Physiological Regulation of Growth during Social Ascension in a Group-Living Fish

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ABSTRACT

In social groups, dominant animals typically are larger and have better access to resources than subordinates. When subordinates are given the opportunity to ascend to a dominant position, they will elevate their rates of growth to help secure dominance. This study investigated the physiological mechanisms facilitating this increased growth. Using the group-living cichlid, *Neolamprologus pulcher*, we investigated whether the insulin-like growth factor (IGF) system—a key regulator of growth—is involved in the regulation of growth during social ascension. We also assessed differences in energy storage and expenditure among dominant, subordinate, and ascending males to determine the energetic costs associated with ascension. Daily growth rates tripled during ascension, and ascending males expended more energy after ascension, owing to higher rates of energetically costly social behaviors, increased locomotor activity, and larger home ranges. Ascenders did not increase food intake to offset increasing energetic costs but had half the liver glycogen energy stores of subordinates. Together, these results indicate a reliance on stockpiled energy reserves to fuel the high energetic demands associated with ascension. Transcript abundance of IGF binding proteins 1 (*igfbp1*) and 2a (*igfbp2a*) were low in ascenders relative to subordinates, suggesting a higher capacity for growth during ascension through increased bioavailability of circulating IGF-1. Our findings provide clear evidence of the energetic costs of social ascension and offer novel insight into the physiological mechanisms modulating the social regulation of growth.

Keywords: Neolamprologus pulcher, cooperative breeding, dominance, energy reserves, feeding, insulin-like growth factor, insulin-like growth factor binding protein.

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Introduction

Body size is a key predictor of competitive ability, with larger individuals typically attaining dominant positions within social hierarchies (Abbott et al. 1985; Rabeni 1985; Tokarz 1985; Forsyth and Alcock 1990; Haley et al. 1994; Schuett 1997). Dominant animals generally secure better access to limited resources, including food, shelter, and reproductive opportunities (Milinski and Parker 1991; Clutton-Brock and Huchard 2013), and often have higher fitness than subordinates (von Rueden et al. 2011; Wilson et al. 2011; Majolo et al. 2012). In social groups, the ability of subordinates to challenge dominants for their social position is largely dependent on body size (Clutton-Brock et al. 2006; Wong et al. 2007; Reddon et al. 2011), and conflict between social ranks increases as subordinates approach the size of dominants (Wong et al. 2007, 2008; Ang and Manica 2010). To avoid such conflict, subordinates can restrict their growth and remain smaller than dominants (Buston 2003; Heg et al. 2004; Buston and Cant 2006; Dengler-Crish and Catania 2007; Wong et al. 2007; Matthews and Wong 2015), a feat that is often accomplished through a reduction in food intake (Wong et al. 2008; Ang and Manica 2010). However, when a subordinate perceives the opportunity to ascend to a dominant position, it can rapidly grow and thus increase its likelihood of becoming dominant (Buston 2003; Russell et al. 2004; Dengler-Crish and Catania 2007; Huchard et al. 2016; Thorley et al. 2018). Such periods of enhanced growth are energetically demanding, and although previous studies have hypothesized that subordinates accumulate energy reserves to meet these demands (Taborsky 1984; Hellmann et al. 2016), energy regulation during periods of social transition has not yet been investigated. In fact, despite the fitness benefits that are typically associated with a large body size, few studies have investigated the physiological mechanisms by which individuals adjust their growth based on social circumstances.

One of the most important regulators of growth and development is the insulin-like growth factor system (IGF; Fuentes et al. 2013). In particular, IGF-1 plays a central role in mediating somatic growth, and high levels of IGF-1 are associated with elevated rates of growth and metabolism in mammals (Swanson and Dantzer 2014) and fishes (Fuentes et al. 2013). Production of IGF-1 occurs in most tissues (Murphy et al. 1987; Daughaday and Rotwein 1989; Duguay et al. 1992; Wood et al. 2005); however, the majority of circulating IGF-1 is produced in the liver (Sjogren et al. 1999; Yakar et al. 1999; Stratikopoulos et al. 2008; Ohlsson et al. 2009). Biosynthesis of IGF-1 is stimulated by growth hormone (Froesch et al. 1985), and secretion of growth hormone from the pituitary is stimulated and inhibited by the actions of somatocrinin (Ling et al. 1984) and somatostatin (Brazeau et al. 1973), respectively. In *Astatotilapia burtoni*—a cichlid

