, by measuring the fluorescence emitted by stained nuclear DNA (Allen 1983). Coulter counter analysis is another common cytological method for determining ploidy, which measures nuclear volume of erythrocytes as a proxy for nuclear DNA content (Wattendorf 1986) and is nearly as accurate as flow cytometry (Fiske et al. 2019). There are other ploidy determination methods (e.g., karyotyping, blood smears), but these may not be suitable when processing multiple samples, as most of these methods are time consuming (Maxime 2008; Fiske et al. 2019).

Using cytological methods to determine ploidy can be logistically challenging, especially when sampling fish in remote field locations. For example, when using erythrocytes for cytological ploidy determination, blood must be collected prior to postmortem clotting, as clotted blood makes ploidy determination difficult or impossible. Additionally, blood samples can rapidly deteriorate if not handled properly, must be stored with an anticoagulant (e.g., heparin), refrigerated, and analyzed 14–30 d post collection. Extending the life of blood samples used for ploidy determinations is possible but requires the use of additional specialized methods (Brown et al. 2000; Jenkins and Thomas 2007; Hubalek and Flajshans 2021). Furthermore, collecting

blood samples requires additional equipment (i.e., syringes and needles, heparinized tubes) and skill on the part of the collector, and the procedure can be stressful to the fish. Fisheries managers, biologists, and researchers could benefit from alternative methods for determining ploidy from fish collected in the field. Molecular-based methods of ploidy determination are relatively new and offer an alternative approach that can overcome the difficulties associated with using cytological methods in the field while also providing additional genetic information that can be used for other analyses. Collection and storage of tissue samples for molecular-based ploidy determination is relatively simple; epithelial tissue from a fin is a preferred source of DNA for extraction (Wandeler et al. 2007) and can be easily and non-lethally collected from a fish using only a clean pair of scissors (Pratt and Fox 2002). Properly stored tissue samples (e.g., stored in ethanol, frozen, or dried overnight at room temperature) have a shelf life measured in years to centuries rather than weeks (LaHood et al. 2008; Hubalek and Flajshans 2021). While cytological approaches, especially when using erythrocytes, typically require fish in relatively good condition (i.e., alive or recently deceased), tissue samples used for molecular-based approaches can be used on specimens that have been dead for decades (Wandeler et al. 2007), eliminating the concern of obtaining high-quality samples from fish rapidly postmortem. In this study we compared paired Coulter counter- and molecular-based ploidy determinations for individual fish to test agreement between these two methods and to assess the utility of molecular-based methods for determining ploidy in a percid. Samples (paired blood and fin tissue) for ploidy determination were collected from a population of mixed-ploidy Walleyes in a southwestern Colorado reservoir within the upper Colorado River basin, where triploid Walleyes are stocked to satisfy local angler demand for high-quality sport fisheries while minimizing the

potential spread (natural and illegal) and establishment of nonnative piscivores into areas deemed important for endangered native fish recovery efforts (Farrell et al. 2022).

1.2 Methods

1.2.1 Fish Sampling

Narraguinnep Reservoir is a 215-ha irrigation supply reservoir in southwest Colorado, USA. Colorado Parks and Wildlife (CPW) stocked diploid Walleye in Narraguinnep on an irregular basis from as early as 1972 until 2004. In 2008, Colorado Parks and Wildlife (CPW) began stocking triploid Walleye into Narraguinnep Reservoir resulting in a mixed population of diploid and triploid Walleye (Farrell et al. 2022). Fish were collected using gillnets configured for fall Walleye index netting (FWIN; Morgan 2002) during spring in 2019, and spring and fall in 2020. Gillnets were set overnight during spring sampling and were set for 2 – 4 h during fall sampling. Water temperatures during both sampling periods were less than 12°C, and limited netting related mortality occurred. Fish condition (i.e., alive/dead) was noted prior to processing for tracking sample quality for Coulter counter analyses. Sampling procedures were approved by the Institutional Animal Care and Use Committee (Protocol # 18-7822A) at Colorado State University.

Sagittal otoliths were collected for age determination and cohort assignment. Because CPW began stocking triploid Walleye in 2008, and Walleyes have been documented to reach >20 years old in this population (Farrell et al. 2022), fish born prior to 2008 could be classified as known diploids. While spontaneous polyploidy is rare in higher teleosts such as order Perciformes (Leggatt and Iwama 2003), fish born prior to 2008 were also screened for ploidy using Coulter counter analyses. Otoliths were sectioned transversely through the core and examined with a compound microscope using reflected light at 40-100x magnification. Ages were assigned, blind to fish length, three times by an experienced reader. When ages disagreed,

the median integer age was used as the final age. Ploidy determinations were performed independently by different laboratories that were blind to fish age/cohort assignment.

Blood samples were collected with syringes via cardiac puncture immediately following euthanasia (Duman et al. 2019), stored in tubes coated with lithium heparin kept at 4°C until Coulter counter ploidy determinations could be performed by the Genomic Variation Laboratory at the University of California Davis. Coulter counter analyses were conducted 3 – 7 days post collection. A Z2 Coulter Particle Count and Size Analyzer (Beckman Coulter, Inc., Brea, CA, USA) was used for cytological ploidy determination (Fiske et al. 2019). One microliter of blood was pipetted from the lithium heparin tubes and placed into a 25 mL cuvette containing 10 mL of Isoton II diluent and 3 drops of Zapoglobin II lytic reagent to measure the volume of the erythrocyte nuclei. Previous research indicated that nuclear volumes exhibit less variance than whole erythrocyte volumes and are more appropriate for ploidy determination (Fiske et al. 2019). Approximately 20,000 erythrocyte nuclei were measured for each sample, and the modal value of the nuclear volume was recorded (Fiske et al. 2019).

A 3-cm2 fin clip was collected for genetic analysis from the lower lobe of the caudal fin using scissors and forceps that were sanitized with 70% ethanol (note: smaller tissue samples [e.g., <3-mm2] are likely still effective for genetic ploidy determination). Fin clips were dried at ambient temperature and stored on Whatman paper until genetic analyses could be completed by the Idaho Department of Fish and Game's Eagle Fish Genetics Laboratory. Samples were genotyped for a panel of 151 loci following the GTseq method of amplicon sequencing (Campbell et al. 2015). These loci were a subsample of those described by Bootsma et al. (2020) that were previously found to be polymorphic within the first 79 bp in either this population of Walleye or those present in Idaho (IDFG, unpublished data). Reads were aligned to reference

amplicon sequences using Bowtie2 (Langmead and Salzberg 2012) with parameters "--rdg 0,5 --rfg 0,5 --score-min L,0,-.76" to help account for high variability within the targeted loci. Read counts for each allele were extracted using microTyper (https://github.com/delomast/microTyper). To compare models of diploidy and triploidy, a critical log-likelihood ratio (LLR) was calculated using the read counts for each SNP allele with the method described by Delomas (2019) and implemented in the tripsAndDipR R package (https://github.com/delomast/tripsAndDipR). For loci with multiple SNPs (i.e., microhaplotypes), a maximum of one SNP per locus was used. In these cases, the SNP closest to the forward primer that passed the binomial test described by Delomas (2019) was selected. We genotyped 231 samples to use as a training set to establish LLRs used for determining ploidy from molecular data following the method of Delomas (2019). The LLRs of the training samples were manually assessed to determine critical values for discerning diploids from triploids. The critical value for diploidy was set at -5, as this value excluded all triploid training samples and aligned with critical values identified from similar panels in salmonids Delomas (2019). The critical value for triploidy was set at 200, as this value excluded all diploid training samples. Fin tissue samples were categorized as either triploid ($LLR \geq 200$), diploid ($LLR \leq -5$) or ambiguous ($200 > LLR > -5$). We then used 118 samples, where Coulter counter-based ploidy determinations were withheld from the genetics laboratory, as a test set to evaluate agreement between the two methods.

1.2.2 Statistical Analysis

All statistical analyses were performed using R 4.0.3 (R Development Core Team 2022). We used Cohen's kappa to assess agreement between the two ploidy determination methods (Cohen 1960). Cohen's kappa adjusts for chance agreement (van Stralen et al. 2012); values ≤

0.2 indicate poor agreement and values ≥ 0.9 indicate excellent agreement (Landis and Koch 1977; Byrt 1996). Cohen's kappa values were calculated using the irr package in R (Gamer et al. 2019). Figures were prepared using the package ggplot2 (Wickham 2016).

1.3 Results

Walleyes in this study ranged in size from 179 – 680 mm TL (Table S1). Diploids from the 1998 – 2020 cohorts, and triploids from the 2011 – 2018 cohorts were present in the training and test sets. Because triploid Walleye stocking at Narraguinnep Reservoir began in 2008, and no triploids were assigned to cohorts born prior to 2011, diploid Walleye belonging to the 1998 – 2007 cohorts were treated as individuals of known ploidy, which comprised 54 individuals in the training set, and 7 individuals in the test set.

The mean modal nuclear volumes of erythrocytes estimated by Coulter counter for Walleyes in both the training and test sets were 12.4 μm3 (SD = 0.733 μm3) for diploids and 17.6 μm3 (SD = 0.952 μm3) for triploids (Figure 1.1A). Mean erythrocyte volume for triploid Walleye was approximately 1.42x larger than their diploid conspecifics. Two Walleyes in the test set had nuclear volumes of 24.3 (age = 5) and 24.8 μm3 (age = 8), much larger than expected for a triploid Walleye (Figure 1.1A). Because the nuclear volume of erythrocytes for these two individuals was approximately 1.40x greater than measured in triploids, they were classified as putative tetraploids, as nuclear volume increases by 50% for each unit increase in ploidy level (Benfey 1999).

Calculated LLRs from molecular data had distinct distributions for triploid and diploid Walleyes. Log-likelihood ratios were not normally distributed (Figure 1.1B); median LLRs were -1,795 (IQR = -3,426 – -1,261) for diploids and 3,297 (IQR = 1,591 – 4,587) for triploids.

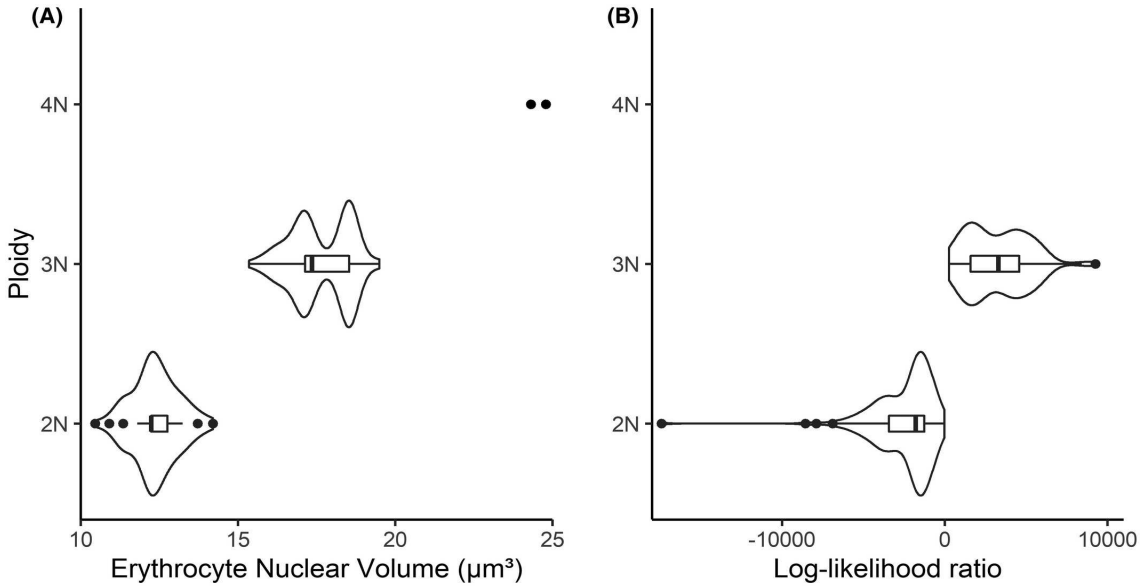

FIGURE 1.1 – (A) Modal nuclear volume of Walleye erythrocytes measured with a Coulter counter. (B) Log-likelihood ratios from genetics using read counts for each SNP allele with the method described by Delomas (2019). Training and test data sets included *n* = 349. 2N = diploid; 3N = triploid; 4N = tetraploid

After applying the critical LLR values established from the training set to the test set, we found 98.3% agreement between the molecular and cytological ploidy determinations. These separate methods disagreed for only two individuals (Table 1). These two individuals were those deemed tetraploid via the Coulter counter method (modal erythrocyte nuclear volumes: 24.3 and 24.8 μm3), but triploid via genetics (LLRs: 4,399 and 4,691). No triploids were misclassified as diploid, nor were any diploids misclassified as triploids. All individuals of known ploidy (i.e., diploids from cohorts born in 2007 and prior) were correctly assigned as diploids by both methods in both the training (n = 54) and test sets (n = 7). Cohen's kappa for the comparison of these two methods was 0.965 (95% CI = 0.917 – 1.00) for the test set, which indicated excellent agreement (Landis and Koch 1977; Byrt 1996).

1.4 Discussion

To our knowledge, this is the first study to examine agreement between cytological and molecular ploidy determination methods for a percid, and to report distributions of erythrocyte nuclear volumes for diploid and triploid Walleye. Molecular techniques have been successfully used to determine ploidy in White Sturgeon *Acipenser transmontanus* (Delomas et al. 2021), Atlantic Salmon *Salmo salar* (Glover et al. 2015; Jacq 2021), Brook Trout *Salvelinus fontinalis*, and Chinook Salmon *Oncorhynchus tshawytscha* (Delomas 2019). Our study further supports that molecular ploidy determination techniques work extremely well and give results indistinguishable from cytological methods. We found 98.3% agreement between molecular and cytological ploidy determinations. The two individuals with non-congruent ploidy assignments were called tetraploid by Coulter counter and triploid by amplicon sequencing. The amplicon sequencing analysis used here assumed all samples were either diploid or triploid, and so misclassification of a true tetraploid is expected. Alternative methods exist that can be used in

TABLE 1.1 – Confusion matrix comparing the ploidy assignments by cytological (Coulter counter) and molecular (genetics) methods of ploidy determination in the test set (*n*= 118).

Cytological Ploidy Calls	Molecular Ploidy Calls		
	2N	3N	4N
2N	73	0	0
3N	0	43	0
4N	0	2	0

situations where ploidies other than diploid and triploid are present (Gompert and Mock 2017; Weiß et al. 2018; Delomas et al. 2021), though a sufficient sample of known polyploids other than triploids would be needed to establish criteria for differentiating higher ploidy levels.

It is unlikely that tetraploids exist in the Narraguinnep Walleye population, and more likely that the Coulter counter misclassified these triploids as tetraploids. Previous attempts to produce tetraploid finfish in species without multiple naturally occurring cytotypes have largely yielded tetraploid embryos that are inviable beyond the larval stages (Arai and Fujimoto 2018) with viable tetraploids being reported for only a few species (Chourrout et al. 1986; Nam et al. 2001). Induction of tetraploidy in Walleye has been demonstrated, but no tetraploids survived past the larval stage (Malison et al. 2001). Furthermore, it is unlikely that tetraploids were accidentally created during the induction process. Colorado Parks and Wildlife uses hydrostatic pressure shocks to induce triploidy in Walleyes by preventing the extrusion of the second polar body (Piferrer et al. 2009). The time of pressure initiation CPW uses for induction of triploidy is 8 min post-fertilization (Fetherman et al. 2015). Malison et al. (2001) used hydrostatic pressure shocks to induce tetraploidy in Walleyes with a time of pressure initiation of 192 min post-fertilization, which suggests that tetraploidy is unlikely to be inadvertently induced in Walleye during triploid production. It is possible that the blood samples for the two fish classified as tetraploid by the Coulter counter were poorly handled in the field and clotting or cellular swelling occurred between collection and processing, causing the erythrocyte nuclei increase in size and appear as tetraploids (Krasznai and Goda 2021). Also, temperature fluctuations can shift estimated nuclear volumes (Schreier et al. 2021). However unlikely, the potential existence of adult tetraploid Walleye suggested by the results of this study warrants confirmation and further investigation.

The choice of ploidy determination method is situationally dependent as each comes with its own advantages and disadvantages. For example, cytological methods can cheaply and accurately measure percent triploidy in batches of larval fish (Jenkins et al. 2017). Also, cytological approaches generally do not require species-specific development, whereas molecular methods require development of a genotyping panel for a given species if a suitable panel does not already exist. In cases where ploidy information is needed immediately (e.g., to inform stocking or production decisions) Coulter counter analysis can provide ploidy determinations results more rapidly than molecular methods (Wattendorf 1986; Fiske et al. 2019). One sample takes only minutes when using the Coulter counter method but can take several hours when using a molecular approach.

However, field researchers and biologists often have other considerations, and a longer delay between sample collection and ploidy determination is acceptable. Fin clips can easily be stored (e.g., in a scale envelope or on Whatman paper), preserved by being dried at ambient temperature overnight, and collected and analyzed at a time convenient for the researcher/biologist. The increased shelf life for molecular-based ploidy determination can allow the researcher/biologist more time to collect more data on individuals (e.g., age) and save money by affording them the opportunity to be more selective with samples sent for ploidy determination. Tissue samples that were collected for other studies where ploidy determination was not the main goal could be used for retroactive analyses. Furthermore, samples can be archived for future studies of populations of mixed ploidy. Obtaining blood samples of satisfactory quality for cytological ploidy determinations requires fish in good condition (i.e., prior to postmortem blood clotting), which can preclude the use of certain fish collection techniques. For example, standardized gillnetting techniques like FWIN or North American

netting can be used to estimate fish density but require soak times of 16 – 24h (Giacomini et al. 2020). Such long soak times can preclude the use of cytological approaches to determine ploidy, as fish can be dead for far too long to obtain quality blood samples. Molecular ploidy determination methods enable the use of these types of approaches for monitoring and researching mixed diploid-triploid populations.

Researchers and managers can also obtain more information for a similar price by using molecular methods to determine ploidy. Cost does vary from lab to lab, but in our case, genetic analyses cost $10.05 USD/ sample while Coulter analyses cost $10.32 USD/ sample. Data obtained via cytological approaches is limited to estimations of DNA content, cellular and nuclear volumes, and ploidy. Alternatively, genetics data obtained for ploidy determination can be used for assessing population structure (Garza et al. 2014), parentage-based tagging (Steele et al. 2013), estimating effective population size (Wang et al. 2016), estimating abundance using close-kin mark-recapture (Bravington et al. 2016), or any of the myriad applications of genetics in fisheries (Carvalho and Pitcher 1995). Additionally, molecular approaches would be able to discriminate hybrids. For example, Montana Fish, Wildlife and Parks have stocked triploid Walleyes in Bighorn Reservoir (spanning the Montana-Wyoming border) since 2009 to reduce potential hybridization and introgression with native Saugers (Dalbey et al. 2016; S. Blackburn, Montana Fish, Wildlife and Parks, personal communication), and a molecular approach to ploidy determination would be able to identify triploid Walleyes and help discriminate between hybrid and pure Saugers. Moreover, tissues collected for DNA extraction can be used for other, non-genetic analyses. For example, fin clips have long been used in mark-recapture studies (VanDeValk et al. 2007), contaminant biomonitoring (Heltsley et al. 2005; Cerveny et al. 2016), and stable isotope analyses (Sanderson et al. 2009). Furthermore, it may be possible to use

molecular-based ploidy determination techniques for retrospective analyses of archived samples for which ploidy determination was not intended (Cuveliers et al. 2009; Price et al. 2019).

While molecular-based methods are appealing from several standpoints, those collecting samples in the field must be aware that cross-contamination is a greater concern than for cytological methods. While DNA sequencing is less sensitive to cross-contamination than other molecular techniques like eDNA (Rodgers 2017), it can occur and render samples useless. Practitioners should ensure that instruments are sufficiently cleaned between each sample that is collected.

Emerging concerns over stocking non-native sportfish or native fish of different lineages than those in recipient systems in some western states and provinces has increased interest in the use of triploids as an alternative stocking strategy to mitigate potential problems associated with releasing diploid conspecifics onto the landscape, especially where there are concerns of their predatory (Farrell et al. 2022), competitive (Budy et al. 2012), or genetic (Koenig et al. 2011) impacts on native species. In addition to their use by Montana Fish, Wildlife and Parks in Bighorn Reservoir, triploid Walleyes are currently being used by Utah Division of Wildlife Resources (UDWR) and CPW to provide alternative sport fisheries while mitigating the potential for Walleye to establish themselves in the Colorado River and undermine endangered native fish recovery efforts, as required by a cooperative agreement among CPW, UDWR, Wyoming Game and Fish, and the U.S. Fish and Wildlife Service (USFWS 1996). This cooperative agreement prohibits the stocking of nonnative, non-salmonid sportfish in the upper Colorado River Basin, with a notable exception that grants state fisheries agencies the ability to stock sterile triploids (USFWS 1996). While the primary intention of stocking of triploid sportfish is to reduce the impact of natural dispersal, providing anglers opportunities to catch these sportfish may also

reduce the extent of illegal stocking (Johnson et al. 2009). With growing interest in using triploid sportfish, other species may be triploidized and studies examining species-specific impacts of triploidization on life-history and population dynamics will be needed for fisheries management purposes. As such, managers, biologists, and researchers should be aware of alternative approaches to ploidy determination that may better suit their needs.

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CHAPTER TWO

Relative performance juvenile triploid Walleye in the wild

2.1 Introduction

The artificial induction of triploidy has been frequently used in finfish and shellfish commercial aquaculture (Benfey 1999; Piferrer et al. 2009) and to produce Grass Carp *Ctenopharyngodon idella* for aquatic vegetation control (Allen and Wattendorf 1987). The primary motivation for using triploid fish is because their reproductive sterility reduces the risk of unwanted reproduction and genetic introgression should fish disperse from their desired locale (Benfey 1999; Piferrer et al. 2009). Interest in using triploid fish has risen among sport fisheries managers because they provide a lower-risk stocking option that is one of few methods to reconcile sportfish management with native species conservation (Martinez et al. 2009; Budy et al. 2012; Cassinelli et al. 2019) and one of few methods to buffer against illegal stocking (Johnson et al. 2009).