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fish in which transitions between territorial and nonterritorial status frequently occur—faster growth by nonterritorial males and males that recently gained a territory likely reflects an increase in IGF-1 production via stimulation of growth hormone secretion, because nonterritorial and recently ascended males produce less somatostatin relative to territorial males (Hofmann et al. 1999; Hofmann and Fernald 2000; Trainor and Hofmann 2007). Indeed, production of IGF-1 differs with social rank in several species, with dominant wild baboons (*Papio anubis*; Sapolsky and Spencer 1997) and dominant pudu deer (*Pudu puda*; Bartoš et al. 1998) displaying higher circulating levels of IGF-1 compared to subordinates. As well, higher hepatic transcript abundance of *igf-1* was associated with higher growth rates in dominant Nile tilapia (*Oreochromis niloticus*; Vera Cruz and Brown 2007). These findings suggest that IGF-1 may play an important role in the social regulation of growth. In addition, bioavailability of circulating IGF-1 is tightly regulated through the combined actions of several binding proteins (IGFBPs; Rajaram et al. 1997; Duan and Xu 2005; Shimizu and Dickhoff 2017). In particular, IGFBP-1 (Lee et al. 1997; Kajimura et al. 2005) and IGFBP-2 (Eckstein et al. 2002; Wheatcroft and Kearney 2009) strongly suppress the growth-promoting actions of IGF-1. Here, we tested the hypothesis that IGF signaling regulates growth during social transitions.

To explore how ascension to dominant status influences the regulation of energy storage and growth, we used *Neolamprologus pulcher*—a cooperatively breeding African cichlid that lives in social groups organized in a size-based hierarchy (Wong and Balshine 2011*a*). Subordinate *N. pulcher* restrict their growth to remain smaller than dominants (Heg et al. 2004), but males grow rapidly when provided the opportunity to ascend within the hierarchy (Hamilton and Heg 2008). To assess the physiological mechanisms regulating growth during ascension, we removed dominant males, creating an opportunity for subordinate males to assume the dominant position within a social group. We predicted that ascension would be energetically demanding, owing to the performance of more social behaviors, increased activity, and elevated growth rates, and that these demands would be met through increased food intake and the utilization of stored energy reserves. Additionally, we predicted that elevated growth rates in ascenders would be associated with increased activation of the IGF system. Therefore, we quantified transcript abundances of IGF-1 (*igf-1*) and IGFBP-1 and 2 (*igfbp-1*, *igfbp-2a*, and *igfbp-2b*) in the livers of dominant, subordinate, and ascending males.

Material and Methods

Experimental Animals

The experiment was conducted between November 2016 and April 2017 using a colony of *Neolamprologus pulcher* housed at McMaster University in Hamilton, Ontario, Canada. All fish used in the experiment were laboratory-reared descendants of wild-caught breeders from Lake Tanganyika, Africa. Social groups ($n = 20$) consisted of a dominant male-female breeding pair, one to three large helpers (standard length [SL] > 4.5 cm), and one to four small helpers (SL < 4 cm). Groups were held in 189-L aquaria filled with carbon-filtered city of Hamilton tap water at 27°C. All social groups had been together for at least a month and had produced young before any experimental manipulation. Each fish was given a unique dorsal fin clip for identification, which does not adversely affect behavior (Stiver et al. 2004). Each aquarium contained two large sponge filters, a heater, 3 cm of coral sand for substrate, two terra-cotta flowerpot halves, two mirrors, and two PVC tubes as shelter. A 13L:11D photoperiod was maintained throughout the experiment. Fish were fed 1% combined group body weight daily with NorthFin floating cichlid pellets (1 mm; Canadian Aquatic Feed, Toronto, Ontario). All experimental protocols were approved by the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol 14-02-05) and were in compliance with the guidelines of the Canadian Council on Animal Care regarding the use of animals in research and teaching.