Triploid Walleye *Stizostedion vitreum* (see Bruner 2021) have been stocked by state and provincial fisheries agencies in Colorado, Utah, Montana, and Alberta since as early as 2008. Walleye management in the Colorado and Utah portions of the upper Colorado River Basin, USA, emphasizes a suite of measures to reconcile nonnative sport fishing with the recovery of threatened and endangered fish endemic to the Colorado River and its tributaries. This includes stocking triploids to diversify recreational angling opportunities, deter illegal fish stocking, limit the possibility of new populations of Walleye in sensitive areas, and possibly interfere with reproduction in unwanted diploid populations (Johnson et al. 2009; Farrell et al. 2022a). Within the upper Colorado River Basin, Colorado Parks and Wildlife stocks triploid Walleyes into

Narraguinnep, Rifle Gap, and Puett reservoirs, and Utah Division of Natural Resources stocks triploid Walleyes into Red Fleet and Big Sand Wash reservoirs (R. Shields, Utah Division of Wildlife Research, personal communication). Outside of the upper Colorado River Basin, triploid Walleyes are typically stocked to limit unwanted introgression with native *Stizostedion* species. In Montana, triploid Walleye have been stocked in Bighorn Reservoir semi-regularly since 2009 to prevent hybridization of Walleyes with native Saugers *Stizostedion canadanse* (Bramblett and Zale 2016; S. Blackburn, Montana Fish, Wildlife, and Parks, personal communication). In Alberta, triploid Walleye were stocked into Lac Ste. Ann in 2021 to reduce the risk of introgression with wild Walleye already present in the system (S. Fithen, Alberta Environment and Parks, personal communication). While this describes most extant triploid Walleye populations and stocking programs, there is interest in expanding triploid Walleye stocking throughout the western U.S. and Canada.

Despite growing interest in stocking triploid Walleye, there is a lack of basic information regarding their post-stocking performance. Managers need to know more about how growth and survival of triploids compares to diploid conspecifics to use triploid Walleye effectively. It has often been hypothesized that triploid fish should grow larger than diploid conspecifics because they do not invest energy towards reproduction (Benfey 1999; Maxime 2008; Piferrer et al. 2009). However, a triploid growth advantage has rarely been demonstrated, as triploid fish typically do not grow larger than diploid conspecifics (Maxime 2008). It should be noted that much of the literature regarding comparisons of growth between diploid and triploid conspecifics is limited to larval and juvenile fish, and one would not expect a growth advantage to appear in triploids until after diploids begin devoting energy towards reproductive development. Farrell et al. (2022a) is the only published information regarding triploid Walleye growth, and they found

that adult triploid Walleyes produced via hydrostatic pressure grew similarly to diploid conspecifics. There is relatively more information for saugeye (Walleye x Sauger). Garcia-Abiado et al. (2002) found that after 40 days, heat-shocked saugeye (62.5 – 86.7% triploids) were significantly larger than control diploids. In the same study, heat-shocked saugeyes (69% triploid) were stocked into O'Shaughnessy Reservoir, Ohio. Triploids were significantly smaller than diploids, by approximately 14%, 153 days post-stocking. Koch et al. (2018) found that in autumn, age-0 triploid saugeye stocked in four Kansas reservoirs were slightly smaller on average than diploid saugeye, but there were no apparent differences in mean total length at age-1 or age-2.

Similarly, there is a paucity of information regarding the relative survival of triploid Walleyes. Overall, the effect of triploidy on survival is mixed, both among and within species. Triploids often exhibit lower survival than diploids in early life stages (Ihssen et al. 1990; Piferrer et al. 2009; Fraser et al. 2012b). Survival of post-larval triploid fish is generally similar to diploid conspecifics, particularly when reared separately (Piferrer et al. 2009; Fraser et al. 2012b). However, larval and juvenile triploids typically have lower survival when reared together in common garden experiments (Piferrer et al. 2009). While somewhat limited in terms of sample size, Ewing (1989) is the only published account of triploid Walleye survival. Survival of heat-shocked triploid Walleye was extremely poor; survival to the fingerling stage was 2.2% (1/48; number survived/ number stocked) compared to 28.4% (44/155) for diploid Walleyes stocked into a 0.05 ha hatchery pond (Ewing 1989). Garcia-Abiado et al. (2002) found survival of heat-socked saugeye fry was 22.4%, compared to 89.4 – 100% for diploid controls Additionally, Koch et al. (2018) found the probability of survival to autumn of triploid saugeye fry approximately 0.25 relative to diploids when stocked at equal densities in the same systems.

However, Garcia-Abiado et al. (2002) found that triploid saugeyes stocked as fingerlings showed similar survival relative to diploid fingerlings.

As there is little information regarding their post-stocking performance, there is a need for detailed investigations on the growth and survival of triploid Walleyes due their increasing use as an alternative stocking method. In this study, we used three years of paired stockings of diploid and triploid Walleye in two Colorado reservoirs to evaluate triploid Walleye post-stocking performance in the wild. Specifically, we investigated relative differences in growth and survival between multiple cohorts of triploid and diploid Walleye.

2.2 Methods

2.2.1 Study site

Jumbo (40.93ºN, 102.65ºW) and Jumbo Annex (40.91ºN, 102.665ºW) Reservoirs are water storage impoundments west of Sedgwick, Colorado, USA. The primary water source for these reservoirs is the South Platte River. Jumbo Reservoir has a maximum surface area of 639 ha and maximum depth of approximately 7 m (at full pool; surface elevation 1,131 m AMSL). Jumbo Annex Reservoir has a maximum surface area of 32 ha and maximum depth of approximately 4.5 m (at full pool; surface elevation 1,125 m AMSL). Both reservoirs have fish communities composed of Walleye, saugeye, Black Crappie *Pomoxis nigromaculatus*, White Crappie *Pomoxis annularis*, Largemouth Bass *Micropterus salmoides*, Smallmouth Bass *Micropterus dolomieu*, Channel Catfish *Ictalurus punctatus*, Yellow Perch *Perca flavescens*, Bluegill *Lepomis macrochirus*, Green Sunfish *Lepomis cyanellus*, Orange spotted Sunfish *Lepomis humilis*, wiper (White Bass *Morone chrysops* x Striped Bass *M. saxatilis*), Gizzard Shad *Dorosoma cepedianum*, and Freshwater Drum *Aplodinotus grunniens* (M. Brandt, Colorado Parks and Wildlife, personal communication).

2.2.2 Stocking procedures

Paired stocking of triploid and diploid Walleye fry and fingerlings of known densities occurred each spring 2018 – 2021, except for 2020, when zero Walleyes were stocked due to the COVID-19 pandemic. Walleyes were produced, ploidy was verified, and stocking densities were estimated according to the methods described by Fetherman et al. (2015). At Jumbo Reservoir, diploid and triploid fry were stocked at 1,902 – 6,293 fish/ha and diploid and triploid fingerlings at 15 - 47 fish/ha. At Jumbo Annex Reservoir, diploid and triploid fry were stocked at 1,563 – 5,859 fish/ha, and diploid and triploid fingerlings at 30 - 63 fish/ha (Table 2.1).

2.2.3 Fish sampling

Walleyes were collected via pulsed-DC shoreline boat electrofishing units and/or overnight gill net sets each fall. Electrofishing commenced 30 min prior to sunset, as Walleyes are most active during the crepuscular period (Bozek et al. 2011). Gill nets used American Fisheries Society standard experimental gill nets or Colorado warmwater gill nets. The American Fisheries Society standard experimental gill nets were 24.4 m long and comprised of eight randomly ordered monofilament panels that are 3.1 m wide by 1.8 m tall, with mesh sizes (stretch measure) of 38-, 50- 64-, 76-, 88- 102-, 114-, and 128-mm (Bonar et al. 2009). The Colorado warmwater gill nets were 45.7 m long and comprised of six graduated monofilament panels that are 7.62 m wide by 1.8 m tall, with mesh sizes (stretch measure) of 38-, 51-, 76-, 102-, 127-, and 152-mm.

Target sizes of fish kept for biological sampling depended on sampling year and prior system-specific knowledge of Walleye growth rate. In 2018, we targeted only age-0 Walleyes, and sampled all Walleyes < 300 mm total length (TL). In 2019, we were targeted age-0 and age-1 Walleyes and sampled all Walleyes < 400 mm. In 2020 and 2021, we targeted up to age-4 Walleyes, sampling all those < 500 mm. Prior to biological sampling, fish were euthanized with

MS-222 (250mg/L). Sampled fish were measured for TL and weighed (WW; g). A blood sample, obtained via cardiac puncture (Duman et al. 2019), and/or a caudal fin clip was collected from each individual for ploidy determination, using methods described by Farrell et al. (2022b). Sagittal otoliths were collected from each fish for age determination.

2.2.4 Age estimation

Otoliths were sectioned transversely through the core, placed on a slide, immersed in mineral oil to enhance readability, and photographed using a camera mounted to a compound microscope at 40 – 100x magnification under reflected light. An experienced reader (CJF) aged each fish three times using RFishBC (Ogle 2019). Fish where all three age assignments corresponded with each other were used to create system-, ploidy-, and year-specific age-length keys to apply to unaged fish and for fish which had disagreeing age assignments using package FSA (Ogle et al. 2022). We calculated biological ages as the time elapsed from birth until capture, assuming fish were born on April 1st of their estimated birth year (Barton and Barry 2011).

2.2.5 Statistical analyses

All statistical analyses were performed using R 4.1.3 (R Development Core Team 2022). We used package brms, a high-level interface to stan, to fit growth and relative survival models in a Bayesian framework (Bürkner 2017; Bürkner 2018; Bürkner 2021). Each model was fit by implementing four chains for 3,000 iterations each using brms and the No-U-Turn sampler (Hoffman and Gelman 2014). For each chain, the first 1,500 iterations were discarded as burn-in, leaving 6,000 draws to make inference on the posterior distribution of each model's parameters. We used default priors provided by brms for all models. Convergence of chains was assessed using potential scale reduction factor ($\hat{r}$) with estimates less than 1.05 considered acceptable and

TABLE 2.1 – Stocking densities (number per ha) of fry and fingerling triploid (3N) and diploid (2N) Walleyes at Jumbo (JUM) and Jumbo Annex (ANX) reservoirs, 2018 – 2021.

			Fry		Fingerling	
Waterbody	Cohort	Ploidy	*n*	Density (*n*/ha)	*n*	Density (*n*/ha)
ANX	2018	2N	50,000	1562.5	1,042	32.6
		3N	50,382	1574.4	974	30.4
	2019	2N	138,692	4334.1	1,998	62.4
		3N	161,353	5042.3	2,002	62.6
	2021	2N	222,664	6958.3	2,017	63.0
		3N	152,336	4760.5	2,006	62.7
JUM	2018	2N	1,584,414	2479.5	10,006	15.7
		3N	1,686,414	2639.1	9,984	15.6
	2019	2N	1,436,181	2247.5	10,004	15.7
		3N	1,751,252	2740.6	10,008	15.7
	2021	2N	4,153,055	6499.3	30,035	47.0
		3N	2,727,559	4268.5	10,029	15.7

Note: No Walleye were stocked in 2020 at Jumbo or Jumbo Annex due to COVID-19.

demonstrating convergence (Vehtari et al. 2021). Figures were created using packages ggplot2 (Wickham 2016) and tidybayes (Kay 2022).

2.2.5.1 Growth comparisons

We compared length-at-age of triploid and diploid Walleye by comparing the predicted posterior distributions of *TL* for each age-class (i.e., 0 – 3) on October 1. To account for multiple sampling events within a given year, we estimated the posterior distribution of *TL* for diploid and triploid Walleyes using the following hierarchical model:

$$\mathrm{TL}_{t_i} \sim lognormal(\mu, \sigma) \quad (2.1)$$

$$\mu = \log(a_i - b_i t) \quad (2.2)$$

$$a_i \sim N(0, \sigma) + (1|cohort + waterbody) \quad (2.3)$$

$$b_i \sim N(0, \sigma) + (1|cohort + waterbody) \quad (2.4)$$

where TL_t is the total length (mm) of ploidy *i* fractional age *t*, with random intercepts (*a*) and slopes (*b*) for each cohort and waterbody, running separate regressions for each age-class. We used posterior draws for *a* and *b* to calculate the posterior predictive distribution for diploid and triploid fish at $t = 0.5, 1.5, 2.5$, and 3.5. We used Full Bayesian Evidence Testing (Pereira and Stern 1999), which is a Bayesian analog to classical *p*-value hypothesis testing to quantitatively test our null hypotheses, that there were no differences in growth between ploidies. Differences < 5% were considered not different. Full Bayesian Evidence Tests produce *e*-values, which is the epistemic value of a hypothesis given the observed data, similar to a *p*-value in the frequentist realm (Pereira and Stern 2022). We used package fbst (Kelter 2022) to calculate e-values to test our hypotheses. We set an interval of ±5% for our null hypothesis that there was no difference in the percent difference (i.e., 100 x [$TL_{triploid} - TL_{diploid}$]/ $TL_{diploid}$) of the posterior means of *TL* at age *t* between triploid and diploid Walleyes.

2.2.5.2 Relative Survival

We calculated relative survival to assess how survival of triploid Walleyes compares to diploids. According to Hilborn and Walters (1992), catch is proportional to abundance:

$$C_t = qE_tN_t \tag{2.5}$$

where C_t is catch at time t, q is the catchability coefficient, E_t is fishing effort at time t, and N_t is abundance at time t:

$$N_t = N_{t-1}s \tag{2.6}$$

where s is the survival rate from t-1 to t. Assuming that q for does not differ by ploidy, and because E did not differ between ploidies of the same cohort and age, we estimated the ratio of survival of triploids relative to diploids as:

$$\frac{S_{3N_{ijt}}}{S_{2N_{ijt}}} = \frac{C_{3N_{ijt}}C_{2N_{ij(t-1)}}}{C_{2N_{ijt}}C_{3N_{ij(t-1)}}} \tag{2.7}$$

where $\frac{S_{3N_{ijt}}}{S_{2N_{ijt}}}$ is the ratio of triploid (3N) to diploid survival (2N) survival for cohort i in reservoir j at age t, $\frac{C_{3N_{ijt}}}{C_{2N_{ijt}}}$ is the ratio of catch for cohort i in lake j at age t, and $\frac{C_{2N_{ij(t-1)}}}{C_{3N_{ij(t-1)}}}$ is the ratio of catch for cohort i in lake j at age t-1. To estimate relative survival at age t = 0.5, we used the relative stocking proportions of diploids to triploids as the ratio of the catch at age t-1. Because we could not account for the origin (i.e., natural versus stocked) or the size-at-stocking (fry versus fingerling), we ran two scenarios to estimate the relative difference of survival from stocking to fall, assuming that all fish captured in the fall were either 100% fry or 100% fingerlings.

We estimated the posterior distributions of relative survival for diploid and triploid Walleyes using the following Bayesian model:

$$\frac{S_{3N_{ijt}}}{S_{2N_{ijt}}} \sim lognormal(\mu, \sigma) \quad (2.8)$$

where μ is analogous to the intercept. Posterior distributions of relative survival that had HDIs overlapping with one were considered not significantly different, whereas those that had HDIs not overlapping with one were considered significantly different.

2.3 Results

Over four years of sampling, we collected 2,788 Walleyes (Table 2.2), of which 320 were unaged due to missing or low-quality otoliths. Age estimation was precise; there were disagreeing ages for 77 fish among otoliths with three age reading replicates (n = 2,391). Cohorts present in our sample ranged from 2009 – 2021, 871 fish belonged to cohorts outside of our target range (i.e., 2009 – 2017), and 1,917 belonged to cohorts within our target range (i.e., 2018 – 2021).

2.3.1 Growth

For age-0 fish, posterior means for the parameters of the hierarchical linear model for diploids were a = 71.7 (σ = 8.9) and b = 290.0 (σ = 17.1), and for triploids were a = 71.4 (σ = 17.3) and b = 259.3 (σ = 35.5). Posterior predicted median TL at t = 0.5 was 217 mm (95% Highest Density Interval [HDI] = 183 – 254 mm) for diploids and 201 mm (95% HDI = 170 – 236 mm; Figure 2.1). The posterior median of the difference of *TL* at t = 0.5 was 15.6 mm (95% HDI = 12.5 – 18.9 mm). Triploids were 7.2% smaller (95% HDI = 5.8 – 8.7% smaller, Figure 2.1) than diploids at t = 0.5, with an e-value < 0.01, meaning that the probability of no difference in the relative percent difference according to our *a priori* criteria was < 0.01.

For age-1 fish, posterior means for the parameters of the hierarchical linear model for diploids were a = 68.1 (σ = 30.3) and b = 174.4 (σ = 21.4), and for triploids were a = 162.7 (σ = 59.6) and b = 100.5 (σ = 40.3). Posterior predicted medians for TL at t = 1.5 was 329 mm (95%

HDI = 286 – 376 mm) for diploids and 313 mm (95% HDI = 271 – 360 mm; Figure 2.1). The posterior median of the difference of *TL* at $t = 1.5$ was 16.3 mm (95% HDI = 11.2 – 21.0 mm). Triploids were 4.9% smaller (95% HDI = 3.4 – 6.5%, Figure 2.1) than diploids at $t = 1.5$, with an *e*-value = 0.528, indicating no significant differences in length at $t = 1.5$.

For age-2 fish, posterior means for the parameters of the hierarchical linear model for diploids were $a = 436.8$ ($\sigma = 166.2$) and $b = -17.1$ ($\sigma = 67.2$), and for triploids were $a = -61.4$ ($\sigma = 519.2$) and $b = 173.2$ ($\sigma = 207.5$). Posterior predicted medians for TL at $t = 2.5$ was 393 mm (95% HDI = 324 – 467 mm) for diploids and 370 mm (95% HDI = 306 – 445 mm; Figure 2.1). The posterior median of the difference of *TL* at $t = 2.5$ was 27.4 mm (95% HDI = 13.2 – 42.6 mm). Triploids were 6.3% smaller (95% HDI = 2.8 – 9.7%, Figure 2.1) than diploids at $t = 3.5$, with an *e*-value = 0.267.

For age-3 fish, posterior means for the parameters of the hierarchical linear model for diploids were $a = -807$ ($\sigma = 698$) and $b = 354$ ($\sigma = 119$), and for triploids were $a = -1109.6$ ($\sigma = 2231$) and $b = 433$ ($\sigma = 636$). Posterior predicted medians for TL at $t = 3.5$ was 436 mm (95% HDI = 368 – 505 mm) for diploids and 408 mm (95% HDI = 345 – 479 mm; Figure 2.1). The posterior median of the difference of *TL* at $t = 3.5$ was 22.5 mm (95% HDI = 13.7 – 31.3 mm). Triploids were 5.7% smaller (95% HDI = 3.6 – 8.1%, Figure 2.1) than diploids at $t = 3.5$, with an *e*-value = 0.230.

2.3.2 Relative Survival

In all years, the proportion of triploids was lower than diploids from stocking to the first fall, but we did not observe changes in the relative catch in subsequent ages (Figure 2.2). When assuming that all age-0 Walleyes captured in the fall were stocked as fry, the median of the posterior distribution for the ratio of survival of triploids to diploids was 0.21

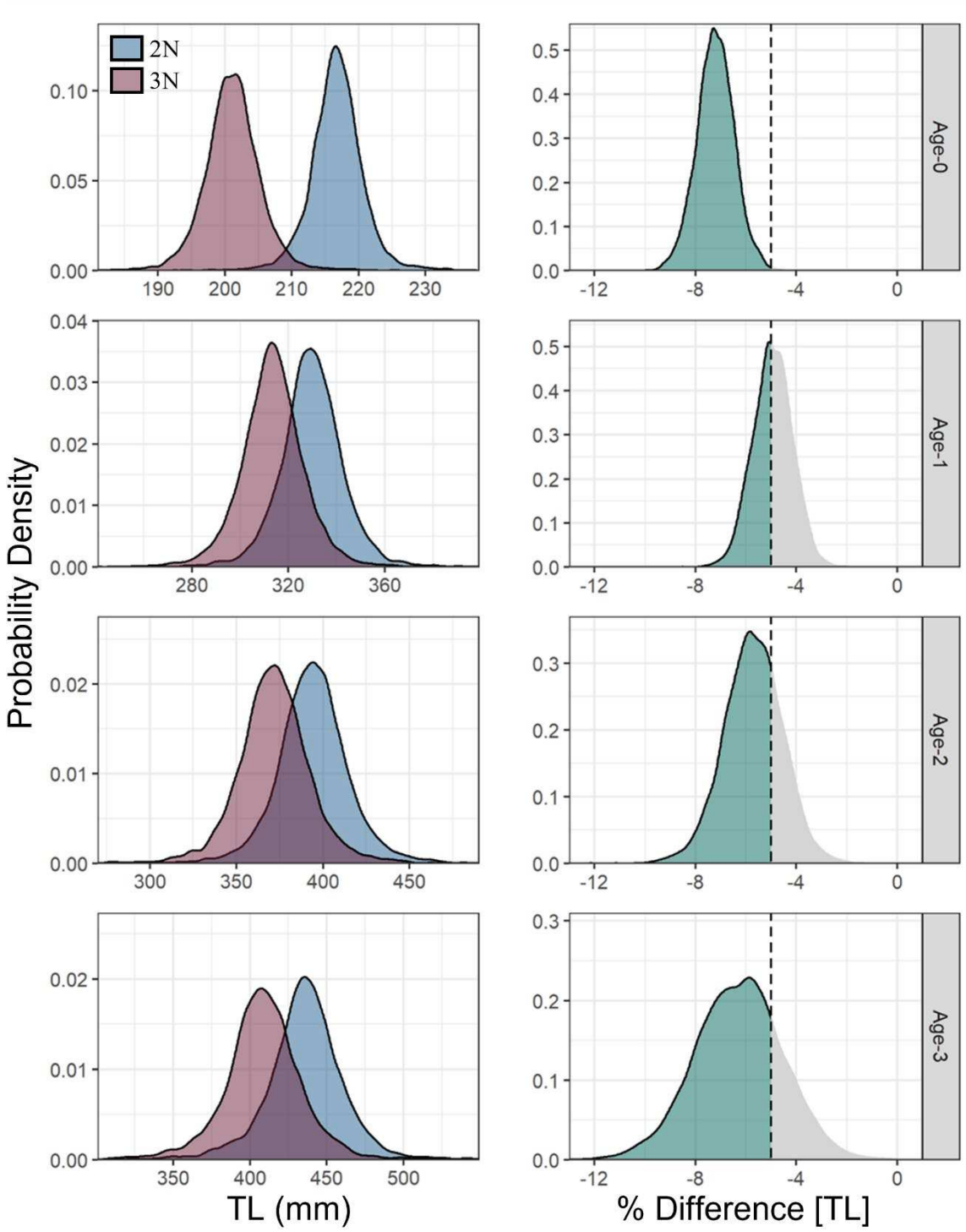

FIGURE 2.1 - Posterior distributions of mean total length (*TL*) of diploid (2N) and triploid (3N) Walleyes, and corresponding relative percent difference (i.e., [3N – 2N]/2N) of mean total length between ploidies of Walleyes (reservoirs combined). Each row corresponds to an age-class (i.e., age-0 – age-3). For percent difference plots, the grey shaded area represents our *a priori* criteria of ±5% for no difference of the posterior means of *TL* at age *t* between triploid and diploid Walleyes and the dashed line is at -5%.

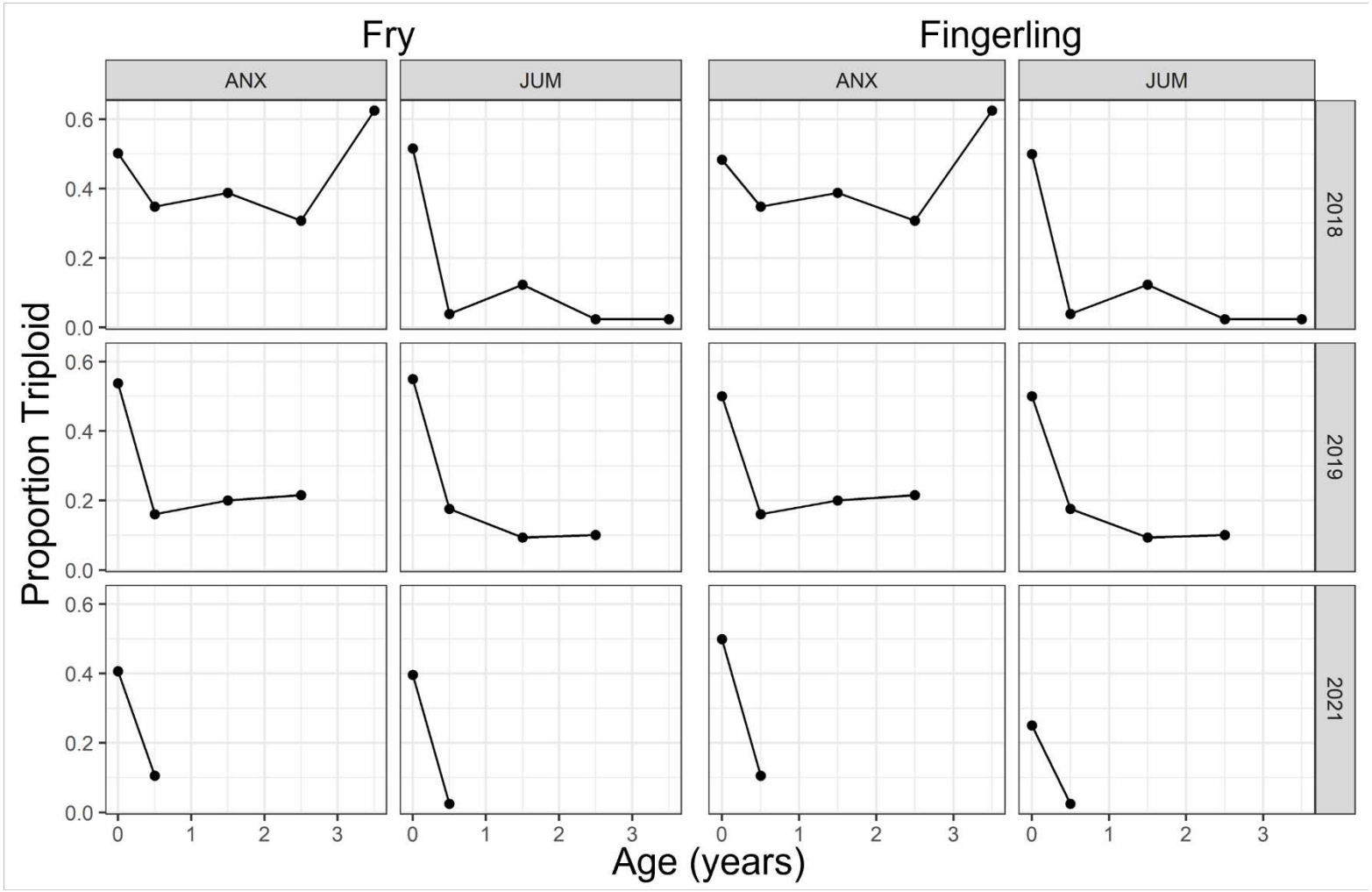

FIGURE 2.2 – Observed catch proportions of triploids by cohort and age-class at Jumbo Annex (ANX) and Jumbo (JUM) reservoirs, all gears combined, 2018 - 2021. The two left columns (FRY) assume that all fish captured at $t = 0.5$ were stocked as fry, and the right two columns (FINGERLING) assume that all fish captured at $t = 0.5$ were stocked as fingerlings. Rows represent cohorts.

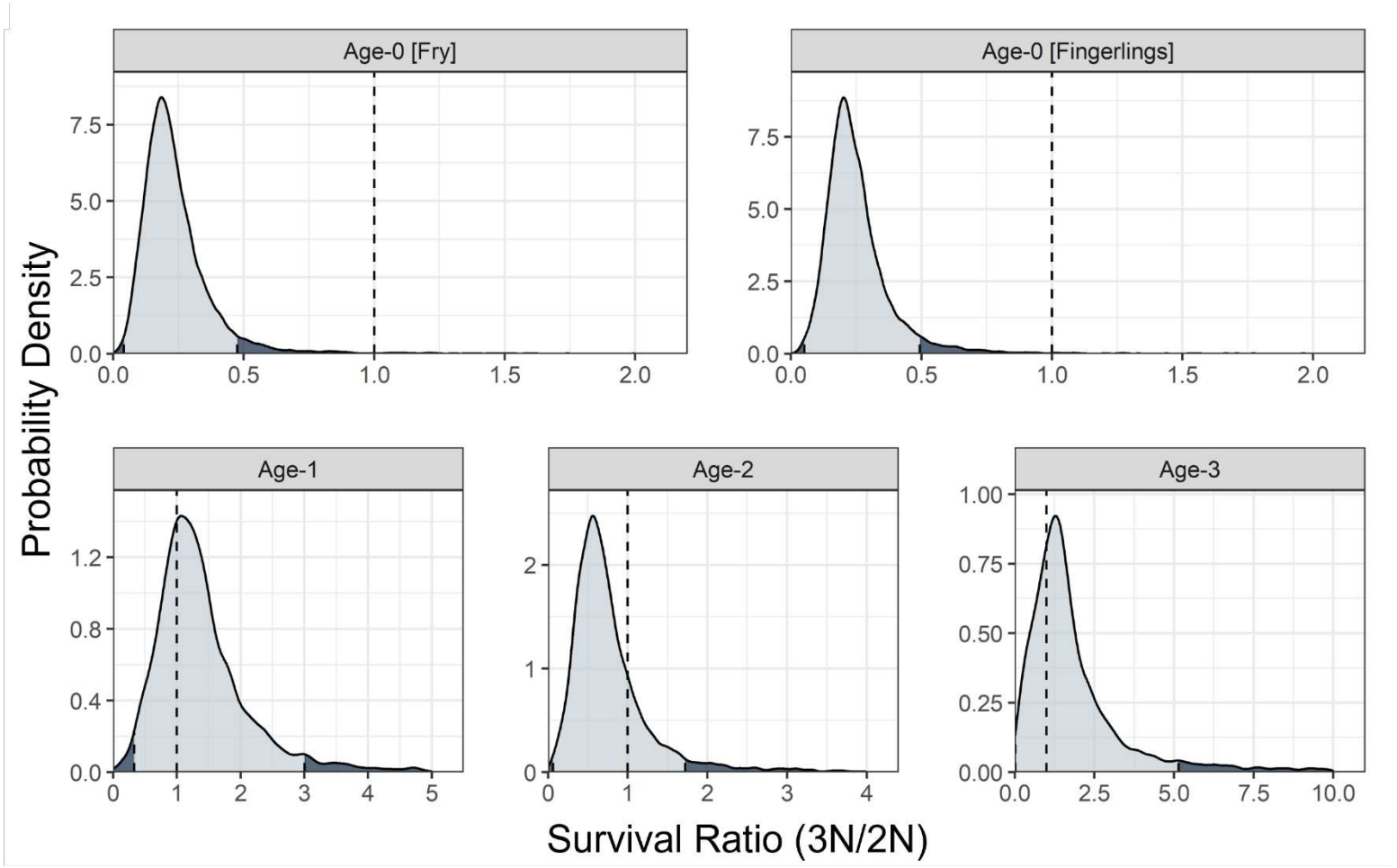

FIGURE 2.3 – Posterior probability density distributions of the estimated survival ratio of triploid (3N) relative to diploid (2N) Walleyes. The light blue shaded area represents the 95% Highest Density Intervals (HDI). The dashed line is at 1, which represents equivalent survival between triploid and diploid Walleyes. Survival ratios that have 95% HDIs which do not overlap the dashed line are considered significantly different.

TABLE 2.2. – Age- and cohort-specific catch (*n*) and percent triploid (*%3N*) summary for all fall sampling events and gear types (i.e., gill netting and electrofishing) targeting diploid and triploid Walleyes in Jumbo and Jumbo Annex reservoirs, Colorado, 2018 - 2021.

		2018 Cohort		2019 Cohort		2020 Cohort		2021 Cohort	
Waterbody	Age	*n*	%3N	*n*	%3N	*n*	%3N	*n*	%3N
ANX	0	23	34.8%	249	16.1%	43	0.0%	161	10.6%
	1	67	38.8%	55	20.0%	35	0.0%	-	-
	2	13	30.8%	102	21.6%	-	-	-	-
	3	32	62.5%	-	-	-	-	-	-
JUM	0	178	3.9%	450	17.6%	3	0.0%	121	2.5%
	1	73	12.3%	75	9.3%	1	0.0%	-	-
	2	42	2.4%	129	10.1%	-	-	-	-
	3	42	2.4%	-	-	-	-	-	-

(95% HDI = 0.04 – 0.48). When assuming that all age-0 Walleyes captured in the fall were stocked as fingerlings, the median of the posterior distribution for the ratio of survival of triploids to diploids was 0.23 (95% HDI = 0.05 – 0.49). Because the HDIs for both post-stocking scenarios did not overlap one, survival for triploids was significantly lower than diploids from stocking to fall (Figure 2.3). The ratio of survival beyond age-0 were not significantly different, as 95% HDIs for the survival ratios for age-classes 1 – 3 overlapped one (Figure 2.3). At age-1, the median of the posterior distribution for the ratio of survival of triploids to diploids was 1.25 (95% HDI = 0.16 – 3.15). At age-2, the median of the posterior distribution for the ratio of survival of triploids to diploids was 0.66 (95% HDI = 0.06 – 1.91). At age-3, the median of the posterior distribution for the ratio of survival of triploids to diploids was 1.45 (95% HDI = 0.01 – 6.83).

2.4 Discussion

This study is the first to assess the growth and relative survival of juvenile triploid Walleyes in the wild. Overall, we found that age-0 triploid Walleyes were significantly smaller (8%) and had significantly lower survival (77 – 79%) than age-0 diploid Walleyes. However, there were no significant differences in size or survival between triploid and diploid Walleyes at ages 1 – 3.

Differences in size between ploidies of age-0 Walleyes may be explained by several possibilities. Growth of fish, especially during the juvenile stages, tends to be shaped by metabolism (Kooijman 2010; Kearney 2021). The Gill-Oxygen Limitation theory (Pauly 1981) posits that metabolism, and thereby growth, in immature water-breathing ectotherms is proportional to oxygen uptake Q:

$$Q = \frac{dP \times U \times GSA}{WBD} \tag{2.9}$$

where *dP* is the difference between the oxygen partial pressure on either side of the gill membrane, *U* is Krogh's diffusion constant, *GSA* is the gill surface area, and *WBD* is the water-blood distance, or the thickness of the gill tissue separating water and blood. Sadler et al. (2001) found that triploid Atlantic Salmon *Salmo salar* had reduced gill surface areas relative to diploid controls, which could correspond to relatively lower oxygen uptake and growth rates for triploids. Also, it may be possible that triploid growth could be limited because they may have a relatively larger water-blood diffusion distance (Benfey 1999). Triploids have 50% more DNA than diploids, and have larger cells to accommodate their larger genome, which would correspond to larger diffusion distances and reduced growth (Benfey 1999).

It is also possible that triploid Walleyes have narrower thermal tolerances than diploid Walleyes, as has been demonstrated for triploids of other species (Altimiras et al. 2002; Fraser et al. 2012b; but see Bowden et al. 2018), which could negatively affect their food consumption and growth rates (Kitchell et al. 1977). Maximum surface temperatures in Jumbo and Jumbo Annex are typically around 26°C, which is higher than the optimal temperature for consumption of larval and juvenile walleye (25ºC) but below their maximum temperature for consumption (28ºC) and respiration (32ºC; Madon and Culver 1993). If triploid Walleye have lower thermal tolerances, surface temperatures at Jumbo and Jumbo Annex could lead to relatively lower growth for triploid Walleye.

Size and survival are linked, as survival of juvenile fish is size-dependent, with smaller fish being more susceptible to predation, cannibalism, and starvation (Miller et al. 1988). Grausgruber and Weber (2020) found that the probability of an age-0 Walleye being preyed up decreased by 2% for every 10 mm increase in total length. According to their findings, we would

expect that triploid Walleye have 3.1% (95% HDI: 2.5 – 3.8%) increased chance of being the victim of predation compared to diploids.

There are several potential explanations for the lower relative survival rates for age-0 triploid we observed in addition to size-dependent mortality. It is possible that triploid Walleyes have more morphological abnormalities compared to diploids. For example, compared to diploids, triploid Atlantic Salmon were more susceptible to deformities of the jaw (Sutterlin et al. 1987) have higher prevalence of vertebral deformities (Fjelldal and Hansen 2010), and altered brain morphology (Fraser et al. 2012a), all of which could negatively affect relative survival of larval triploid fish. It is also possible that triploids are less well-suited to dealing with the natural environment, as triploids can be less aggressive than diploids, and more susceptible to thermal stress, both of which would likely negatively affect survival of triploids (Fraser et al. 2012b). For *Stizostedion spp.,* Czesny et al. (2002) demonstrated that triploid saugeye were less aggressive and less successful predators than diploids in controlled feeding experiments. As hypothesized by Koch et al. (2018), less successful foraging by triploids could expose them to more predation pressure, and negatively affect growth and survival.

Additionally, producing triploid fish is time sensitive and production-related issues could explain low post-stocking survival. For example, Fetherman et al. (2015) found that a difference of 3.5 minutes for the time of initiation of pressurization of eggs used for triploid Walleye production led to nearly three times higher hatching rates. While unknown, it is possible that differences in post-fertilization time to pressure initiation affects post-stocking survival. Also, Taylor et al. (2011) demonstrated that egg quality was more indicative of hatching success in Atlantic Salmon than ploidy status. Post-ovulatory oocyte ageing has been identified as the most important factor affecting fish egg quality (Samarin et al. 2019) and egg quality may explain

variability in recruitment in the wild (Kjørsvik et al. 1990). Thus, increased handling time required for triploid production may increase oocyte ageing and could be an explanation for low survival of age-0 triploid Walleye.

However, despite the lower relative survival rate of age-0 triploid Walleye, relative survival rates for older triploids in our study indicate that there may be an early period of high mortality for triploid Walleye during their first year of life that could act as a recruitment bottleneck. In fact, triploid fish generally show similar if not lower mortality rates than diploids beyond the larval stages (Fraser et al. 2012b), like we observed in this study. It is possible that the relatively lower survival rates we observed for triploid Walleyes are driven by stage-specific differences in survival; survival for triploid fingerlings may be similar to diploid fingerlings, and differences in fry survival between ploidies may explain our observations. Post-stocking survival of diploid Walleyes typically increases with age-at-stocking (Fielder 1992; Johnson et al. 1996; Weber et al. 2020). Stocking of older juvenile triploids, like fingerlings or advanced fingerlings, could lead to improved triploid Walleye recruitment (Fraser et al. 2012b). Diploid Walleyes stocked as fry typically experience high rates of mortality, with < 0.2% surviving until fall (McWilliams and Larscheid 1992; Brooks et al. 2002). Fingerling Walleye typically have higher survival rates than those stocked as fry (Fielder 1992; Koppelman et al. 1992; Grausgruber and Weber 2020). Johnson et al. (1996) found that, for Walleyes stocked as fingerlings, survival to their first autumn was approximately 2.5% on average. Existing studies on triploid *Stizostedion spp.* fry found that triploids had low relative survival rates (Ewing 1989; Garcia-Abiado et al. 2002; Koch et al. 2018), but post-stocking survival for triploid saugeyes stocked as fingerlings was similar to diploids (Garcia-Abiado et al. 2002). Walleye fry are typically stocked within days of hatching, and prior to the onset of exogenous feeding (Barton and Barry 2011; Kerr

2011). Larval triploid fish often exhibit higher rates of deformities and reduced sensory capacity relative to diploids (Maxime 2008), both of which would reduce survival relative to diploids. As such, triploid Walleyes stocked as fry may experience much higher mortality rates in the days or weeks following stocking, whereas triploid Walleyes stocked as fingerlings may have already gone through this "mortality filter". Unfortunately, we were unable to determine the age-at-stocking for sampled fish and unable to disentangle its impact on this experiment. Future studies should focus on evaluating potential stage-specific differences in survival for triploid Walleyes.

However, our estimates or relative survival may be biased against triploid Walleyes, as we could not account for the origin of diploid Walleyes (i.e., natural versus hatchery). If there was any natural reproduction in our study sites, relative survival estimates would be biased against, because there could potentially be more diploids present at the time of sampling than expected. We have evidence that natural reproduction occurred in both of our study reservoirs in 2020, as no Walleyes (diploid or triploid) were stocked, but we captured 3 age-0 diploid Walleyes at Jumbo Reservoir and 43 age-0 diploid Walleyes at Jumbo Annex Reservoir during the fall of 2020.

Overall, our results are encouraging for the use of triploid Walleyes as an alternative stocking method. Growth and survival for age-1+ triploid Walleyes was not significantly different than diploid Walleyes. Triploid Walleyes are useful in situations where unwanted natural reproduction is a concern (Bramblett and Zale 2016; Koch et al. 2018; Farrell et al. 2022a). Additionally, using triploid fish to provide anglers an opportunity to target sportfish they desire, illegal stocking occurrences are likely to decrease and less likely to create new populations of nonnative fish in undesired areas due to their sterility (Benfey 1999; Johnson et al. 2009). Adult triploid Walleyes are more efficient predators than diploid Walleyes. The trophic

efficiency of triploid Walleyes decreases contaminant bioaccumulation, making triploid Walleyes especially appealing for stocking in systems where contaminant problems exist (Farrell et al. 2022a). Furthermore, the trophic efficiency of triploid Walleyes could potentially allow for a system to support higher Walleye densities, meaning more large fish for anglers to catch. While our results are encouraging, this study also indicates that more research is needed to clarify potential differences in survival during their first year of life. More detailed studies are needed to address a potential bottleneck in triploid Walleye recruitment by investigating stage-specific survival of larval triploid Walleyes.

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CHAPTER THREE

Induced triploidy reduces mercury bioaccumulation in a piscivorous fish[3]

3.1 Introduction

Induced triploidy is commonly used in aquaculture operations (Piferrer et al. 2009), for biocontrol of aquatic vegetation (Allen and Wattendorf 1987), and increasingly as a stocking option for recreational fisheries (Teuscher et al. 2003; Koch et al. 2018; Cassinelli et al. 2019), primarily because triploid fish are reproductively sterile (Benfey 1999). Triploid females typically have small ovaries incapable of producing viable ova, while triploid males in many species develop normally but produce aneuploid spermatozoa (Thorgaard 1983; Benfey 1999). Yet, triploid males may still attempt to spawn with diploid females (Benfey 1999) and can act as a control on natural reproduction (Piferrer et al. 2009). Despite increased interest in triploidy as a stocking option for recreational fisheries, studies examining the ecology of triploids and diploids in sympatry are rare.

Sterility confers several potential advantages for the use of triploids as a stocking option in recreational fisheries, most notably for reproductive containment and the potential for increased growth rates (Piferrer et al. 2009). It is commonly hypothesized that triploids should grow faster and reach larger body sizes relative to their diploid counterparts because of the reduced energetic requirements associated with gonadal development, particularly for females (Leary et al. 1985; Tiwary et al. 2004; Maxime 2008). However, potential differences in growth performance between ploidies remain inconclusive (Benfey 1999; Maxime 2008). Although

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growth has not been fully reconciled, differential energetic requirements associated with spawning could modify consumption dynamics and the bioaccumulation of contaminants between diploid and triploid fish – an overlooked element that may have important implications for the use of triploids in recreational fisheries.

Mercury (Hg) is a globally pervasive contaminant and potent neurotoxin. Once released into the atmosphere, Hg can disperse far from its source (Wentz et al. 2014) before being deposited in terrestrial, freshwater, and marine ecosystems (Morel et al. 1998; Chen et al. 2008; Driscoll et al. 2013). Thus, controlling atmospheric deposition of Hg is challenging. Under certain conditions (Paranjape et al. 2017), microbial processes transform Hg into methylmercury (MeHg), the most toxic and bioavailable form of Hg, which bioaccumulates in organisms and biomagnifies in food webs. Concentrations can reach levels that are hazardous or lethal to fish, wildlife, and humans (Morel et al. 1998; Boening 2000; Scheuhammer et al. 2007). Mercury is fully or partially responsible for the vast majority (>80%) of fish consumption advisories in the U.S. and Canada (Eagles-Smith et al. 2016b). A better understanding of factors that affect bioaccumulation of Hg in fish could help identify new mitigation strategies that protect the health of humans and piscivorous fish and wildlife species.

Several studies have investigated how MeHg bioaccumulation affects reproduction (Crump and Trudeau 2009), but the effects of reproductive investment (i.e., the cumulative energetic investment into gamete production over a fish's lifetime) on MeHg bioaccumulation are not well understood. Spawning is energetically costly for both males and females and requires fish to meet this energetic demand by consuming prey (Diana 1983; Trudel et al. 2000). Since food consumption is the primary pathway of Hg uptake in predators (Hall et al. 1997), the energetic costs associated with spawning should at least partially regulate MeHg

bioaccumulation (Nicoletto and Hendricks 1988). Therefore, we would expect a positive correlation between MeHg concentrations (i.e., [MeHg]) and reproductive investment. Reproductive investment was correlated with higher [MeHg] in sharks (Coelho et al. 2010; Pethybridge et al. 2010) and ray-finned fishes (Nicoletto and Hendricks 1988; Son et al. 2014). However, these studies only examined the relationship between gonadosomatic index (GSI) and [MeHg] or compared concentrations between mature and immature fish. As GSI, maturation, and [MeHg] are collinear with age (Grieb et al. 1990; Shatunovskii and Ruban 2009), the effects of reproductive investment could not be isolated. Given that the development of ovaries is typically more energetically costly than the development of testes (McBride et al. 2015), one would expect that reproductive females would consume more food, and therefore bioaccumulate more MeHg than reproductive males. However, several studies demonstrated that reproductive males have similar or higher [MeHg] relative to reproductive females of the same age (Gewurtz et al. 2011; Bastos et al. 2016; Madenjian et al. 2016). Differences in resource use (Lepak et al. 2012a), activity levels (Henderson et al. 2003), and standard metabolic, Hg elimination, or growth rates (Trudel and Rasmussen 2006; Madenjian et al. 2014; Madenjian et al. 2016) between males and females complicate studies using between-sex comparisons to examine the effects of reproductive investment on [MeHg]. No study has effectively accounted for age or other potential sex-dependent variables to discern the relative importance of reproductive investment in governing the bioaccumulation of Hg. Differential gonad development arising from ploidy manipulation gives us the ability to isolate the effects of reproductive investment on Hg bioaccumulation and overcome previous limitations.