Experimental Protocols

Thirty-two focal males were targeted in this experiment. At the start of the experiment (day 0), body mass (to the nearest milligram) and standard length (to the nearest 0.1 mm) of all individuals within the social groups of each focal fish were recorded. To ensure precision, all body measurements throughout the study were taken three times, and averages of these measures are reported. Each social group was randomly assigned to be either a control ($n = 8$; average of 6.8 ± 0.5 group members) or a treatment group ($n = 12$; average of 6.8 ± 0.4 group members). On the morning of day 11, dominant males ($n = 12$; mass $= 7.45 \pm 0.35$ g, SL $= 6.73 \pm 0.18$ cm, mean $\pm$ SEM) were removed from treatment groups, and subordinate males ($n = 12$; mass $= 3.79 \pm 0.31$ g, SL $= 5.23 \pm 0.12$ cm) were removed from control groups. These fish were euthanized, measured, and dissected (see "Tissue Sampling"). The remaining fish in each group were measured, and groups were returned to their respective tanks. In treatment groups, dominant removal provided an opportunity for large subordinate males to ascend. On the morning of day 14, males that had ascended to the dominant position were sampled as above ($n = 8$; mass $= 5.21 \pm 0.38$ g, SL $= 5.86 \pm 0.17$ cm). In four of the 12 treatment groups, a clear dominant male had not emerged by day 14, and therefore, target ascending males were not collected from these groups.

Behavioral Analyses

All social groups were video-recorded twice (days 10 and 11) to assess the behavior of each focal fish. Additionally, treatment groups were recorded twice after dominant removal (days 13 and 14) to assess behavioral changes of ascending fish. On days 11 and 14, groups were recorded immediately before sampling. After placement of a video camera (Canon; VIXIA HF S200) in front of each tank, fish were given 5 min to acclimate and then recorded continuously for 10 min. Previous studies have used similar methods to assess behavior in this species (Fitzpatrick

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et al. 2008; Wong and Balshine 2011*b*). From the 10-min video recordings, we scored all aggressive (chases, bites, rams, opercular flares, aggressive postures, and lateral displays), submissive (flees and submissive postures and displays), and territory maintenance (digs and carries—the act of picking up and moving substrate with their mouths) behaviors performed by each focal fish. Locomotor activity was measured by recording the proportion of time that focal fish were in motion during each video. To assess the size of the home range of focal fish (Werner et al. 2003), tanks were visually split into 12 quadrats using a grid, and the number of unique squares that focal fish entered during the observation period was counted and expressed as a proportion of the total squares. Additionally, the proportion of time that focal fish spent in the upper third of the tank was recorded, because this zone represents a more risky and less preferred area for these substrate-bound cichlids (Konings 2015). Behaviors are reported as averages of the two observation periods (i.e., days 10/11 or days 13/14).

Food intake of focal fish was measured from 5-min video recordings of feedings on days 5, 7, and 9. To determine whether ascending males adjusted their feeding after dominant removal, treatment groups were also video-recorded on days 11, 12, and 13. Feeding rates are expressed as the total number of feeding acts performed by each focal fish while food was present. Observations concluded when all pellets had been consumed (average duration = 2.88 ± 0.63 min), and feeding rates are reported as averages of the three observation periods (i.e., days 5/7/9 or days 11/12/13). All groups were fed between 1300 and 1400 hours.

Tissue Sampling

Fish were rapidly netted and euthanized via terminal anaesthesia (0.5 g L^{-1} ethyl-*p*-aminobenzoate; Sigma-Aldrich, Oakville, Ontario), and mass and standard length were recorded. Gonads and livers were removed and weighed, flash frozen, and stored at −80°C. Half of each liver was used to measure liver glycogen levels (Keppler and Decker 1974), and the remaining liver tissue was used to measure transcript abundance of IGF system components by semiquantitative real-time reverse transcription polymerase chain reaction (RT-PCR).

Transcript Abundance Analysis by Real-Time RT-PCR

Livers were homogenized on ice in TRIzol reagent (Invitrogen, Burlington, Ontario) using a sonicator (Sonic Dismembrator model 100; Thermo-Fisher Scientific), and total RNA was extracted according to the manufacturer's protocol. Extracted RNA was quantified (NanoDrop 2000c UV-Vis Spectrophotometer; Thermo-Fisher Scientific), and complementary DNA (cDNA) was generated using a QuantiTech Reverse Transcription Kit (Qiagen, Toronto, Ontario) following the manufacturer's protocol.

Gene specific primers (table 1) were used to assess changes in transcript abundance by semiquantitative real-time RT-PCR. Previously published primers were used for the reference gene *18s*. Primers for target genes in the IGF system (*igf-1*, *igfbp-1*, *igfbp-2a*, and *igfbp-2b*) were designed using Primer-BLAST (NCBI; Ye et al. 2012) based on predicted sequences. Pooled PCR products were purified using a QIAquick PCR purification kit (Qiagen) and sequenced (Génome Québec, Montreal, Quebec) to confirm primer specificity.