In this study, we used field sampling to compare Hg dynamics in diploid and triploid Walleye *Stizostedion vitreum* (see Bruner 2021) co-occurring in the wild, and bioenergetics

modeling to elucidate potential explanations for observed patterns in [MeHg] by quantifying prey consumption and dietary Hg exposure among the four sex-by-ploidy groups. Reservoir fisheries management in the upper Colorado River Basin, USA, emphasizes a suite of measures to reconcile nonnative sport fishing with the recovery of endangered fish endemic to the Colorado River and its tributaries. This includes stocking triploid Walleye to diversify recreational angling opportunities in sensitive locations, deter illegal fish stocking, and possibly interfere with diploid reproduction in unwanted populations (Johnson et al. 2009; Fetherman et al. 2015). The relatively long history of triploid Walleye stocking by the state of Colorado offered a unique opportunity to evaluate the ecology of diploid and triploid fish in sympatry, including Hg dynamics. Although originally stocked with diploid Walleye as early as 1972, management of Narraguinnep Reservoir in southwest Colorado shifted to triploid Walleye stocking in 2008 to support native fish conservation efforts downstream. Colorado Parks and Wildlife has stocked triploid Walleye in Narraguinnep Reservoir every year since 2008, except for 2009 and 2020, when Walleye were not stocked. This has resulted in a mixed population of diploid and triploid Walleye with sufficient diversity in age-classes to quantify Hg dynamics and account for potential differences in resource use, growth, or other factors between sexes and ploidies. Thus, Narraguinnep Reservoir served as an ideal study system to test various hypotheses of how reproductive investment influences Hg bioaccumulation. We hypothesized that (1) diploid female Walleye would have significantly higher [MeHg] than triploid females, driven by greater prey consumption associated with supporting the production of energetically costly eggs, (2) diploid and triploid males would have similar [MeHg], since triploid males of other species often develop normal testes (Benfey 1999), and (3) males of both ploidies would have lower [MeHg] relative to diploid females because the development of testes is less

energetically costly than the development of ovaries (McBride et al. 2015). We tested our hypotheses by pairing empirical data on [MeHg], energy density of somatic and gonadal tissue, diet composition, and growth with bioenergetics model simulations to discern the role of sex-dependent reproductive investment in MeHg bioaccumulation.

3.2 Methods

3.2.1 Study site

Narraguinnep Reservoir is a 215-ha irrigation water storage reservoir in southwest Colorado, USA (Figure 3.1). The primary water supply is the Dolores River. The reservoir has a maximum depth of 25 m at full pool (surface elevation of 2037 m above mean sea level). Total dissolved solids average 240 $mg \cdot L^{-1}$. The reservoir is polymictic and oxygen is present throughout the water column. As is typical of reservoirs in the region, large annual water-level drawdowns during the irrigation season, and rising water levels during refilling periodically inundate terrestrial vegetation (Gray et al. 2005) and contribute organic matter that may stimulate MeHg production (Sorensen et al. 2005; Selch et al. 2007). The fish community is comprised of diploid and triploid Walleye, Northern Pike *Esox lucius*, Channel Catfish *Ictalurus punctatus*, Smallmouth Bass *Micropterus dolomieu*, White Sucker *Catostomus commersonii*, Brown Trout *Salmo trutta*, Rainbow Trout *Oncorhynchus mykiss*, Black Crappie *Pomoxis nigromaculatus*, and Yellow Perch *Perca flavescens*. Elevated [MeHg] were found in several fish species from the reservoir, and fish consumption advisories for MeHg have been in place at Narraguinnep Reservoir since 1991 (Butler et al. 1995).

3.2.2 Fish sampling

Walleye were collected with standard fall Walleye index netting gillnets (Morgan 2002) during spring and summer 2018 - 2019, and spring 2020. Spring sampling coincided with the spawning

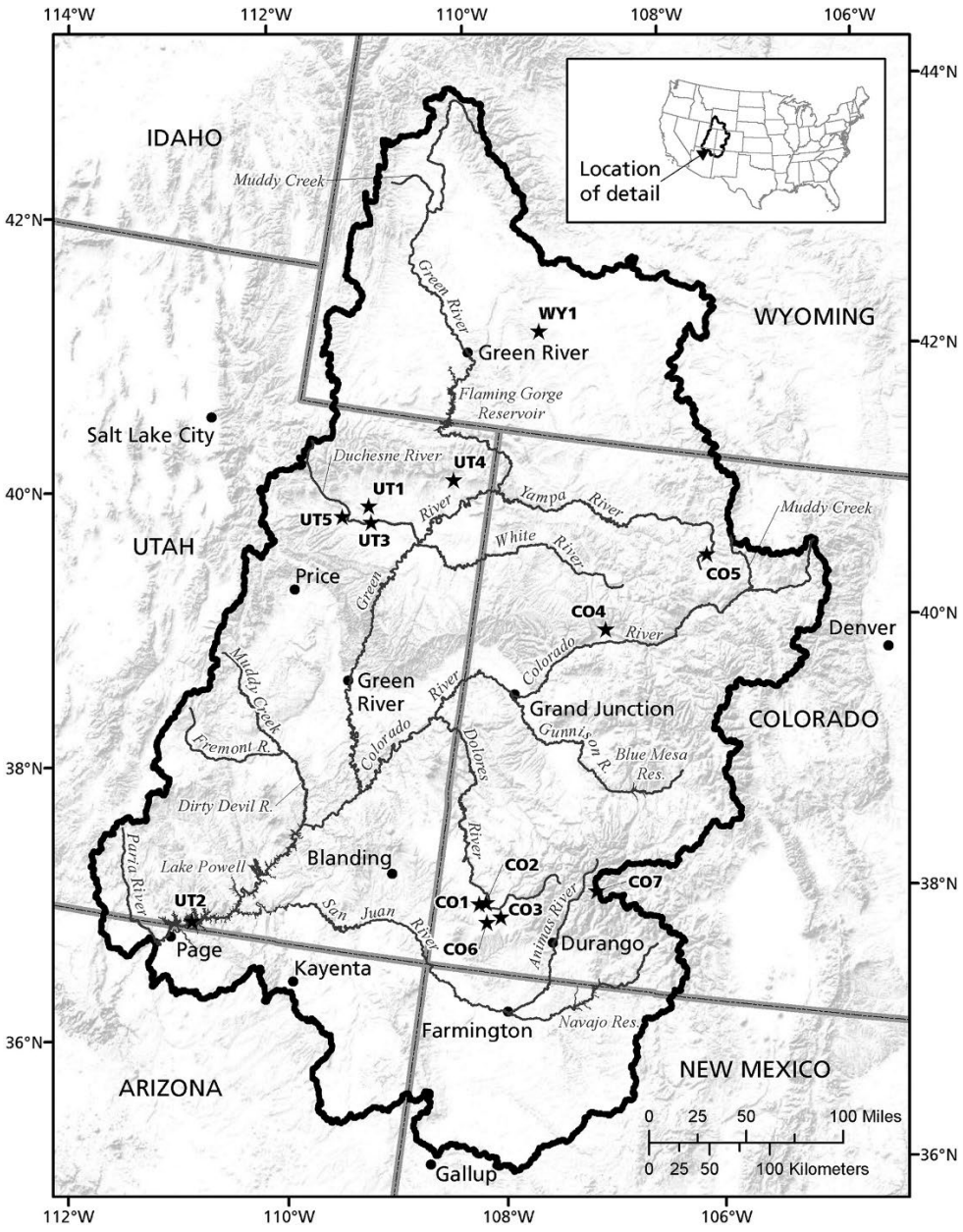

FIGURE 3.1 – Map of the Upper Colorado River Basin (UCRB) in Arizona, Colorado (CO), New Mexico, Utah (UT), and Wyoming (WY). Stars show reservoirs within the UCRB with Walleye present. CO1 = Narraguinnep Reservoir, CO2 = McPhee Reservoir, CO3 = Puett Reservoir, CO4 = Rifle Gap Reservoir, CO5 = Stagecoach Reservoir, CO6 = Totten Lake, CO7 = Vallecito Lake, UT1 = Big Sand Wash Reservoir, UT2 = Lake Powell, UT3 = Midview Reservoir, UT4 Red Fleet Reservoir, UT5 = Starvation Reservoir, WY1 = Jim Bridger Pond. Letters of the index code indicate the US state in which the waterbody resides. Map created in ArcMap 10.8 (Environmental Systems Research Institute 2020) with data from the National Elevation Dataset (US Geological Survey 2012), National Hydrography Dataset (US Geological Survey 2020a, 2020b, 2020c, 2020d), Geographic Names Information System (US Geological Survey 2017), and state boundaries (US Census Bureau 2020).

period for Walleye. We recorded total length (TL, mm), total wet weight (WW, g), and gonad WW (g) of each fish. Sex, maturity, and gonad condition were classified according to Duffy et al. (2000). We characterized gonadal development for each fish using GSI:

$$\text{GSI} = \frac{\text{gonad WW}}{\text{total WW}} \times 100 \quad (3.1)$$

Sagittal otoliths were collected for age determination and growth estimation. To determine ploidy, blood samples were collected via cardiac puncture (Duman et al. 2019), stored in tubes coated with lithium heparin (anticoagulant), and chilled until ploidy analysis could be performed at the Genomic Variation Laboratory at the University of California-Davis. Ploidy was determined for each fish captured in 2019 and 2020 from blood samples using a Coulter Counter with methods described by Fiske et al. (2019). A skinless fillet, a 1-cm^3 epaxial muscle sample with skin removed, and one gonad from each fish was collected, frozen, and held at -20°C until subsequent analyses could be completed. Stomachs were removed and either frozen or preserved in 10% formalin for later analysis. Potential prey items were collected opportunistically for stable isotope analysis and food web characterization. Sampling procedures were approved by the Institutional Animal Care and Use Committee (Protocol # 18-7822A) at Colorado State University.

3.2.3 Observed somatic [T-Hg]

Skinless fillets from captured fish were analyzed for total mercury (T-Hg) as a surrogate for MeHg, as T-Hg analyses are less expensive and MeHg typically comprises approximately 95% of T-Hg in fish (Bloom 1992). Somatic [T-Hg] ($\mu g \cdot g^{-1}$ WW) were estimated for 56 diploid and 34 triploid Walleyes captured during spring and summer 2019 by the Colorado Department of Public Health and Environment using EPA method 7473. Samples included nearly equal

numbers of males and females for each ploidy. Walleye ranged from 248 to 680 mm TL. Triploids ranged in age from two to eight years, and diploids from two to 21 years. Linear models were used to test for differences in [T-Hg] between each sex and ploidy after accounting for age. We used Akaike Information Criterion with small sample size correction (AIC_C) to identify plausible models that best described patterns in [T-Hg] (Burnham and Anderson 2002). The full model was parameterized with a three-way interaction among age, sex, and ploidy (i.e., AGE × SEX × PLOIDY), and all models ranging in complexity from the full to the null model were tested (n = 19). Regressions were restricted to age-classes represented by both ploidies in the catch (i.e., ≤ age-8), which reduced the number of diploids included in the analysis to 34. Due to model selection uncertainty, comparisons of [T-Hg] among sex-by-ploidy groups were made using model-averaged estimates of regression coefficients (Burnham and Anderson 2002). The 95% confidence set of best models (i.e., cumulative AIC_c weight, $\omega_i \leq 0.95$) was used for model averaging (Symonds and Moussalli 2011). One outlier, an age-3 diploid female (0.467 µg T-Hg·g^{-1}), had 107-123% higher [T-Hg] than the other age-3 diploid females and was removed from this analysis.

All statistical analyses were performed using R 4.0.3 (R Development Core Team 2022) and figures were created using package ggplot2 (Wickham 2016). Package MuMIn was used for AIC_c model selection and model averaging (Bartoń 2020). Package emmeans was used to obtain estimated marginal means, and to compute contrasts and pairwise differences (Lenth 2020). We used $\alpha = 0.05$ to determine statistical significance, and Tukey-adjusted *p*-values for pairwise comparisons.

3.2.4 Energy density of somatic and gonadal tissue

Epaxial muscle and gonadal tissue samples were weighed and then dried to remove water at 60°C to a constant weight and homogenized with a mortar and pestle. We computed dry matter content (DM, %) for each tissue type as:

$$\mathrm{DM}(\%) = \frac{\text{dry mass (g)}}{\mathrm{WW}} \times 100 \tag{3.2}$$

Since muscle comprises the majority of soma for zander *Stizostedion lucioperca* (Jankowska et al. 2003), we used DM to estimate somatic energy densities (ED, $J \cdot g^{-1}$ WW) for each individual with the model of Hartman and Brandt (1995):

$$\mathrm{ED} = 45.29 \times \mathrm{DM}^{1.507} \tag{3.3}$$

We used AIC_c to identify plausible models (i.e., $\Delta AIC_c < 2$) that best described patterns in somatic ED. The full model was parameterized with a three-way interaction among age, sex, and ploidy.

Unlike somatic ED, we used a semimicro bomb calorimeter to directly measure gonadal ED, given uncertainty in the appropriateness of using existing ED:DM models that were developed by homogenizing whole fish (Johnson et al. 2017). Gonadal ED ($J \cdot g^{-1}$ dry weight) was measured with a Parr Instrument Company Model 6752 semimicro bomb calorimeter. A subsample of dried gonadal tissue (0.25 ± 0.05 g) was pelletized and placed into the combustion chamber, which was charged to 30 atmospheres with pure oxygen (Parr Instrument Company 2013). Three subsamples were combusted from each gonad, and we only used data from subsamples that underwent complete combustion. Replicate measurements were averaged for each fish. A benzoic acid standard (Gundry et al. 1969) was used to verify accuracy and precision of the calorimeter. Standards were combusted at the beginning and end of each session,

as well as after every tenth combustion cycle. Estimates of ED on a dry weight basis were converted to WW using corresponding estimates of percent water content. Linear regression was used to test for and characterize age-dependency in male and female gonadal ED for diploids.

3.2.5 Diet and stable isotope analysis

We estimated the diet composition of Walleye to inform prey proportions as input into the bioenergetics model. Stomachs preserved in the field were dissected and prey items were identified to the lowest possible taxonomic classification. The lengths of ingested prey were measured directly if intact or reconstructed from diagnostic bones. Diet composition was quantified using the displacement volume method of Hazzard and Madsen (1933). Each prey item was blotted to remove excess liquid and displacement volume was measured to the nearest 0.1 mL. Diet composition was expressed as the mean proportion of each prey taxon by volume. We used quantile regression to test whether maximum (95^{th} percentile) and median (50^{th} percentile) prey size increased with predator size (Chipps and Garvey 2007), as this could influence the ingestion of Hg by different age-classes of Walleye.

Stable isotope analysis was used to generate more time-integrated measures of resource use by each sex-by-ploidy group and to compliment stomach content analyses. Dried and homogenized epaxial muscle tissue samples from Walleye and potential prey items were sent to the Cornell University Stable Isotope Laboratory for determination of $\delta^{13}C$, $\delta^{15}N$ and C:N ratios. Carbon signatures were corrected for lipid content if necessary (Post et al. 2007; Skinner et al. 2016). Trophic position and dietary sources for different groups of Walleye were inferred based on expected isotopic fractionation and corresponding increases in $\delta^{15}N$ (3.4‰) and $\delta^{13}C$ (≤1.0‰) values when moving from prey to predator (Post 2002).

3.2.6 Growth estimation

We estimated the age and growth of Walleye from sagittal otoliths. Otoliths were sectioned transversely through the core and examined through a compound microscope using reflected light at 40-100x magnification. Ages were assigned, blind to fish length, independently by two readers. When assigned ages disagreed, both readers examined the structure together to reach a consensus-based age. Annulus radius measurements were made on magnified images with the RFishBC package in R (Ogle 2019). Measurements were from the nucleus of the otolith to each annulus along the distal growth axis adjacent to the sulcus.

Individual lifetime growth trajectories were estimated by back-calculation of length-at-age using otolith annulus radius measurements. Heidinger and Clodfelter (1987) found that Walleye have an asymptotic otolith radius-total length (OR-TL) relationship, and Schirripa (2002) demonstrated that a Weibull cumulative function was the best model formulation for characterizing this relationship:

$$L_{c_i} = \gamma \left(1 - e^{-\left[\frac{R_{c_i}}{\alpha}\right]^{\beta}} \right) \tag{3.4}$$

where L_{c_i} is the TL at capture for fish *i*, R_{c_i} is otolith radius at capture for fish *i*, and α, β, and γ are estimated parameters. We fit the Weibull function to data separately for each sex using maximum likelihood estimation with lognormal error structure to account for increasing variation in R_c as age increased. Statistical model fitting was performed using the bbmle package in R (Bolker and R Development Core Team 2020).

We then applied the corresponding back-calculation model Schirripa (2002) developed for fish with an asymptotic OR-TL relationship:

$$L_{it} = \left(\gamma \left[\frac{L_{c_i}}{L_{p_i}} \right] \right) \left(1 - e^{-\left[\frac{R_{c_i}}{\alpha} \right]^{\beta}} \right) \quad (3.5)$$

where L_{it} is the back-calculated TL for fish *i* at age *t*, and L_{p_i} is the theoretical length of fish *i* according to its otolith radius as predicted by the fitted OR-TL relationship. Back-calculated length-at-age estimates are repeated measurements of size over time for individual fish, which have a hierarchical structure. Therefore, we fit the parameters of the von Bertalanffy growth model to these data for each sex-by-ploidy group using hierarchical nonlinear maximum likelihood estimations following the procedures recommended by Ogle et al. (2017):

$$L_i = L_{\infty_h} \left(1 - e^{K_h \left[t - t_{0_h} \right]} \right) + \varepsilon_{h_i} \quad (3.6)$$

where L_i is the TL for fish *i*, *h* is an integer that identifies the population (i.e., sex-by-ploidy group), L_∞ is the asymptotic mean length, *K* is the Brody growth coefficient, t_0 is the theoretical age at zero length, and ε_{h_i} are within-population random errors, assumed normally distributed with a mean of 0 and standard deviation of σ. Lastly, length-at-age estimates from the fitted von Bertalanffy growth curves were linked to WW-TL regressions (Table 3.1) derived for each sex-by-ploidy group during early summer (when gonadal tissue was minimal) to characterize somatic WW-at-age for incorporation into the bioenergetics model

3.2.7 Bioenergetics simulations

We used a bioenergetics model informed by field data and implemented in Fish Bioenergetics 4.0 (Deslauriers et al. 2017) to estimate prey consumption, dietary T-Hg exposure (i.e., the amount of Hg ingested), and resulting [T-Hg] in somatic tissue for triploid and diploid Walleyes of each sex for comparison to empirical [T-Hg] observations. Consumption and respiration.

TABLE 3.1 – Parameter estimates for weight–length regressions (WLR; ln WW = a + b × ln TL) and von Bertalanffy growth functions (VBGF) estimated for each sex-by-ploidy group of Walleye in Narraguinnep Reservoir.

		WLR parameters			von Bertalanffy Parameters					
Ploidy	Sex	*a*	*b*	R^2	L_∞		*K*		t_0	
2N	Male	1.48×10^{-6}	3.28	0.976	495	(11.43)	0.373	(0.026)	-0.074	(0.109)
	Female	4.12×10^{-6}	3.10	0.979	573	(10.76)	0.285	(0.024)	-0.210	(0.101)
3N	Male	3.68×10^{-6}	3.12	0.965	514	(19.75)	0.322	(0.043)	-0.294	(0.171)
	Female	9.55×10^{-7}	3.35	0.987	534	(15.00)	0.305	(0.032)	-0.192	(0.131)

Note: Corresponding standard errors for the VBGF parameter estimates are listed in parentheses. 2N diploid, 3N = triploid.

parameters were taken from Kitchell et al. (1977), and egestion and excretion parameters from Stewart et al. (1983). The Walleye bioenergetics model assumed that standard metabolic rate (SMR) and activity did not vary between the sexes. Simulations were structured to align with the phenology of gonadal development from immediately post-spawn (somatic WW only) to immediately pre-spawn (somatic + full gonad WW) the following year. Simulations, fit to observed annual growth in total WW, were conducted for an average individual from each age-class (age-2 to age-8), sex, and ploidy to estimate daily prey consumption rates and resulting dietary T-Hg exposure accumulated over one year.

Walleye typically spawn when surface water temperatures reach 5 – 10°C in spring (Barton and Barry 2011). Therefore, we defined day one of each simulation as the day after presumed spawning on April 1, when surface temperatures reached 7.0°C in Narraguinnep Reservoir. The final day was set to that of presumed spawning on March 31 the following year. Daily thermal experience was estimated from surface temperatures recorded with Onset HOBO Pendant UA-002-08 temperature loggers placed in Narraguinnep Reservoir during 2020. Simulations assumed a diet of 100% crayfish *Orconectes spp*. based on results from diet and stable isotope analyses (see below). The indigestible proportion of crayfish was set to 0.19 (Stein and Murphy 1976). Crayfish ED (3,706 $J \cdot g^{-1}$ WW) was derived from a study on another reservoir in southwestern Colorado (Pate et al. 2014). Dietary T-Hg exposure was calculated from the mean T-Hg value estimated for crayfish (0.061 $\mu g \cdot g^{-1}$) from previous work on Narraguinnep and other nearby reservoirs (Colorado Department of Public Health and Environment 2021) multiplied by the total estimated annual consumption. Annual somatic gross growth efficiency (GGE_S) was calculated for each simulation as the change in predator somatic WW divided by the WW of prey consumed.