Real-time RT-PCR reactions were performed in duplicate using a Rotor-Gene SYBR Green PCR Kit (Qiagen) and a Rotor-Gene Q real-time PCR system (Qiagen), following the manufacturer's protocol with the exception that reaction volumes were scaled to 10 μL from 25 μL. Each reaction contained 5 μL SYBR 2× PCR mix, 1 μL of combined forward and reverse primers (10 μM of each), 3 μL of RNase/DNase free water, and 1 μL of cDNA template. Cycling parameters consisted of a 5-min activation step at 95°C, followed by 40 cycles consisting of a 5-s denaturation step at 95°C and a combined 10-s annealing and extension step. Standard curves were developed for each primer

Table 1: Gene-specific primers used for real-time reverse transcription polymerase chain reaction

Gene	Primer sequence (5′ to 3′)	Amplicon size (bp)	Efficiency (%)	Annealing temperature (°C)	Accession no.	Reference
18s	F: ACAAGAAGAGACCTTCACCTGG R: CTCAATCTCGTGTGGCTGAA	146	91	60	AF337051	O'Connor et al. 2013
igf-1	F: ATGGCCGTTCTTAGTTGGTG R: TGCTGGGCATTTGTCCATTT	130	96	60	XM_006780878	
igfbp-1	F: TGGACACCATAGCCACCTCT R: GATGACTCGCACTGCTTGG	109	104	60	XM_006786434	
igfbp-2a	F: GGCTTTGAGTACACCTGGCT R: TTACGGTCATGTCCTTCGGC	104	98	60	XM_006800269	
igfbp-2b	F: TATCTGCCAAGGTGCTCCAC R: GTGTTTAGAGGCGGTCTCCC	194	92	60	XM_006793717	

Note. *18s*, 18S ribosomal RNA; *igf-1*, insulin-like growth factor 1; *igfbp-1*, insulin-like growth factor binding protein 1; *igfbp-2a*, insulin-like growth factor binding protein 2a; *igfbp-2b*, insulin-like growth factor binding protein 2b.

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set using serial dilutions (4 ×) of cDNA pooled from each individual, and conditions were adjusted to optimize the efficiency of each reaction. Negative controls, including no template controls (where cDNA was replaced with water) and no reverse transcriptase controls (where reverse transcriptase was replaced with water in the synthesis of cDNA), were included. Melt curves were performed at the end of each run to confirm the presence of a single product, as well as the absence of primer dimers. Transcript abundance was calculated relative to the subordinate group using the modified $2^{-\Delta\Delta Ct}$ method (Pfaffl 2001), normalizing to mRNA abundance of the reference gene *18s*, which did not vary among groups.

Statistical Analyses

Specific growth rate was calculated as $[\ln(SL_{final}) - \ln(SL_{initial})] \times 100/D$, where SL is the standard length of the fish in centimeters and D is the number of days elapsed between measurements (Ricker 1975).

Statistical analyses were conducted using R (ver. 3.3.2; R Core Team 2018). All data are presented as means ± 1 SEM, and a significance level (α) of 0.05 was used for all tests. Data were tested for normality and homoscedasticity using Shapiro-Wilk and Levene's tests, respectively. Data that did not meet these assumptions were either log or logit (for data presented as proportions) transformed. To investigate differences among dominant, subordinate, and ascending males, general linear mixed models (LMMs) were fitted using the lmer function in the "lme4" package (Bates et al. 2015). Group ID was included as a random factor in all models to account for the fact that in four social groups, two subordinate males were sampled. When overall differences were detected using the Anova function in the "car" package (Fox and Weisberg 2011), Tukey's honest significant difference post hoc analysis was performed using the glht function in the "multcomp" package (Hothorn et al. 2008). Final standard length (taken immediately before dissection) was included as a covariate in LMMs of daily growth (total change in body length divided by number of days between measurements) to account for differences in somatic growth due to body size (Taborsky 1984). Final body mass was included as a covariate in LMMs of gonadal and liver investment, as well as food intake. The residuals of log liver mass against log body mass (determined using least square linear regression) were included as a covariate in LMMs of hepatic glycogen reserves to account for individual differences in relative liver investment. To assess changes in behavior and growth of ascending fish before and after removal of the dominant male, LMMs were performed including individual ID as a random factor.

Results

Ascenders Increased Their Growth Rates

Specific growth rates of ascenders (fig. 1*A*; LMM, $\chi^2 = 9.99$, df = 2, $P = 0.007$) were higher than those of both dominants ($P = 0.005$) and subordinates ($P = 0.04$). Ascenders also had higher absolute growth per day (fig. 1*B*; LMM, $\chi^2 = 7.55$, df = 2, $P = 0.02$) compared to dominants ($P = 0.02$) but not relative to subordinates ($P = 0.64$). Growth increased with body length ($\chi^2 = 8.12$, df = 1, $P = 0.004$).

After dominant removal, ascending males grew faster in terms specific growth rate (fig. 1*A*; $\chi^2 = 15.62$, df = 1, $P < 0.001$), as well as absolute growth per day (fig. 1*B*; $\chi^2 = 4.45$, df = 1, $P = 0.03$). In contrast, nonfocal fish (dominant females and nonascending large helpers) did not increase their growth after dominant removal (table 2).

Ascenders Utilized Energy Reserves but Did Not Increase Gonadal Investment

Ascenders had lower liver glycogen reserves (fig. 2*A*; $\chi^2 = 42.65$, df = 2, $P < 0.001$) than subordinates ($P < 0.001$). Glycogen stores did not differ between ascending and dominant males ($P = 0.22$) and did not vary with residual liver mass ($\chi^2 = 0.09$, df = 1, $P = 0.76$). Liver investment did not differ across social ranks ($\chi^2 = 3.19$, df = 2, $P = 0.20$), but absolute liver mass increased with body mass ($\chi^2 = 6.30$, df = 1, $P = 0.01$). Ascending males did not adjust their gonadal investment (fig. 2*B*; LMM, $\chi^2 = 12.26$, df = 2, $P = 0.002$), which did not differ from that of subordinates ($P = 0.64$). Gonadal investment of ascenders was lower than that of dominant males ($P = 0.003$). Absolute gonad mass increased with body mass ($\chi^2 = 4.55$, df = 1, $P = 0.03$).

Ascenders Increased Their Performance of Social and Locomotor Behaviors

Dominant and ascending males performed more aggressive acts (fig. 3*A*; LMM, $\chi^2 = 14.84$, df = 2, $P < 0.001$) and fewer submissive acts (fig. 3*B*; $\chi^2 = 36.66$, df = 2, $P < 0.001$) than subordinates. Ascending males performed levels of territory maintenance (fig. 3*C*; $\chi^2 = 10.80$, df = 2, $P = 0.004$) intermediate to that of dominants ($P = 0.13$) and subordinates ($P = 0.57$). Ascenders and dominants were more active (fig. 4*A*; $\chi^2 = 48.11$, df = 2, $P < 0.001$) and spent less time in the top third of the tank (fig. 4*B*; $\chi^2 = 24.59$, df = 2, $P < 0.001$) compared to subordinates. Ascending males had larger home ranges (fig. 4*C*; $\chi^2 = 44.02$, df = 2, $P < 0.001$) than subordinates ($P = 0.003$), although the home ranges of ascenders remained smaller than those of dominants ($P = 0.02$). Feeding rates did not differ across social ranks (fig. 4*E*; $\chi^2 = 1.58$, df = 2, $P = 0.45$) but tended to increase with body mass (data not shown; $\chi^2 = 3.42$, df = 1, $P = 0.06$).

After dominant removal, ascending males became more aggressive (fig. 3*A*; LMM, $\chi^2 = 16.67$, df = 1, $P < 0.001$) and less submissive (fig. 3*B*; $\chi^2 = 6.53$, df = 1, $P = 0.01$). No change in territory maintenance behaviors was detected (fig. 3*C*; $\chi^2 = 1.75$, df = 1, $P = 0.19$). Ascenders became more active (fig. 4*A*; $\chi^2 = 34.69$, df = 1, $P < 0.001$), spent less of their time in the upper third of the tank (fig. 4*B*; $\chi^2 = 6.19$, df = 1, $P = 0.01$), and expanded their home ranges (fig. 4*C*; $\chi^2 = 13.73$, df = 1, $P < 0.001$). However, ascending males did not adjust their feed-