3.2.7.1 Simulating differential reproductive investment

We accounted for differential reproductive investment in estimates of prey consumption and dietary T-Hg exposure from the bioenergetics model by manipulating daily inputs for the somatic and gonadal ED of Walleye to reflect observed differences in seasonal gonadal development and energy content among sexes and ploidies. Linear models developed above provided age-specific estimates of mean somatic and gonadal ED for each sex-by-ploidy group. Next, we used a local polynomial regression model fit to monthly GSI values estimated for male and female Walleye from Henderson et al. (1996) to characterize the daily progression of gonad development and corresponding changes in gonad WW for each age-class of mature diploid Walleye over the one-year simulation period. Age-at-maturity was defined as the first age-class where >50% of diploid fish sampled during the spawning season were deemed reproductive (≥ age-3 for males and ≥ age-5 from females). Conversely, for male and female triploid Walleye of mature age—based on observations from their diploid counterparts—we used the mean GSI observed in spring to estimate their maximum gonad WW and mean GSI observed in summer to estimate their minimum gonad WW. These values were linearly interpolated to estimate daily GSIs and gonad WW over the simulation interval. Daily gonad and somatic WW were summed to generate daily total WW, and to then compute a mean composite whole-body ED weighted by the relative proportion of each tissue type. For diploid fish, simulations were conducted with (i.e., spawning diploids) and without gonadal development (i.e., non-spawning diploids) to separate the relative effect of reproductive investment from somatic growth and ED on model-predicted [T-Hg].

3.2.7.2 Translating dietary T-Hg exposure into somatic [T-Hg]

Converting the bioenergetics-based estimates of dietary T-Hg exposure to expected [T-Hg] in somatic tissue enabled relative comparisons to the empirical data. Annual T-Hg

accumulation was calculated by multiplying T-Hg exposure by the T-Hg assimilation efficiency of fish consuming contaminated crayfish (0.94; Bowling et al. 2011) and subtracting the amount of T-Hg lost to spawning. Spawning losses were assumed zero for triploids and immature diploids, 0.043 μg Hg·g^{-1} gonadal tissue for mature diploid females, and 0.055 μg Hg·g^{-1} gonadal tissue for mature diploid males (Mauk and Brown 2001). Since Mauk and Brown (2001) measured whole testes rather than milt, we assumed that males lost 100% of the T-Hg in their testes due to spawning. Cumulative somatic T-Hg burdens adjusted for losses from spawning were then divided by ending somatic WW to obtain expected [T-Hg] for each age-class. We used the average observed [T-Hg] for age-2 Walleye from Narraguinnep Reservoir as the starting value in our calculation of predicted [T-Hg] from the bioenergetics model. Because the metabolic elimination rate of MeHg is variable and poorly understood (Yao and Drouillard 2019), our conversions between dietary T-Hg exposure and [T-Hg] did not include metabolic elimination and therefore should be viewed as overestimates.

3.3 Results

A total of 473 Walleyes were sampled from Narraguinnep Reservoir between March 20, 2018, and March 20, 2020. Diploid females, diploid males, triploid females, and triploid males made up 11.0%, 67.4%, 10.2%, 11.4% of the catch in spring samples and 26.4%, 31.3%, 23.6%, 18.8% in summer samples, respectively. We sampled diploid Walleye from the 1998 – 2017 cohorts, and triploids from the 2011 – 2017 cohorts. While triploid stocking at Narraguinnep Reservoir began in 2008, it appears the first two cohorts (2008 and 2010) failed to recruit.

3.3.1 Observed somatic [T-Hg]

Somatic [T-Hg] ranged from 0.13 to 0.95 μg T-Hg·g^{-1} across age-classes and sex-by-ploidy groups. The results of our linear modelling revealed that triploids had lower [T-Hg] than

diploids after accounting for age. The top two models, AGE × PLOIDY + SEX ($F_{3,57}$ = 69.79; R^2 = 0.833; p < 0.0001; ω_i = 0.350) and AGE × PLOIDY ($F_{2,57}$ = 89.7; R^2 = 0.825; p < 0.0001; ω_i = 0.317), were the only models with $\Delta AIC_c < 2$, and accounted for 66.8% of the Akaike weights (Table 3.2). Two other models, AGE × SEX × PLOIDY ($F_{7,53}$ = 42.1; R^2 = 0.848; p < 0.0001; ω_i = 0.107) and AGE × SEX + AGE × PLOIDY ($F_{5,55}$ = 54.9; R^2 = 0.833; p < 0.0001; ω_i = 0.101) were included in the average model (Table 3.2; Figure 3.2). At age-8, Diploid females had 30.8% (95% CI = 24.1 – 37.5%, $p < 0.0001$) higher [T-Hg] than triploid females, and diploid males had 28.3% (95% CI = 20.3 – 36.2%, $p = 0.0002$) higher [T-Hg] than triploid males (Table 3.3). Within each ploidy, males had higher [T-Hg] than females, but these contrasts were not significantly different (Table 3.3).

3.3.2 Gonadosomatic Index

Gonadal development as characterized by seasonal GSI values varied by sex and ploidy. Diploids of both sexes exhibited much higher GSI values than their triploid counterparts during the spawning period (Figure 3.3). Mean GSI for spawning diploid females was 12.6% (n = 17, SD = 4.77) compared to 0.4% (n = 11, SD = 0.20) for similarly aged triploid females. Mean GSI for spawning diploid males was 2.3% (n = 157, SD = 0.76) compared to 0.3% (n = 11, SD = 0.03) for triploid males. Mean GSI values observed for mature diploids decreased by more than 80% from spring to summer. In the summer, mean GSI for mature diploid females was 1.5% (n = 20; SD = 0.35), and 0.3% (n = 25, SD = 0.25) for mature diploid males. Mean GSI values observed for mature aged triploids during summer were only slightly lower than those during spring for both females (0.2%, SD = 0.14, n = 2) and males (0.1%, SD = 0.005, n = 2).

TABLE 3.2 – Models retained (cumulative $\omega_i \leq 0.95$) for predicting [T-Hg] based on sex, ploidy, and fish age for Walleyes captured at Narraguinnep Reservoir (March and June 2019).

Model structure	R^2	k	loglik	AIC_c	ΔAIC_c	ω_i
AGE × PLOIDY + SEX	0.833	6	92.1	-170.6	0.000	0.350
AGE × PLOIDY	0.825	5	90.7	-170.4	0.197	0.317
AGE × PLOIDY × SEX	0.846	9	94.7	-168.2	2.381	0.107
AGE × SEX + AGE × SEX	0.833	7	92.1	-168.1	2.492	0.101

Note: R^2 = coefficient of determination, k = number of parameters, loglik = model log-likelihood, AIC_c = Akaike's information criterion adjusted for small sample sizes, ΔAIC_c = difference in AIC_c between the given model and the most parsimonious model, ω_i = Akaike weights. The full model was AGE × SEX × PLOIDY.

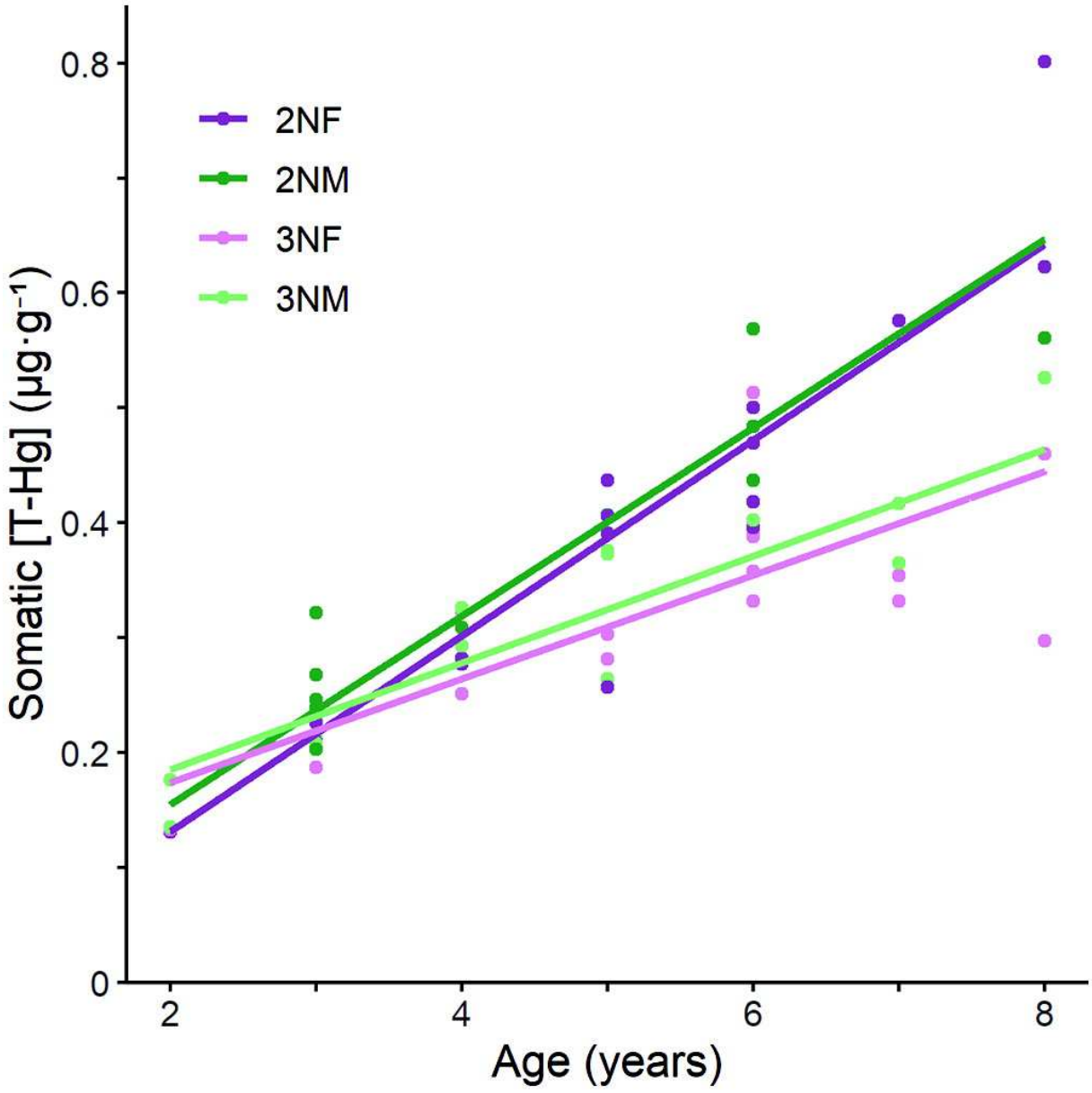

FIGURE 3.2 - Observed somatic mercury concentrations [T-Hg] by age for Walleye from Narraguinnep Reservoir (points). Lines represent model-averaged estimates of regression coefficients for retained models (cumulative $\omega_i \leq 0.95$). 2N = diploid, 3N = triploid, F = female, M = male.

TABLE 3.3 – Relative pairwise contrasts of [T-Hg] at age-8 derived from the average model.

Contrast	Percent difference (95% CI)	*t* ratio	*p* value
2NF - 3NF	30.8% (24.1% - 37.5%)	5.769	<0.0001
2NF - 2NM	0.7% (-5.7 - 7.1%)	-0.137	0.9991
2NF - 3NM	27.8% (20.9% - 34.7%)	5.048	<0.0001
3NF - **2NM**	31.2% (23.9% - 38.6%)	-5.399	<0.0001
3NF - **3NM**	4.2% (-1.3% - 9.7%)	-0.686	0.9019
2NM - 3NM	28.3% (20.3% - 36.2%)	4.491	0.0002

Note: *p* values are Tukey-adjusted. Bolded sex-by-ploidy group indicates the group the percent difference is relative to. 2N = diploid, 3N = triploid, F = female, M = male.

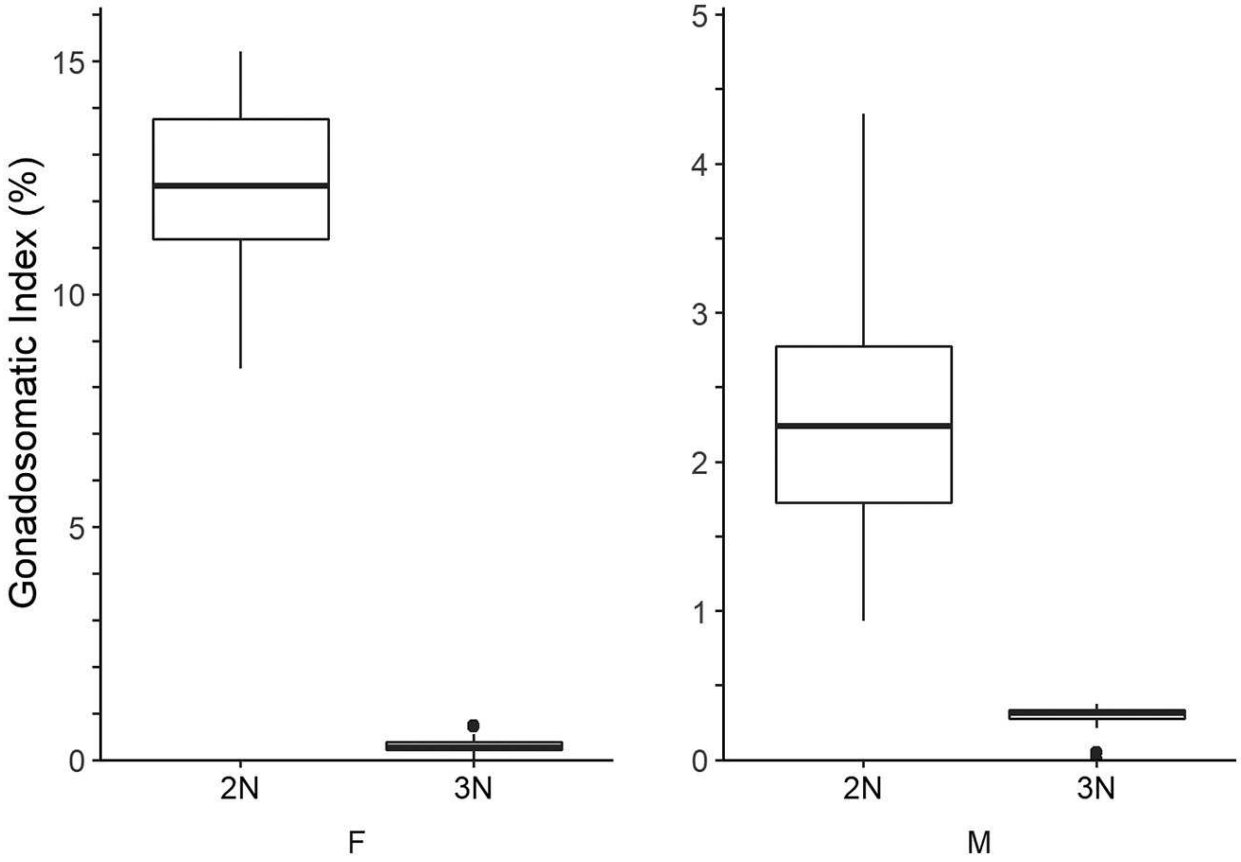

FIGURE 3.3 - Summarized distributions of gonadosomatic index values (%) by sex and ploidy for adult Walleye captured during the spawning period in March 2019 and March 2020 in Narraguinnep Reservoir. Boxes show the median (thick line), first and third quartiles (lower and upper hinges). Box whiskers extend from the hinges to the most extreme value no further than 1.5 ± the interquartile range from the hinge. 2N = diploid, 3N = triploid, F = female, M = male.

3.3.3 Energy density of somatic and gonadal tissue

The best model as determined by AIC_c model selection demonstrated a significant effect of sex and ploidy on somatic ED after accounting for age, and this difference was driven by diploid females. The full model (i.e., AGE × SEX × PLOIDY) was the only model with a ΔAIC_c value < 2 ($F_{7,165} = 9.71$, $R^2 = 0.292$, $p < 0.001$). Estimated marginal mean somatic EDs were similar among the sex-by-ploidy groups (diploid males = 3,983 J·g^{-1} WW, triploid males = 3,951 J·g^{-1} WW, triploid females = 3,890 J·g^{-1} WW, diploid females = 3,811 J·g^{-1} WW). However, the effect of age on somatic ED was not the same for all groups, ED increased significantly with age for all except diploid females. The slope for diploid females (β_{age} = -40.8 J·year^{-1}, SE = 41.7) was significantly different from triploid females (β_{age} = 111.5 J·year^{-1}, SE = 27.6, $p = 0.014$) and diploid males (β_{age} = 132.1 J·year^{-1}, SE = 24.9, $p = 0.003$), but not from triploid males (β_{age} = 86.1 J·year^{-1}, SE = 37.0, $p = 0.107$). All other pairwise contrasts of slopes among sex-by-ploidy groups for somatic ED were not significant ($p \geq 0.73$). Furthermore, the ED of fully developed diploid testes increased significantly with age (β_{age} = 88.9 J·year^{-1}, SE = 26.3, $R^2 = 0.289$, p = 0.0022), and averaged 3,875 J·g^{-1} WW (SD = 750, n = 30) across age-classes. No age-dependency was observed for the ED of fully developed diploid ovaries ($F_{1,8} = 0.251$, $p = 0.63$), which averaged 10,048 J·g^{-1} WW (SD = 973, n = 10). Gonadal energy densities estimated for triploids averaged 3,680 J·g^{-1} WW (SD = 624, n = 7) for females and 2,991 J·g^{-1} WW (SD = 892, n = 3) for males.

3.3.4 Diet and stable isotope analysis

Stomach content analysis demonstrated minimal variation in diet composition among sexes and sizes of Walleye. Overall, we analyzed 60 stomachs, of which 16 were empty, from Walleye ranging in length from 233 – 593 mm TL captured during summer 2018. By volume,

crayfish comprised 94.4% of the diets, Yellow Perch accounted for 5.3%, and fish of unknown species comprised 0.2%. Diet composition was similar for males (96% crayfish by volume; n = 24) and females (94% crayfish by volume; n = 36). Carapace lengths of crayfish found in Walleye stomachs ranged in size from 13 – 54 mm and averaged 34 mm (SD = 9.48, n = 38). Yellow perch found in Walleye stomachs ranged in size from 33 – 81 mm TL and averaged 52 mm (SD = 13.5, n = 12). There was no evidence of a relationship between predator TL and maximum ($F_{1,52} = 0.91$, $p = 0.541$) or median ($F_{1,52} = 0.03$, $p = 0.878$) prey size. Unforeseen issues regarding laboratory sample analyses precluded ploidy determination for fish captured in 2018. However, the consistent and strong presence of crayfish indicated minimal differences in diet between ploidies.

Stable isotope analyses confirmed minimal differences in diet among age-classes of Walleye (n = 173) and sex-by-ploidy groups and supported the notion that Walleye in Narraguinnep Reservoir prey primarily on crayfish (Figure 3.4). Mean δN^{15} and δC^{13} values for each sex-by-ploidy group were indistinguishable (Table 3.4). In addition, the linear model with age only best described variation in δN^{15} ($F_{1,171} = 168$, $R^2 = 0.495$; $p < 0.001$) based on AIC_c. However, the estimated rate of change in δN^{15} with age was low ($\beta_{age} = 0.165$ ‰·year^{-1}), reflecting a negligible shift in trophic position from age-2 to age-8. Conversely, the top model for δC^{13} was SEX + AGE × PLOIDY ($F_{5,167} = 19.9$, $R^2 = 0.36$, $p < 0.001$). Only the pairwise contrasts between marginal means for diploid males and triploid females ($p = 0.001$), and diploid and triploid males ($p = 0.031$) were significantly different. These differences were < 0.312 ‰, and not ecologically meaningful given the relatively large range in δC^{13} present in the food web.

TABLE 3.4 – Mean $\delta^{15}N$ and $\delta^{13}C$ (‰) values and corresponding standard deviations estimated for each sex-by-ploidy group of Walleye (age ≤ 8) captured at Narraguinnep Reservoir (March and June 2019).

			Total length (mm)			$\delta^{15}N$‰		$\delta^{13}C$‰	
Ploidy	Sex	n	Range	Mean	SD	Mean	SD	Mean	SD
Diploid	Male	57	310-519	425	49.6	12.3	0.44	-25.3	0.53
	Female	26	258-537	428	70.6	12.4	0.37	-25.2	0.45
Triploid	Male	31	248-479	357	63.1	12.2	0.45	-25.4	0.54
	Female	59	269-506	392	71.3	12.3	0.39	-25.2	0.57

The position of Walleye relative to potential prey and isotopic baselines in δ-space aligned with a diet predominated by crayfish based on the expected enrichment of δN^{15} and δC^{13} when moving from prey to predator (Figure 3.4). Great pond snails (*Lymnaea stagnalis*) (n = 51; δN^{15} = 5.30 ± 0.87‰; δC^{13} = -32.0 ± 1.38‰; mean ± SD) and bulk zooplankton samples (n = 2; δN^{15} = 5.1 ± 0.07‰; δC^{13} = -36.5 ± 0.22‰) represented the benthic and pelagic baselines, respectively. Crayfish sampled (n = 69; δN^{15} = 8.79 ± 0.95‰; δC^{13} = -25.2 ± 1.20‰) ranged in size from 22 - 65 mm carapace length (mean = 49 mm), which aligned well with the range of sizes observed in Walleye diets. Potential fish prey, represented by Yellow Perch (n = 6; δN^{15} = 11.1 ± 1.27‰; δC^{13} = -26.5 ± 1.73‰), ranged in size from 50 – 265 mm total length (mean ± SD = 131 ± 89.2 mm). The trophic positions of Walleye, Yellow Perch, and crayfish relative to the benthic baseline were 3.09, 2.71, and 2.03, respectively. Thus, Walleye and crayfish approximately differed by one full trophic level and remained within the expected range for δC^{13}, whereas the difference in trophic position between Walleye and Yellow Perch was too small to suggest that Yellow Perch were common prey for Walleye in Narraguinnep Reservoir.