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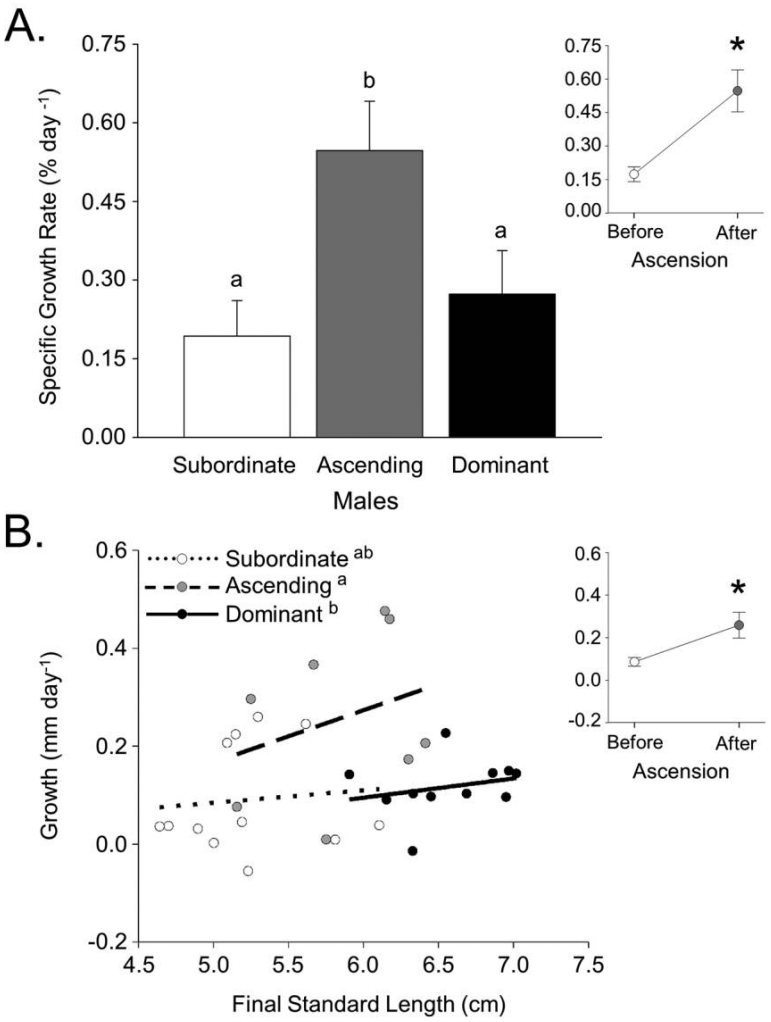

Figure 1. Specific growth rates (*A*) and daily growth rates (*B*) of subordinate ($n = 12$), ascending ($n = 8$), and dominant ($n = 12$) male *Neolamprologus pulcher*. Symbols represent individual fish, and lines indicate linear trends within each social rank. Significant differences among social ranks are indicated on the figures using letters. The insets show growth rates of ascending males before and after ascension ($n = 8$). An asterisk indicates a significant difference as a result of ascension. Values are means ± SEM.

ing rates after dominant removal (fig. 4*D*; $\chi^2 = 0.01$, df = 1, $P = 0.92$).

Subordinates Had Higher Transcript Abundance of IGFBPs

No differences in hepatic *igf-1* transcript abundance were detected across social ranks (fig 5*A*; LMM, $\chi^2 = 4.05$, df = 2, $P = 0.13$). However, subordinates had elevated transcript abundance of *igfbp-1* (fig. 5*B*; $\chi^2 = 6.80$, df = 2, $P = 0.03$) compared to dominants ($P = 0.02$) and elevated transcript abundance of *igfbp-2a* (fig. 5*C*; $\chi^2 = 10.55$, df = 2, $P = 0.005$) compared to ascenders ($P = 0.01$) and dominants ($P = 0.01$). Subordinates also tended to have higher transcript abundance

Table 2: Specific growth rates (SGR) and absolute growth rates of nonfocal fish before and after removal of the dominant male

		SGR (% d^{-1})				Growth (mm d^{-1})			
	N	Before	After	χ^2	*P*	Before	After	χ^2	*P*
Dominant females	8	.15 ± .04	.12 ± .11	.09	.76	.09 ± .02	.08 ± .07	.03	.87
Nonascending helpers	9	.19 ± .03	.14 ± .08	.67	.41	.10 ± .02	.06 ± .04	.46	.50

Note. Values are means ± SEM. No significant differences were detected.