3.3.5 Predicted somatic [T-Hg] from bioenergetics model

After integrating field data on ED, GSI, diet and growth, the bioenergetics simulations showed that the energetic costs of spawning increased [T-Hg] for females, but not males, due to differences in prey consumption required to meet somatic and gonadal growth. On a cumulative basis from age-2 to age-8, spawning diploid females consumed the most prey (22,648 g) and T-Hg (1,382 µg), followed by non-spawning diploid females (17,182 g; 1408 µg), triploid females (16,442 g; 1003 µg), spawning diploid males (15,499 g; 945 µg), non-spawning diploid males (15,049 g; 918 µg), and triploid males (14,649 g; 894 µg). From age-at-maturity to age-8, diploid males required 405.4 kJ of energy to develop testes, while diploid females

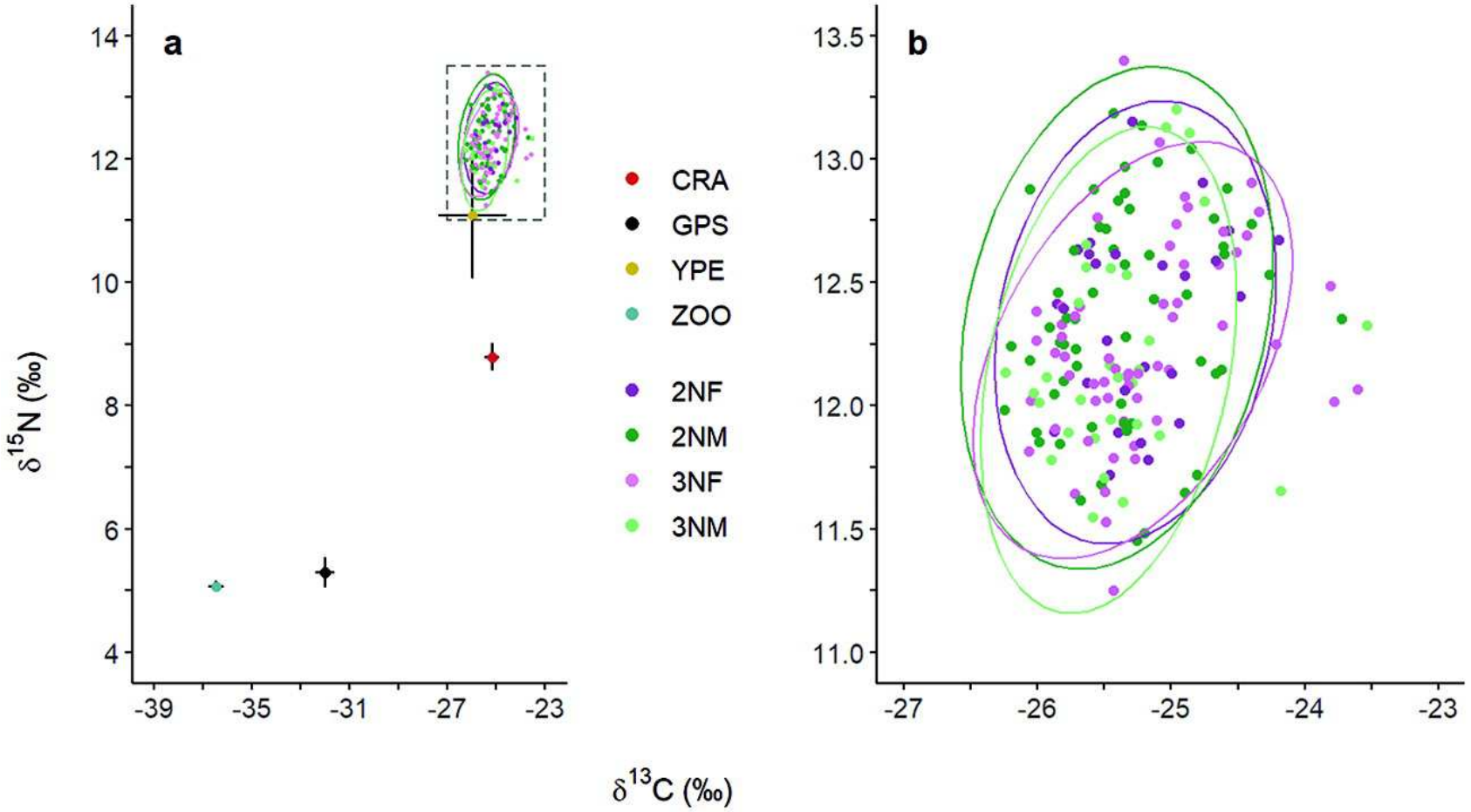

FIGURE 3.4 - (A) Bi-plot of stable isotope values from individual Walleye (age ≤ 8) and potential prey items in Narraguinnep Reservoir. Lines represent 95% confidence ellipses for Walleye by sex and ploidy, and error bars for mean values from prey items represent 95% confidence intervals. (B) Magnified view for Walleye only from panel (A). CRA = Crayfish, GPS = Great Pond Snail, YPE = Yellow Perch, ZOO = Zooplankton, 2NF = diploid female Walleye, 2NM = diploid male Walleye, 3NF = triploid female Walleye, 3NM = triploid male Walleye.

required 7,525.9 kJ to develop ovaries. Predicted [T-Hg] for spawning diploid females diverged from all other groups beginning at age-5, when they began devoting energy toward egg development (Figure 3.5). At age-8, [T-Hg] for spawning diploid females (0.900 $\mu g \cdot g^{-1}$) was 25% higher than non-spawning diploid females (0.719 $\mu g \cdot g^{-1}$), 20% higher than triploid females (0.748 $\mu g \cdot g^{-1}$), and 8% higher than spawning diploid males (0.836 $\mu g \cdot g^{-1}$). Spawning diploid males had 2% higher [T-Hg] than non-spawning diploid males (0.820 $\mu g \cdot g^{-1}$ T-Hg), and 4% higher [T-Hg] than triploid males (0.806 $\mu g \cdot g^{-1}$ T-Hg). Triploid males had 7% higher [T-Hg] than triploid females.

Annual patterns in total WW-at-age and corresponding model-derived GGEs for each sex-by-ploidy group helped demonstrate the effects of allocating energy to gamete production on somatic growth and why [T-Hg] for spawning diploid females diverged from all other groups. While spawning diploid females had higher GGEs prior to maturity relative to their triploid counterparts, their GGEs decreased following maturation (Figure 3.6). By age-8, the energetic costs of spawning decreased GGEs for diploids of both sexes, but more so for females than males. Non-spawning diploid females had 46% higher GGEs (0.347) than spawning diploid females (0.238), and non-spawning diploid males (0.321) had 4% higher GGEs than spawning diploid males (0.310). Triploid females (0.329) had 38% higher GGEs than spawning diploid females, and triploid males (0.321) had 4% higher GGEs than spawning diploid males

3.4 Discussion

This is the first study to investigate the role of reproductive investment in Hg bioaccumulation using triploid fish as reproductive controls. As hypothesized, triploid females had significantly lower observed somatic [T-Hg] than diploid females. This difference could not be explained by diet and somatic growth alone. Relative comparisons of predicted [T-Hg] among spawning.

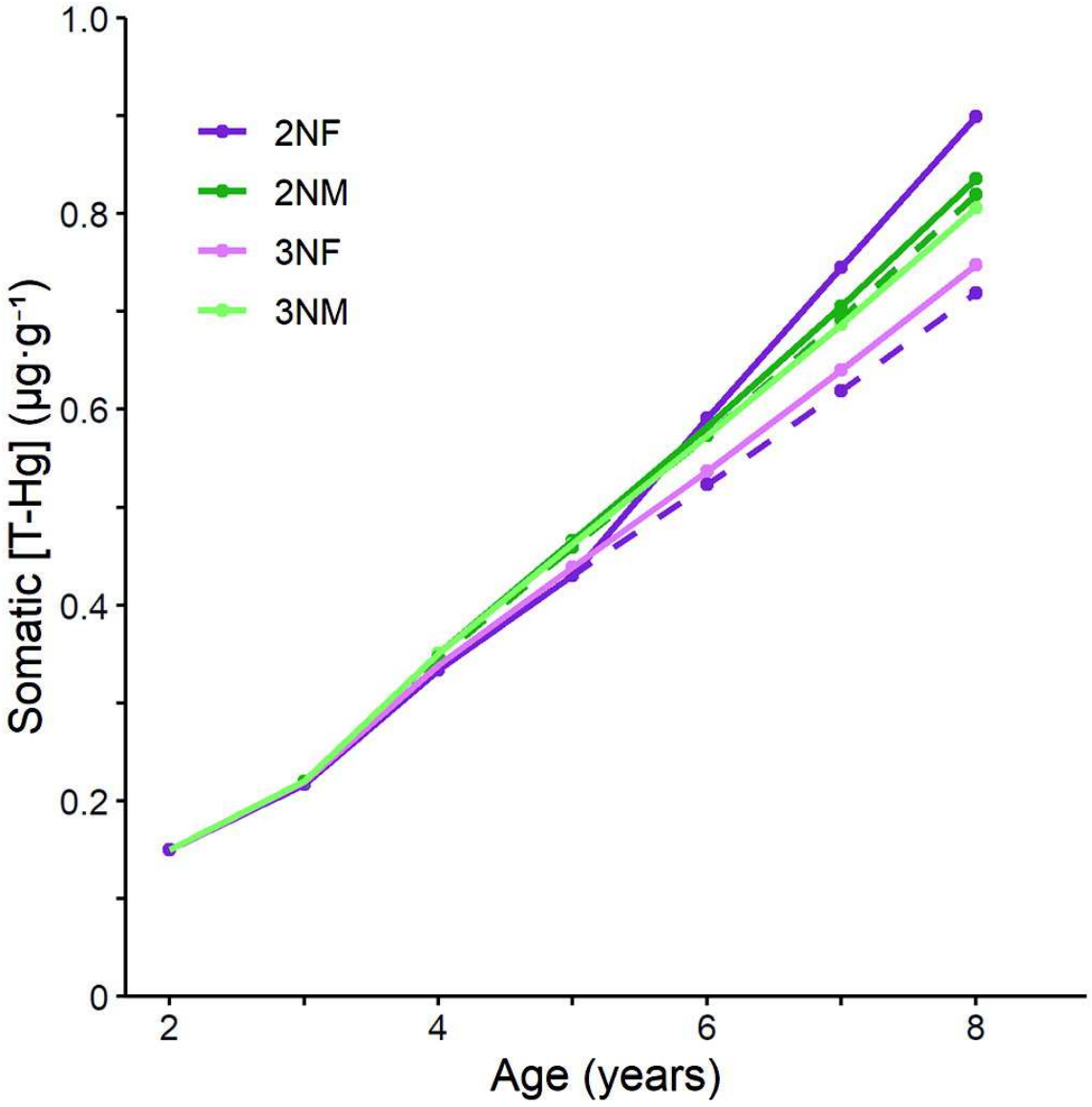

FIGURE 3.5 – Somatic total mercury concentrations ([T-Hg]; $\mu g \cdot g^{-1}$) by age for Walleye estimated by the bioenergetics model. 2N = diploid, 3N = triploid, F = female, M = male. For diploid Walleye, solid lines represent spawning fish (full gonadal development observed in the field), while dashed lines represent hypothetical non-spawning fish (no gonadal development)

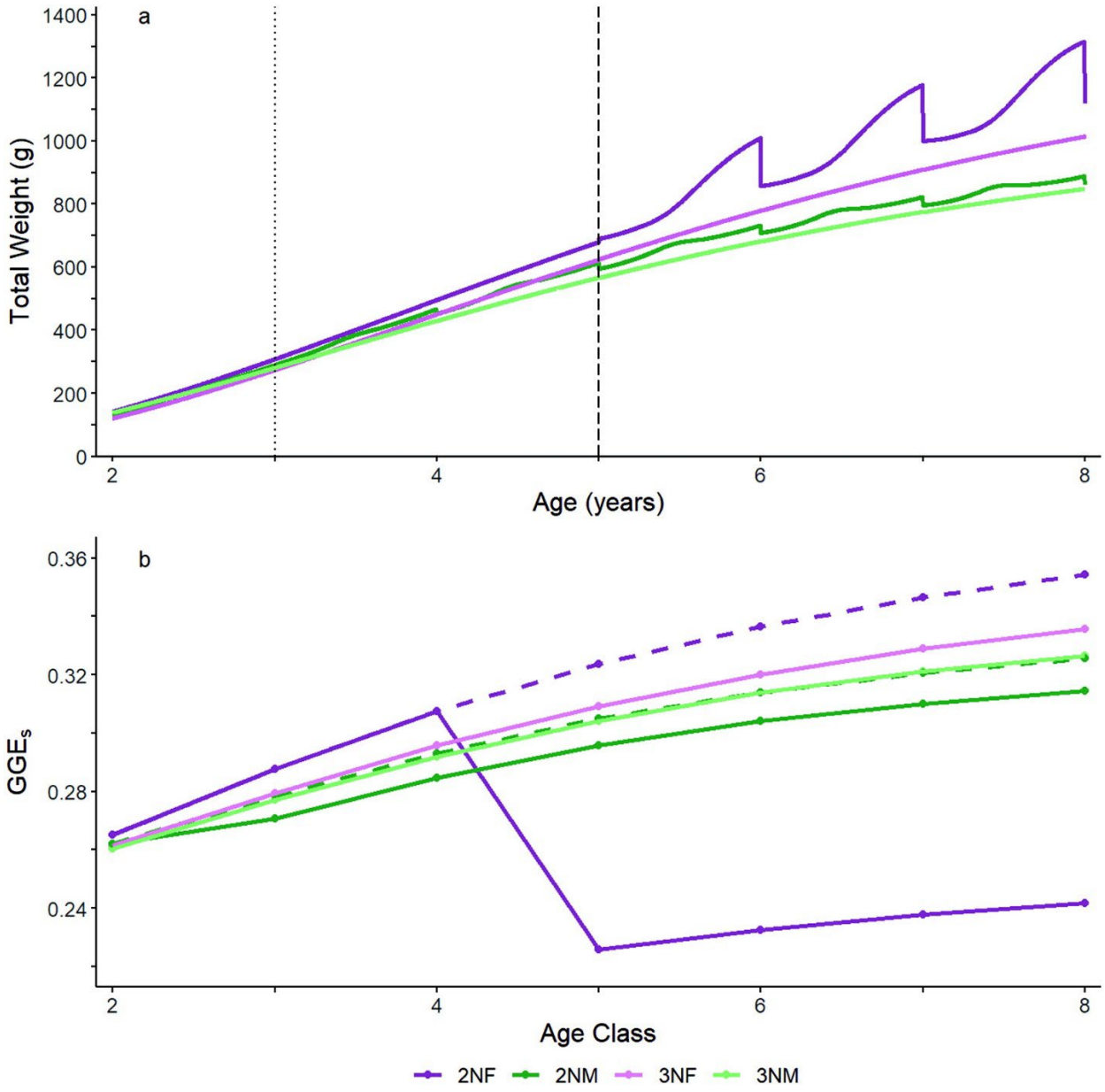

FIGURE 3.6 - (A) Total wet weight-at-age (somatic + gonadal tissue) and (B) annual somatic gross growth efficiency (GGEs) estimated from the bioenergetics model for Walleye in Narraguinnep Reservoir. For diploid Walleye, solid lines represent spawning fish (full gonadal development observed in the field), while dashed lines represent hypothetical non-spawning fish (no gonadal development). Total wet weight-at-age was used to fit the bioenergetics models. The dotted and dashed vertical lines show observed age-at-maturity for diploid males and females, respectively.

diploid, non-spawning diploid, and triploid females from the bioenergetics model indicated that the observed difference in somatic [T-Hg] was mostly driven by increased prey consumption required by diploid females to meet the energetic demand of ovarian development. Contrary to the premise of our second hypothesis, triploid males exhibited reduced testicular development. In addition, our second hypothesis was not supported, as triploid males showed lower [T-Hg] than diploid males despite similar diet and somatic growth rates. However, unanticipated differential testicular development could not explain observed differences in [T-Hg] between ploidies for males in the bioenergetics model. Lastly, our third hypothesis was only partially supported. We expected that males of both ploidies would exhibit lower [T-Hg] than diploid females because testes are typically less energetically costly to produce than ovaries. As anticipated, our bioenergetics modelling confirmed that testes are less energetically costly to produce than ovaries. Differences in the energetic costs of reproductive development likely drove the disparity in observed [T-Hg] between diploid females and triploid males. However, observed [T-Hg] for diploid males did not differ from diploid females despite reduced consumption requirements. Thus, processes unrelated to investment of energy into gonadal tissue was driving elevated [T-Hg] in diploid males relative to a priori expectations.

We hypothesized that reproductive investment was an important process driving differential [T-Hg] between diploid and triploid females because the energetic demand of ovarian development would require additional prey consumption. Since fish primarily uptake Hg via food (Hall et al. 1997), the need for additional prey should lead to increased dietary Hg exposure and decreased GGEs for diploid females. Essentially, reproductive investment should "distill" Hg from food which accumulates in somatic tissue because the biochemical composition of eggs is not conducive to the binding of MeHg and resulting [MeHg] in eggs are typically low (Niimi

1983). Methylmercury primarily binds to sulfhydryl groups in muscle protein (Wiener and Spry 1996), whereas teleost eggs are primarily composed of lipids and yolk proteins (Brooks et al. 1997). For example, mean [T-Hg] measured in eggs from seven Walleye populations across the United States and Canada was only 2.1% of that in muscle (Johnston et al. 2001). Thus, eggs are not a meaningful route of elimination of Hg in Walleye.

Results from the bioenergetics simulations supported the "distillation" hypothesis for females. The relative magnitudes of the difference in model-predicted [T-Hg] at age-8 between spawning diploids and triploids (20%) and between spawning and non-spawning diploids (25%) were similar to that observed between diploid and triploid females in the field (31%). Walleye eggs were more than 2.5 times as energy dense as somatic tissue. Since ovaries comprised ~15% of the total body WW when fully developed, diploids had higher annual energetic requirements relative to triploids and ingested more T-Hg. On a cumulative basis, spawning diploid females consumed 38% and 32% more prey by WW and their GGEs was 27.7% and 31.4% lower than triploid and non-spawning diploid females, respectively. Assuming the bioenergetics model characterized Hg dynamics perfectly, the inclusion of differential reproductive investment explained 53-104% of the observed difference in [T-Hg] for females.

The premise of our second hypothesis, namely that triploid males in Narraguinnep Reservoir would exhibit normal testicular development, was not supported. We did not capture any triploid males that expressed milt, nor find strong evidence of sexual maturation, during the spawning season in 2019 or 2020. Triploid males from a diverse set of species typically show normal testicular development (Benfey 1999), but there are exceptions (Tiwary et al. 2004). We are unsure why triploid Walleye may be another exception to the norm, but Tiwary et al. (2000) suggested that low levels of sex steroids may impair testicular development in triploid Stinging

Catfish *Heteropneustes fossilis*. Alternatively, it is possible that testicular development is severely delayed for triploid male Walleye, and the population of triploid males in Narraguinnep Reservoir has not yet reached sexual maturity.

Furthermore, we did not find support for our second hypothesis that diploid and triploid males would have similar [T-Hg]. Rather, diploid males had 28.3% higher observed [T-Hg] than triploid males at age-8. However, differences in testicular development likely did not contribute to the differences in T-Hg bioaccumulation between triploids and diploids, as the investment of energy into reproductive tissue had little influence on estimated consumption rates for males. At age-8, the bioenergetics model predicted relatively small differences in [T-Hg] among triploid, spawning diploid, and non-spawning diploid males; all pairwise comparisons of predicted [T-Hg] among these groups were less than 4%. Thus, while triploid males did not develop testes to the same extent as mature diploid males, similarities in diet, body composition, and somatic growth resulted in similar consumption requirements according to the bioenergetics model.

Our third hypothesis postulated that males of both ploidies would have lower [T-Hg] than diploid females due to differences in the energetic costs of developing testes versus ovaries. As expected, the energetic cost of producing testes was low relative to the cost of producing ovaries. On a cumulative basis from age-2 to age-8, ovarian development required 18.6-times more energy intake than testicular development, despite males reaching maturity two years earlier. As a result, the bioenergetics model predicted that [T-Hg] for all male groups (i.e., spawning diploids, non-spawning diploids, and triploids) would fall below diploid females by 7-10%. However, only the triploid males had significantly lower observed [T-Hg] than diploid females at age-8, while diploid males exhibited similar observed [T-Hg] as diploid females of the same age.

Differences in swimming activity among the sex-by-ploidy groups could explain why diploid male Walleye exhibited elevated [T-Hg] relative to a priori expectations and corresponding predictions from the bioenergetics model. As in many species of fish (Lucas 1992; Altimiras et al. 1996; Bruch and Binkowski 2002; Acolas et al. 2004; Dean et al. 2014), male Walleyes are more active during the spawning period than females (Becker 1983). Female Walleye participate in spawning for a few days at most, while males roam the spawning grounds for up to four weeks (Becker 1983). In addition, scramble competition among male Walleyes for mating opportunities substantially increases activity and associated metabolic costs (Henderson et al. 2003). Catch rates for diploid males were much higher than those for any other sex-by-ploidy group during the spawning season in Narraguinnep Reservoir; diploid males represented 67% of the catch, whereas all other groups only represented 10-11% of the catch. During summer however, when spawning should not influence behavior or distribution, our catch was more evenly distributed among the sex-by-ploidy groups, and diploid males comprised 26% of the catch. The relative proportions of diploid males caught with passive gear during and outside of the spawning period suggest that diploid males are likely more active than the other sex-by-ploidy groups.

Likewise, differences in standard metabolic rates (SMRs), in addition to higher swimming activity, could also explain why diploid males exhibited elevated [T-Hg] relative to a priori expectations and corresponding predictions from the bioenergetics model. Standard metabolic rates are generally higher for male fish than for female fish, and this pattern is evident for a wide range of vertebrates (Madenjian et al. 2016). Malison et al. (1985) found that administered doses of 17α-methyltestosterone proportionally inhibited growth in Yellow Perch, which suggests that testosterone may increase SMRs in percids. The minimal testicular

development we observed for triploid male Walleye could have resulted in lower testosterone, and therefore lower SMR, leading to higher growth efficiencies and lower [T-Hg] relative to diploid males. While previous laboratory studies have not found differences in SMRs between triploid and diploid fish (see Maxime 2008), these studies used immature fish, and we would not expect ploidy-specific differences in SMRs to emerge until diploids reach sexual maturity. It is also important to note that observed patterns in [T-Hg] among diploid males, triploid males and diploid females did not solely reflect differences in energy intake or expenditure, as diploid males likely eliminated Hg at a faster rate than any other sex-by-ploidy group (Madenjian et al. 2016). Although unexpected, reduced testicular development and [T-Hg] in triploid males offered more direct insight than previously possible into potential behavioral and physiological mechanisms for why similarly aged diploid males exhibited similar [T-Hg] as larger-bodied diploid females in this study and elsewhere (Henderson et al. 2003; Selch et al. 2019). Further comparisons between diploid and triploid Walleye could help elucidate the role of testosterone in Hg elimination (Madenjian et al. 2014), quantify sex-dependent standard metabolic rates and activity costs associated with spawning, and improve energetics-based Hg bioaccumulation models.

Trophic position affects predator [T-Hg] because Hg biomagnifies in food webs (Lavoie et al. 2013). For example, [T-Hg] in Lake Trout *Salvelinus namaycush* was positively correlated to $\delta^{15}N$, a suitable indicator of trophic position, in seven Canadian shield lakes (Cabana and Rasmussen 1994). Similarly, Taylor et al. (2020) found that an increase of trophic position for lake trout following a dietary shift from invertebrates to prey fish increased their [T-Hg]. In this study, stable isotope analyses indicated that the trophic position of Walleye did not differ among sex-by-ploidy groups. A difference in δN^{15} of 3.4‰ denotes a full shift in trophic position (Post

2002). The maximum difference in mean δN^{15} among sex-by-ploidy groups for Walleye in Narraguinnep Reservoir was only 0.3‰ and did not contribute to observed variation in [T-Hg] among groups.

Diet composition influences Hg bioaccumulation because prey consumption is the dominant pathway of MeHg uptake in predators (Hall et al. 1997). Lepak et al. (2012a) found that prey selection differed by sex for Walleye in two Colorado reservoirs: males consumed smaller, lower quality prey (i.e., resident fish and invertebrates), while females consumed larger, higher quality prey (i.e., stocked Rainbow Trout). Males exhibited higher [T-Hg] than females because of their dietary differences. While typically piscivorous, Walleye consume invertebrate prey in systems with depauperate prey fish assemblages (Chipps and Graeb 2011), which is the case for many Colorado reservoirs (Johnson et al. 2015; Wolff et al. 2017). While we acknowledge that our diet analysis was temporally limited and we were not able to include ploidy in our comparisons, stable isotope analyses confirmed that Walleye in Narraguinnep Reservoir were primarily invertivorous, and that all sex-by-ploidy groups were consuming similar prey items. Thus, dietary differences are not a plausible explanation for patterns of observed [T-Hg] among groups.

Under equivalent assimilation efficiency and elimination, Hg bioaccumulation is largely controlled by the contamination level in prey, the amount of prey consumed, and the efficiency with which the predator allocates consumed energy to growth (Trudel and Rasmussen 2006). Faster growing fish may consume less food to reach a given size than slower growing fish if feeding on higher quality prey or occupying more suitable thermal habitat, reducing contaminant concentrations through growth dilution (Simoneau et al. 2005). Indeed, growth dilution can contribute to differences in [T-Hg] between sexes in diploid Walleye (Madenjian et al. 2016).

Neither diet composition nor thermal regime varied among the four combinations of sex and ploidy in our study. Thus, differences in [T-Hg] among these four groups were not attributable to differences in diet composition or thermal experience. Our results demonstrated that investment of energy into ovaries could account for a substantial portion of the difference in [T-Hg] between diploid and triploid female Walleye. Further, our results suggested that higher energy expenditure by diploid male Walleye, stemming from higher SMR and greater swimming activity, compared with triploid male Walleye could account for the higher [T-Hg] observed for diploid male Walleye.

Regardless of the potential mechanisms, the significantly lower mean [T-Hg] exhibited by triploid Walleye in our study suggests that ploidy manipulations for stocked fish could help address widespread MeHg contamination problems in fish destined for human consumption. Reducing [MeHg] in fish and wildlife by regulatory actions is challenging. Atmospheric Hg levels have risen steadily with humanity's reliance on fossil fuels (Eagles-Smith et al. 2016a) and are forecasted to increase further with the expansion of coal-fired electricity generation in the developing world (Driscoll et al. 2013). The long occupancy and dispersal of Hg in the atmosphere (Boening 2000) decouples point-source pollution from loading to aquatic systems. Thus, deposition from distant sources may undermine local efforts to reduce Hg inputs. Furthermore, recycling of legacy Hg can prolong bioaccumulation in marine and freshwater ecosystems even when external sources are controlled (Amos et al. 2013; Driscoll et al. 2013). While global actions to reduce anthropogenic Hg loading to the environment are essential, alternative management interventions at the local scale are needed to protect fish, wildlife, and humans from elevated Hg exposure. Methylmercury concentrations in fish can be manipulated by several common fisheries management strategies (Sharma et al. 2008; Lepak et al. 2012a;

Lepak et al. 2012b). The use of triploid fish in stocking programs alone or in conjunction with other strategies could prove useful for reducing [MeHg] in fish and subsequent exposure to wildlife and humans. Furthermore, since triploid fish grew more efficiently and consumed less prey, they may serve as a viable alternative stocking option in areas where the predatory impact of piscivorous sport fish is of concern.

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CHAPTER FOUR

Induced sterility in an iteroparous teleost illuminates the effects of reproductive investment on growth

4.1 Introduction

It is thought that growth and reproduction are linked, and the assumed tradeoff between them is fundamental to life history theory (Reznick 1985; Lika and Kooijman 2003; Sibly et al. 2015). The nature of this tradeoff has stimulated intense debate in the ecological and evolutionary literature (Kooijman and Lika 2014; Marshall and White 2019) and is regarded as one of the most critical theoretical issues in ichthyology (Pauly 2021). According to evolutionary theory, reproductive effort—the proportion of an organism's energy budget devoted to reproductive processes—is selected for in a way that maximizes fitness, but at a cost to growth in body size (Hirshfield and Tinkle 1975). The most common energy budget models underpinning life history theory assume energy is diverted from potential somatic growth to fuel reproduction (Schaffer 1974; Hirshfield and Tinkle 1975; Tuomi et al. 1983; Perrin and Sibly 1993). This idea, coined as the Reproductive Drain Hypothesis (RDH) by Iles (1974), implies a fixed energy budget (Hirshfield and Tinkle 1975; Tuomi et al. 1983), and is considered responsible for indeterminate growth patterns (i.e., growth deceleration) in multiple taxa (Roff 1983; Day and Taylor 1997; Charnov et al. 2001; Quince et al. 2008a).

Several growth models arising from life history theory include RDH as a central tenet (Wilson et al. 2017). Biphasic models are among these and assume that growth trajectories differ between the juvenile (sexually immature) and adult phase (sexually mature), and that the onset of reproductive development is responsible for the changing growth pattern. Some argue that a single, uniphasic curve is not appropriate to describe the growth of an organism because it cannot

account for metabolic differences between juveniles and adults (Wilson et al. 2017). Additionally, biphasic models have outperformed uniphasic models in describing empirical data (Quince et al. 2008b; Armstrong and Brooks 2013; Minte-Vera et al. 2016), lending support for RDH.

The validity of RDH has been questioned because the synchrony between the onset of growth deceleration and sexual maturation does not imply causation. For example, The Gill-Oxygen Limitation Theory (GOLT) supposes that reproductive development is triggered at a critical oxygen limitation threshold, and is therefore the result, rather than the cause, of growth deceleration (Pauly 1981). Additionally, Pauly (2019) points to the logical conundrum that female fish, which typically invest more energy into reproductive development, are often larger than male conspecifics, counter to expectations under RDH.

There are several alternative growth models that do not assume RDH. This family of growth models are based on metabolic theory and stem from the original ideas of Pütter (Pütter 1920) where growth is limited by the metabolic interplay of surface area-dependent anabolism/assimilation and volume-dependent catabolism/maintenance (Kearney 2021). The von Bertalanffy growth model (von Bertalanffy 1938) is among the most commonly used, but is essentially the same as Pütter's, with minor modifications. To acknowledge Pütter's priority (Kearney 2021), we refer to this model as the Pütter-von Bertalanffy Growth Model (P-VBGM). The Ontogenetic Growth Model (OGM; West et al. 2001) builds on Pütter's ideas, and assumes that growth is limited by supply network constraints under quarter-power scaling relationships of metabolic rates (Kearney 2021). Dynamic Energy Budget (DEB) theory (Kooijman 2010) extends Pütter's ideas to include effects of food availability, body condition, temperature, and reproduction on growth. In DEB theory, growth is modelled with strict adherence to

thermodynamic principles (Kooijman 2010; Kearney 2021). Rather than assuming RDH, DEB uses an assimilation energy budget, which assumes energy is first stored in reserve then mobilized as needed for different functions (Kooijman 2010). Under some assumptions, like constant food and temperature, DEB simplifies to P-VBGM (Kooijman 2010). GOLT is a mechanistically explicit version of P-VBGM, in that the surface-area of gills in water-breathing ectotherms is what limits anabolism, resulting in growth deceleration.

Here, we test RDH in a novel manner, by comparing the average adult body sizes of a sterile freshwater fish to fertile conspecifics co-occurring in the wild. We examined Walleye *Stizostedion vitreum* (see Bruner 2021) effectively sterilized via induced triploidy (Fetherman et al. 2015). Like most fish species, Walleye grow indeterminately (Charnov et al. 2001; Henderson et al. 2003). Additionally, Walleyes are iteroparous, capital spawners that typically spawn once per year (Barton and Barry 2011; McBride et al. 2015). Triploid Walleye do not allocate energy toward the production of gametes (Figure 4.1), and are likely less active during the spawning period than fertile diploids (Farrell et al. 2022a). Thus, comparisons of growth patterns and maximum body size between triploid and diploid Walleyes cohabiting under the same physical, chemical, and ecological conditions are well suited for directly testing RDH (Pauly 2021). Specifically, we fit length-at-age data collected from sympatric sterile and fertile Walleye to the P-VBGM and the Lester Biphasic Growth Model (LBGM; Lester et al. 2004) in a hierarchical Bayesian framework using informative prior information. We hypothesized that, if reproduction

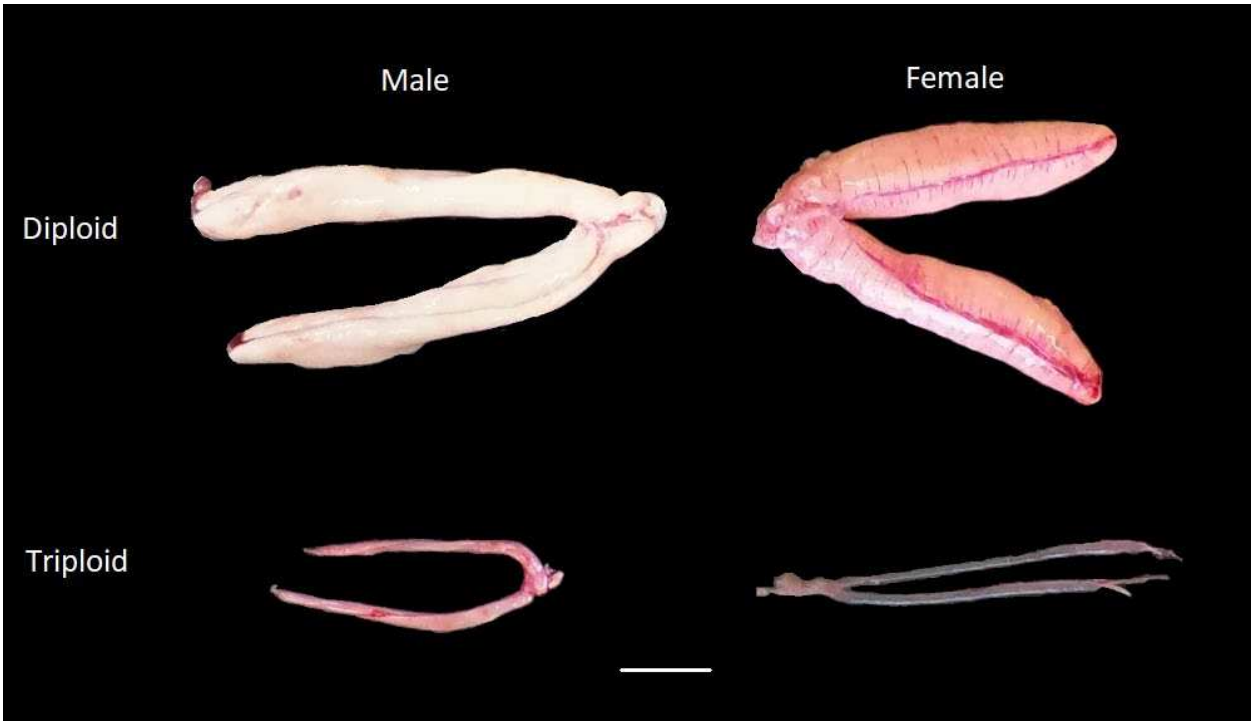

FIGURE 4.1 - Gonads typical for sterile and fertile walleyes captured immediately prior to peak spawning date. For each sex, gonads belong to fish of similar size and age. Scale bar = 25 mm

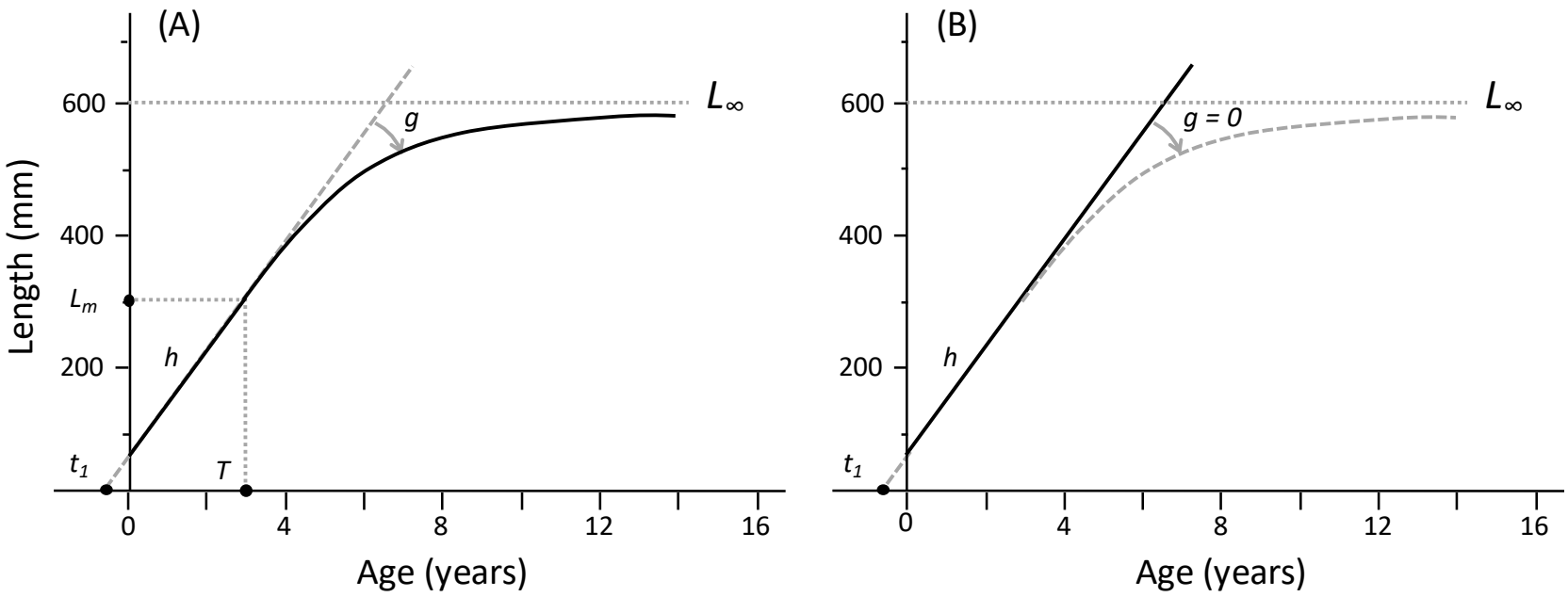

FIGURE 4.2 - Hypothetical fits of the Lester biphasic growth model for (A) fertile and (B) sterile Walleye, h is the maximum potential growth rate, t_1 is the theoretical age at zero length, g is the joint energetic cost of gamete production and activity associated with reproduction, T is the age when gamete production begins, L_m is the length-at-maturity, and L_∞ is the average maximum size. According to the Lester Biphasic growth model, sterile fish are expected to grow linearly throughout their life because they do not invest any energy into gamete production or reproductive activity. Figure adapted from Wilson et al. (2017).

contributes to growth deceleration, then (1) sterile individuals would grow larger than fertile ones, and would have significantly higher estimates for L_∞, a shared model parameter corresponding to the average maximum total length, and (2) LBGM would receive more model weight for sterile fish than P-VBGM because LBGM explicitly accounts for the energetic costs of reproduction and RDH (Figure 4.2).

4.2 Methods

4.2.1 Study Site

Narraguinnep Reservoir (Latitude: 37.49°N, Longitude: 108.62°W) is a 215-ha irrigation water storage reservoir located within the upper Colorado River Basin in southwest Colorado, USA. Sportfish management in the upper Colorado River Basin utilizes several strategies to support the recovery of threatened and endangered fish, which includes the stocking of sterile nonnative piscivores. Although originally stocked with nonnative walleye as early as 1972, Colorado Parks and Wildlife began stocking Narraguinnep Reservoir with sterile triploid walleye in 2008, to continue to provide a highly sought-after sportfishing opportunity while mitigating the risk of natural and illegal dispersal of reproductively capable individuals that could harm downstream recovery efforts (Fetherman et al. 2015; Farrell et al. 2022a). As a result, Narraguinnep Reservoir contains a population of fertile and sterile walleyes of a wide range of sizes and ages and presented a unique opportunity to assess RDH.

4.2.2 Fish sampling

Walleye were collected with experimental gillnets periodically from 2018 to 2021. We recorded total length (TL, mm) and wet weight (W, g) for each individual. Sex and maturity were classified according to Duffy et al. (2000). We collected blood and/or fin tissue samples to determine ploidy using methods described in (Farrell et al. 2022b). All sampling procedures

were approved by the Colorado State University Institutional Animal Care and Use Committee (Protocol # 18-7822A).

4.2.3 Age estimation

We estimated the age and growth of Walleye using sagittal otoliths (Long and Grabowski 2017). Otoliths were embedded in epoxy and sectioned transversely through the core, photographed using a camera mounted to a compound microscope at 40 – 100x magnification under reflected light. An experienced reader (author CJF) aged each fish, blind to size, ploidy, and sex, three times using the RFishBC package in R (Ogle 2019). Fish were assigned fractional ages for each otolith read, which was calculated as the time elapsed in years from birth until capture, assuming fish were born on April 1st of their estimated birth year. Ageing replicates were incorporated into hierarchical Bayesian fits of growth models to incorporate observation uncertainty into parameter estimates.

4.2.4 Growth modelling

All statistical analyses were performed using R 4.1.3 (R Development Core Team 2022). We used package brms to fit the nonlinear growth models in a hierarchical Bayesian framework (Bürkner 2017; Bürkner 2018; Bürkner 2021). This package is a high-level interface to Stan that allows users to specify hierarchical Bayesian models using formula syntax that is similar to package lme4, a popular package used to fit frequentist mixed models in R (Bates et al. 2015). The package loo (Vehtari et al. 2017; Yao et al. 2018) was used to perform leave-one-out cross validation to compute model weights for model comparison. Figures were created using packages ggplot2 (Wickham 2016) and tidybayes (Kay 2022).

We characterized length-at-age for each sex and ploidy group separately using both the P-VBGM and LBGM. Ageing error was incorporated in parameter estimates by using a random

effect of otolith reading (Cope and Punt 2007). We used the Beverton and Holt (1957) parameterization of P-VBGM. Below is a semi-formal model specification for P-VBGM implemented in brms:

$$y_i \sim lognormal(\mu_{ij}, \sigma) \tag{4.1}$$

$$\mu_{ij} = log\left(L_{\infty i}\left(1 - e^{-k_i\left(t_j - t_{0_i}\right)}\right)\right) \text{ for } j = 1,2,3 \tag{4.2}$$

$$L_{\infty_i} \sim uniform(0,1041) + (1|reading) \tag{4.3}$$

$$K_i \sim uniform(0,5) + (1|reading) \tag{4.4}$$

$$t_{0_i} \sim uniform(-3,3) + (1|reading) \tag{4.5}$$

$$\sigma \sim uniform(0,7) \tag{4.6}$$

where y_i is TL at fractional age t for sex-by-ploidy group i and otolith reading j, L_∞ is the asymptotic mean TL, K is the Brody growth coefficient, and t_0 is the theoretical age at zero length. The lognormal distribution was used to represent length-at-age because length is strictly positive. We used informative uniform priors for L_∞, K, t_0 and σ. Because L_∞, K, and σ are strictly positive, the lower bound of the uniform prior for these parameters was zero. We set the upper bound for L_∞ to 1041, which corresponds to the largest recorded walleye length (Gabelhouse 1984). We set the upper bound for K to 5, far greater than most estimates of K for walleye, which rarely exceeds 1 (Quist et al. 2003). Similarly, we set conservative bounds for t_0 ranging from -3 and 3 (Quist et al. 2003). For σ, we set an upper bound of 7 to allow enough variation within the distribution so that it covered the largest recorded walleye length (Gabelhouse 1984).

We chose LBGM (Lester et al. 2004) to represent the RDH because of its prevalence in the fisheries literature, similarity to other biphasic growth models, and relative simplicity (Lester et al. 2014; Wilson et al. 2017). Below is a semi-formal model specification for LBGM implemented in brms:

$$y_i \sim lognormal(\mu_{ij}, \sigma) \tag{4.7}$$

$$\mu_{ij} = \begin{cases} h_i(t_j - t_{1_i}) when\ t\ \leq\ T \text{ for } j = 1,2,3 \\ L_{\infty_i}\left(1 - e^{-K_i[t - t_{0i}]}\right) when\ t\ >\ T \text{ for } j = 1,2,3 \end{cases} \tag{4.8}$$

$$t_{1_i} \sim uniform(-3,3) + (1|reading) \tag{4.9}$$

$$h_i \sim uniform(0,350) + (1|reading) \tag{4.10}$$

where h is the maximum growth rate (length per unit time), t_1 and t_0 are the hypothetical ages at zero length for the juvenile and adult portions of the model, respectively, and T is the age when allocation of energy to gonads begins, one year prior to age-at-50% maturity. We used the same priors for L_∞, K, t_0, and σ as we did for equations 4.3 – 4.6. Similarly, we conservatively set bounds for t_1 to -3 and 3 like we did for its analog t_0 (Quist et al. 2003). We used a uniform prior for h that conservatively encompassed the fastest documented growth rates for juvenile walleye (Bozek et al. 2011). We estimated T, a derived parameter, for diploids by:

$$T = -\frac{c}{d} - 1 \tag{4.11}$$

where c and d are the intercept and slope of the logistic maturity-at-age regression:

$$logit(p) = c + dt \tag{4.12}$$

where p is the probability of maturity at age t and $-\frac{c}{d}$ is the age-at-50% maturity (Chen and Paloheimo 1994). Since triploid walleyes do not develop gonads (i.e., $g = 0$; Farrell et al. 2022a), we assumed they do not mature and were modelled using only the linear, juvenile portion of the LBGM.

Each model was fit by implementing three chains of length 10,000 using brms and the No-U-Turn sampler, an improvement upon Hamiltonian Monte Carlo, which eliminates the need to define the 'number-of-steps' parameter required for Hamiltonian Monte Carlo sampling (Hoffman and Gelman 2014). For each chain, the first 1,000 iterations were discarded as burn-in,

leaving 27,000 draws to make inference on the posterior distribution of each model's parameters. Convergence of chains was assessed using the scale reduction factor $\hat{r}$, with estimates less than 1.05 considered acceptable (Gelman and Rubin 1992).

We used Full Bayesian Significance Tests (Pereira and Stern 1999) to quantitatively test our first hypothesis, that if RDH governs body size, L_∞ for sterile fish would be larger than L_∞ for fertile fish. Full Bayesian Significance Tests are a Bayesian analog to classical *p*-value hypothesis testing, and instead produce *e*-values, the epistemic value of a hypothesis given the observed data (Pereira and Stern 2022). We used package fbst (Kelter 2022) to calculate e-values to test our first hypothesis. Specifically, we specified that sterile fish needed to have L_∞ estimates 25 mm greater than those for fertile fish to be considered larger than fertile fish.

To test our second hypothesis, that if RDH governs body size, LBGM would receive more model weight than P-VBGM for sterile fish, we used leave-one-out cross validation (Vehtari et al. 2017) for model comparison. Ploidy-specific model weights were computed using the stacking method (Yao et al. 2018). Leave-one-out cross validation does not penalize models for the number of parameters included, which could potentially bias the model comparisons against LBGM, as it has two more parameters than P-VBGM (Vehtari et al. 2017). Stacking computes model weights by combining all models and maximizing the leave-one-out predictive density of the combination distribution, and has been shown to outperform other model averaging methods (Yao et al. 2018).

4.3 Results

Each sex-by-ploidy group exhibited asymptotic growth over the range of sizes (179 – 692 mm) and ages (0.54 – 13.31 years old) examined. Females of both ploidies obtained larger body sizes than males, a common pattern observed for walleye that may reflect evolutionary pressures

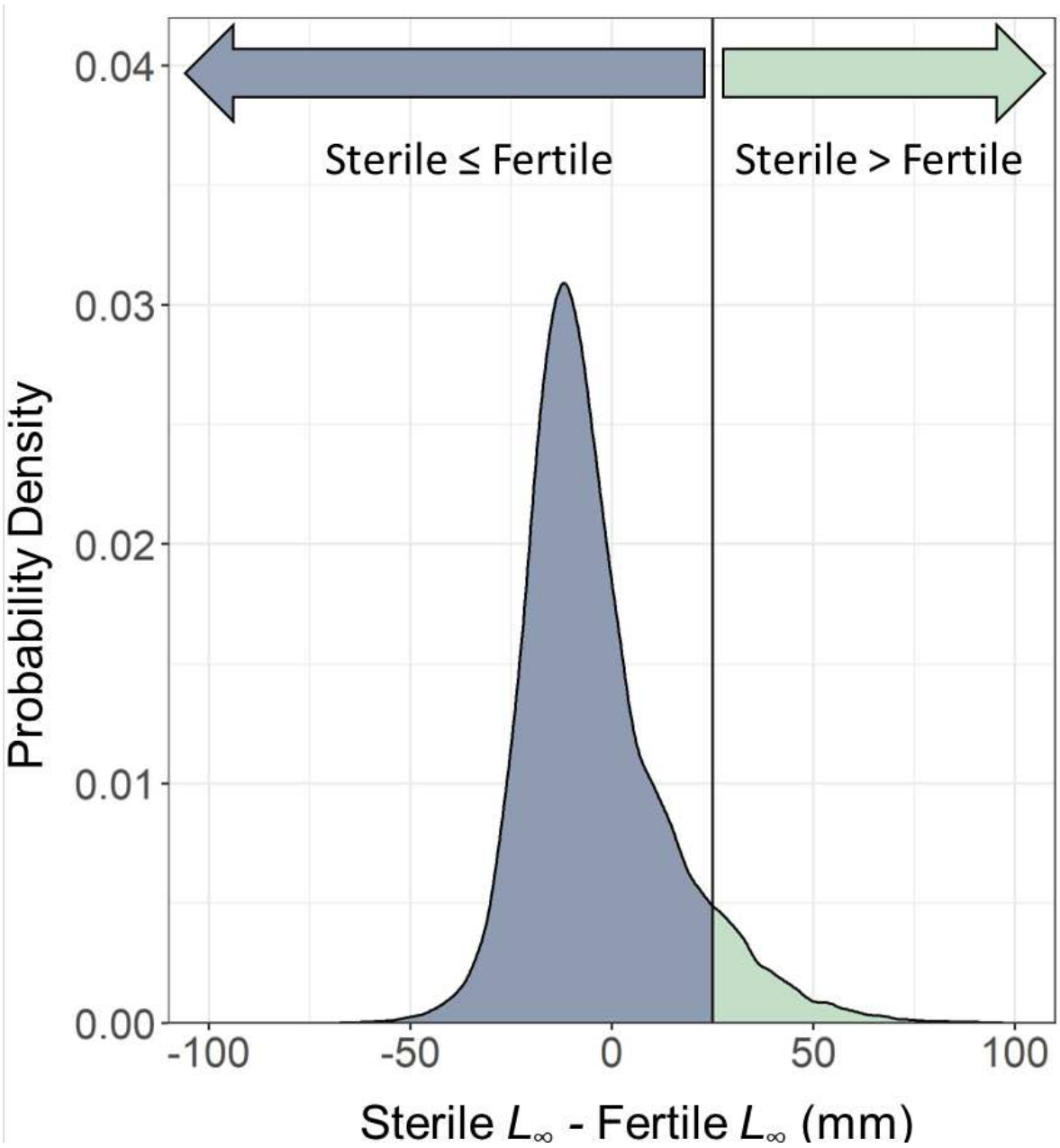

FIGURE 4.3 - Posterior probability density plot showing the probability of the absolute difference in mean maximum size (L_∞, mm) between sterile and fertile Walleyes. The vertical line (25 mm) shows the a priori criterion we set for sterile fish to be considered relevantly larger than fertile fish

TABLE 4.1 – Estimated means (μ) and standard deviations (σ) of posterior distributions for parameters of the Pütter-von Bertalanffy growth model fit to diploid and triploid length-at-age data. Walleyes were aged using sagittal otoliths.

	Females				Males			
	Diploid		Triploid		Diploid		Triploid	
Parameter	μ	σ	μ	σ	μ	σ	μ	σ
L_∞	604.4	8.97	607.8	19.76	498.5	3.76	486.8	8.01
K	0.026	0.029	0.177	0.022	0.316	0.029	0.350	0.031
t_0	-1.652	0.499	-1.801	0.464	-1.242	0.494	-0.461	0.464
σ	0.081	0.001	0.071	0.002	0.081	0.001	0.071	0.002

TABLE 4.2 – Estimated means (μ) and standard deviations (σ) of posterior distributions for parameters of the Lester biphasic growth model fit to diploid and triploid length-at-age data. Walleyes were aged using sagittal otoliths.

	Females				Males			
	Diploid		Triploid		Diploid		Triploid	
Parameter	μ	σ	μ	σ	μ	σ	μ	σ
L_∞	590.5	13.57	-	-	494.8	3.82	-	-
K	0.238	0.168	-	-	0.386	0.031	-	-
t_0	-1.342	0.731	-	-	-0.692	0.303	-	-
t_1	-3.6	0.93	-8.0	0.37	-1.5	0.25	-10.1	0.60
h	56.8	8.46	31.9	1.04	103.2	9.86	26.6	1.17
σ	0.079	0.001	0.082	0.002	0.079	0.001	0.082	0.002

to maximize reproductive output with minimal parental care (Hirshfield and Tinkle 1975; Bozek et al. 2011). We did not find evidence to support our first hypothesis that, if RDH governs body size, sterile fish would be larger than fertile fish (*e*-value = 0.08; Figure 4.3). Estimates of L_∞ for P-VBGM were similar between fertility statuses within each sex, but females were larger than males regardless of fertility (Tables 4.1 and 4.2).

Overall, P-VBGM fit all groups well, whereas LBGM fit fertile fish well, but not sterile fish (Figures 4.4 and 4.5). We found that LBGM (model weight = 0.795) described the length-at-age of fertile walleyes better than P-VBGM (model weight = 0.205). This finding aligns with previous work showing that biphasic models outperform uniphasic ones for fertile fish (Quince et al. 2008b; Armstrong and Brooks 2013; Minte-Vera et al. 2016), which has lent credence to RDH and helped fuel the ongoing debate over the role of reproduction in limiting growth. However, we found that RDH does not explain growth for sterile walleye (LBGM model weight = 0.049), which brings into question the validity of RDH and points to the importance of alternative energy budget models, like assimilation models. Residual plots (Figure 4.4) showed that LBGM overestimated the size of younger and older fish. Counter to expectations under RDH, our results indicate that the growth of sterile fish is asymptotic and better characterized by P-VBGM (model weight = 0.951).

4.4 Discussion

Our findings indicate that reproduction does not drive growth deceleration, contrary to RDH. The LBGM, a model with RDH as a key assumption, could not characterize the asymptotic nature of body size observed for sterile walleye that invest little to no energy into reproduction. Under RDH, we expected L_∞ estimated for sterile walleye to exceed fertile walleye, but estimates were nearly identical for fish growing under equivalent physical, chemical, and

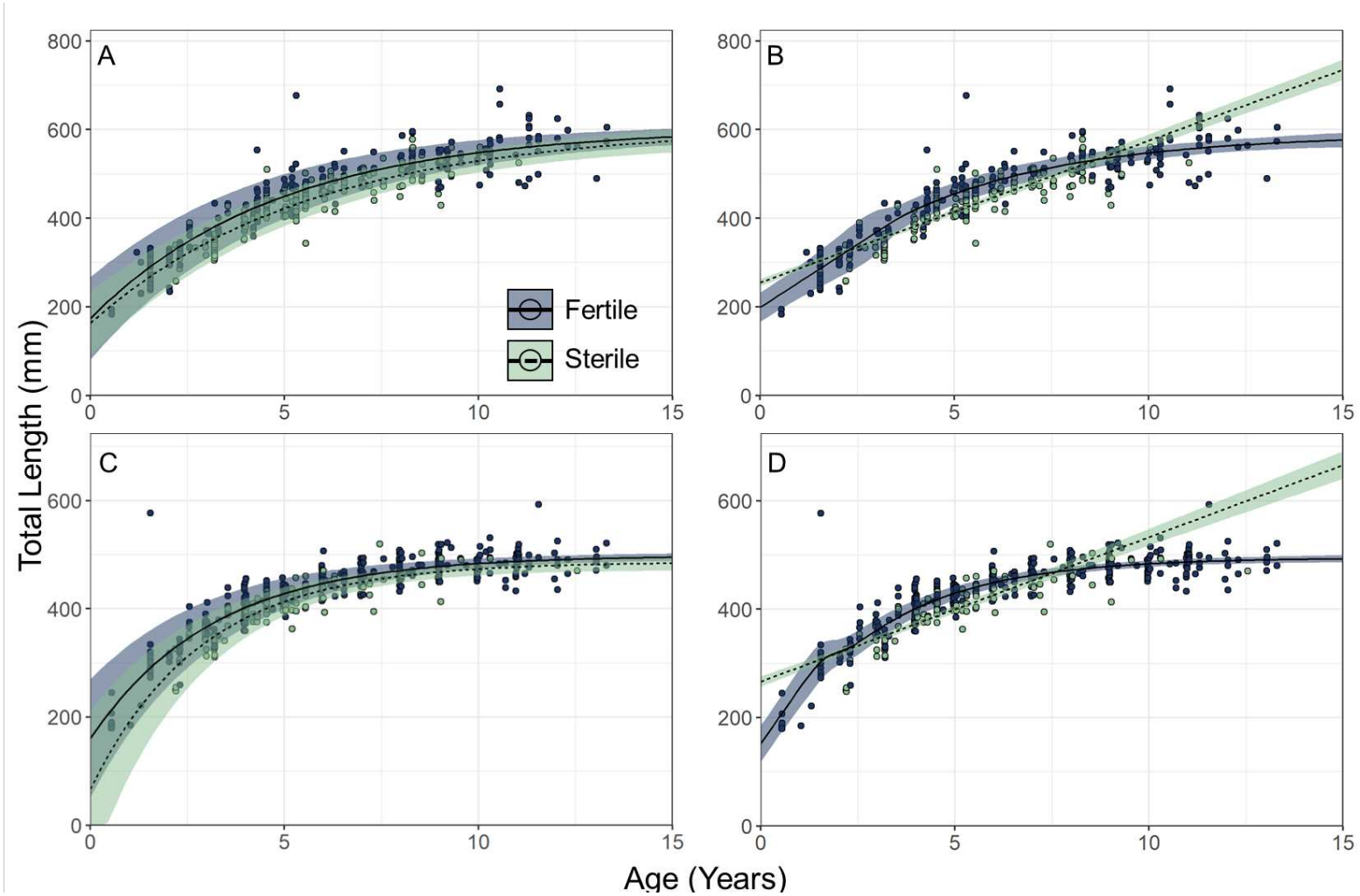

FIGURE 4.4 - Growth model fits to length-at-age data for fertile and sterile Walleye. Panel (A) shows Pütter-von Bertalanffy growth model fits for females, (B) shows Lester biphasic growth model fits for females, (C) shows Pütter-von Bertalanffy growth model fits for males, and (D) shows Lester biphasic growth model fits for males. Solid lines show mean predicted total length for fertile Walleye, and dashed lines for sterile Walleye. Ribbons show 95% credible interval.

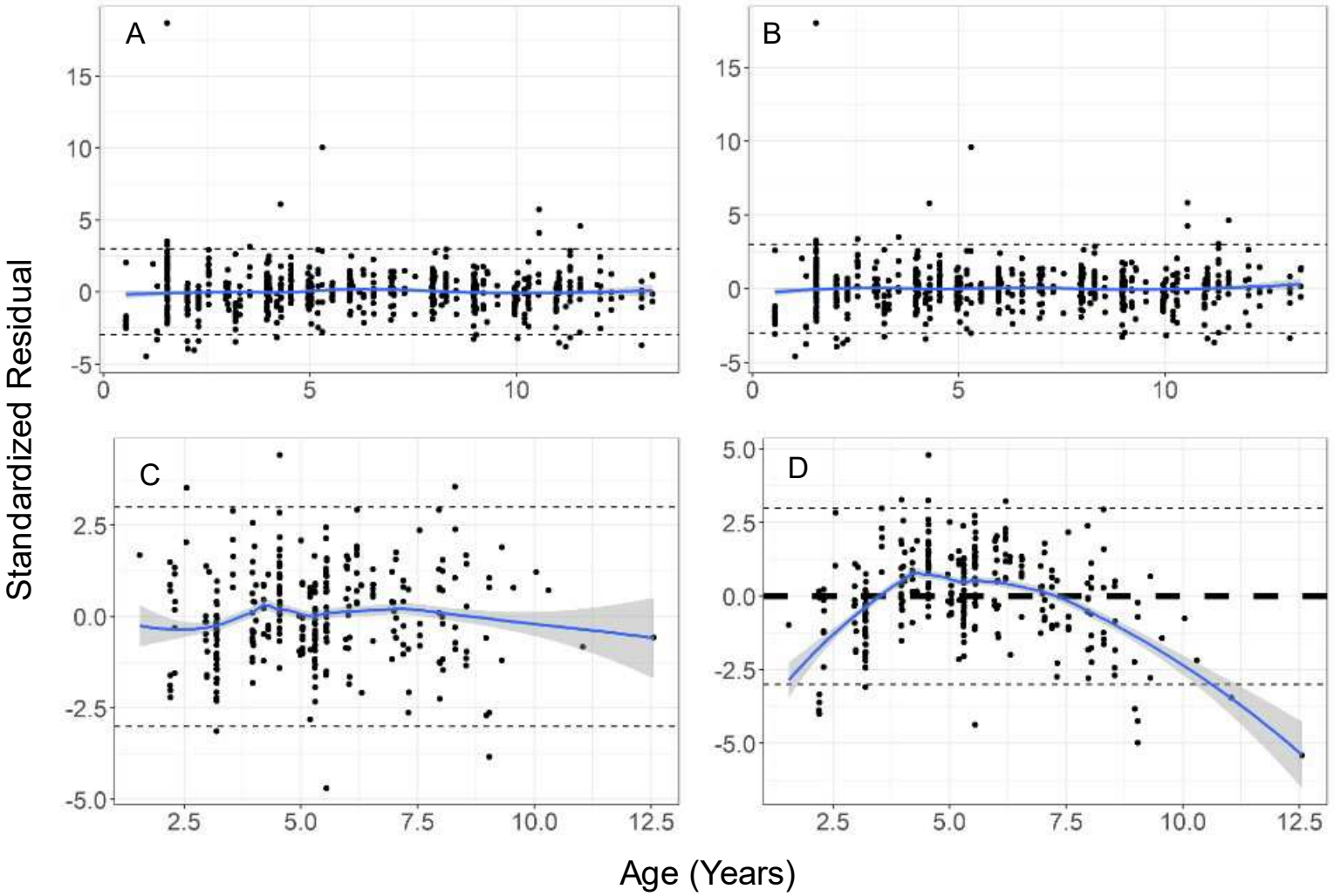

FIGURE 4.5 - Standardized residual plots vs. age for sterile and fertile Walleye fit to the Pütter-von Bertalanffy and Lester biphasic growth models. (A) Fertile Pütter-von Bertalanffy, (B) Fertile Lester Biphasic, (C) Sterile Pütter-von Bertalanffy, (D) Sterile Lester Biphasic. Solid blue line was fit with a LOESS Local Quadratic Regression with a spanning parameter of 0.75. Grey band shows 95% Confidence Interval for LOESS regression. Thick dashed line is at zero, and thin dashed lines at 3 and -3.

ecological conditions. Furthermore, the LBGM represented the growth patterns of sterile walleye poorly, as it received only 4.1% of model weight in contrast to 95.9% assigned to P-VBGM. Lastly, female walleye, regardless of fertility, obtained larger maximum body sizes than males. This finding is difficult to explain under the assumption of RDH (Pauly 2019; Pauly 2021), as female walleyes in this population allocate, on average, 1,756% more energy to gamete production than males by 8 years old (Farrell et al. 2022a). Since sexually dimorphic growth was also conserved in sterile walleye, processes other than reproduction must drive ultimate body size. Thus, growth trajectories of sterile fish are likely better explained by a combination of inheritance and metabolic theory (White et al. 2022).

We speculate that rather than causing growth deceleration, reproduction stimulates food consumption to compensate for its costs. According to DEB, which reduces to P-VBGM under the assumption of constant temperature and food availability (Kooijman 2010):

$$L_\infty = \frac{\kappa f\{\dot{p}_{Am}\}}{[\dot{p}_M]} \tag{4.13}$$

where κ is the fraction of energy spent on maintenance plus growth, f is the scaled functional response ($f = \frac{X}{X+K}$, X = food density, K = half saturation coefficient), $\{\dot{p}_{Am}\}$ is the surface-area-specific maximum assimilation rate, and $[\dot{p}_M]$ is the specific volume-linked somatic maintenance rate. While we lack information specific to walleye, triploid fish generally have similar metabolic rates as diploid conspecifics (Benfey 1999; Maxime 2008), suggesting that $\{\dot{p}_{Am}\}$ and $[\dot{p}_M]$ are likely similar for each ploidy. Logically, this suggests that the fraction of energy spent on reproductive development and maturity maintenance (i.e., $1 - \kappa$) is proportional to feeding. If reproductive investment is proportional to feeding like we hypothesize, DEB and OGM can easily accommodate reproductive hyperallometry (Barneche et al. 2018), eliminating the necessity for new growth models (Kearney 2019; Marshall and White 2019).

There is evidence to support the hypothesis that reproduction drives consumption. Food consumption is the primary pathway of mercury uptake in fish (Hall et al. 1997), and mercury concentrations have been used to reconstruct food consumption rates (Trudel et al. 2000). Farrell et al. (2022a) found that mature fertile walleye from the same population examined here had significantly higher (by 20.3-37.5%) somatic mercury concentrations than sterile walleye of the same age, despite each group having similar diet compositions and experiencing the same thermal conditions. Because there were no fertility-specific differences in growth trajectories, it follows that fertile walleyes required approximately 32% higher food consumption rates than sterile walleyes to support heightened levels of reproductive investment (Farrell et al. 2022a). Because impaired gonadal development in sterile fish corresponds to decreased gonadocorticoid production (Benfey 1999), and food consumption is primarily regulated by hormones produced in the hypothalamus (Volkoff 2016), food uptake may be mediated by the hypothalamic-pituitary-gonadal endocrine axis. Further research is needed to assess this hypothesis and elucidate the biochemical and physiological mechanisms underlying differential food uptake by fertile and sterile fish.

While assessments of sterile conspecifics are useful for examining the effects of reproduction and maturity on a wide variety of response variables, such comparisons are not without complications when sterility is induced via triploidy. Triploids have 50% more DNA than diploids and therefore larger cells to maintain the ratio of nuclear to cytoplasmic volume (Benfey 1999). This means that triploid cells have a smaller surface area to volume ratio compared to diploids, which could affect processes limited by surface area, like oxygen uptake (Pauly 1981). However, disadvantages associated with a smaller surface area to volume ratio may be offset by the fact that triploids have fewer cells, thus decreasing overall maintenance

costs (Benfey 1999; Maxime 2008; Piferrer et al. 2009). Likewise, triploidy affects hematology in complex ways, which could affect respiratory efficiency (Benfey 1999). It appears that changes in cell size and hematology for triploids are compensated for since metabolism, aerobic capacity, and other physiological processes remain similar between ploidies (Benfey 1999; Maxime 2008; Piferrer et al. 2009). Further work on triploid walleye addressing metabolism, assimilation, and maintenance costs, and how these processes compare to diploid walleye, particularly in larger fish, is needed to support or refute our conclusions. Alternatively, germ cell elimination is a promising new technique for producing sterile fish that do not have the cytological complications of triploids (Zohar 2021). Comparisons using fish sterilized in this way should also be useful for studying the potential tradeoff between growth and reproduction. Further, the generality of our findings could be tested with comparisons of sterile and fertile conspecifics using species with different life history types.

Our findings cast doubt on the validity of RDH and its relevance for mechanistic descriptions of fish growth. Metabolically-based growth models, which include P-VBGM, DEB, OGM, and GOLT, do not assume that reproduction hampers growth in body size (Kearney 2021), and may be more appropriate for describing patterns of indeterminate growth. Because survival and reproductive output are highly size-dependent in fishes (Ebenman and Persson 1988; Barneche et al. 2018), interpreting differences in life history strategies and predicting the fitness-related consequences of rapid environmental change require a mechanistic understanding of the hierarchy of energy allocation. By refining the mechanistic understanding of growth, we will be more able to accurately predict and interpret the effects of perturbations such as climate change, exploitation, pollution, invasive species, and habitat degradation (Arthington et al. 2016)

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