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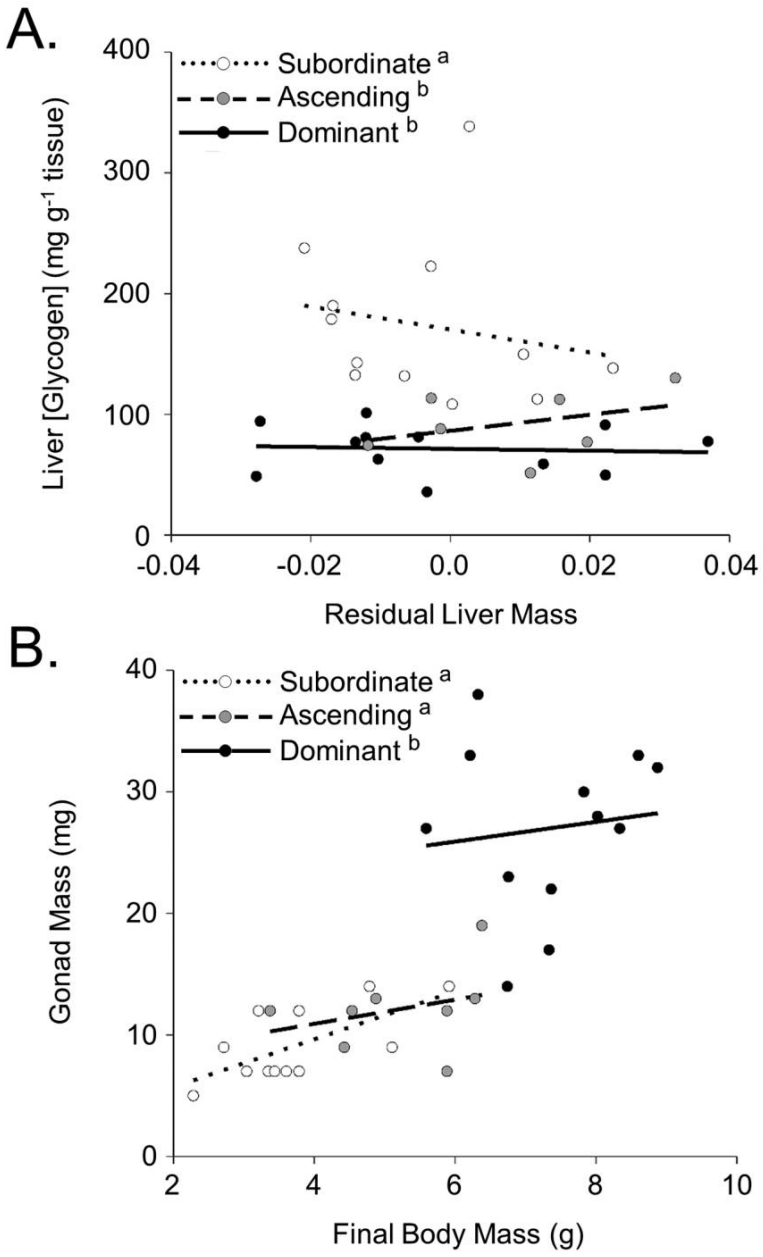

Figure 2. Liver glycogen reserves (*A*) and gonad mass (*B*) of subordinate ($n = 12$), ascending ($n = 8$), and dominant ($n = 12$) male *Neolamprologus pulcher*. Symbols represent individual fish, and lines indicate linear trends within each social rank. Significant differences among groups are indicated on the figures using letters.

of *igfbp-2b*, but this difference did not reach significance (fig. 5*D*; $\chi^2 = 4.84$, df $= 2$, $P = 0.08$).

Discussion

Ascending to a dominant position within a social group is generally assumed to be a socially and energetically demanding life-history transition, but few studies have tested this prediction empirically. Previous studies have shown that subordinate *Neolamprologus pulcher* often have larger livers than dominants relative to their body size (Sopinka et al. 2009; Hellmann et al. 2016), as well as greater muscle energy reserves (Hellmann et al. 2016), suggesting that subordinates may store energy to prepare for energetically demanding events, such as ascension to dominance.

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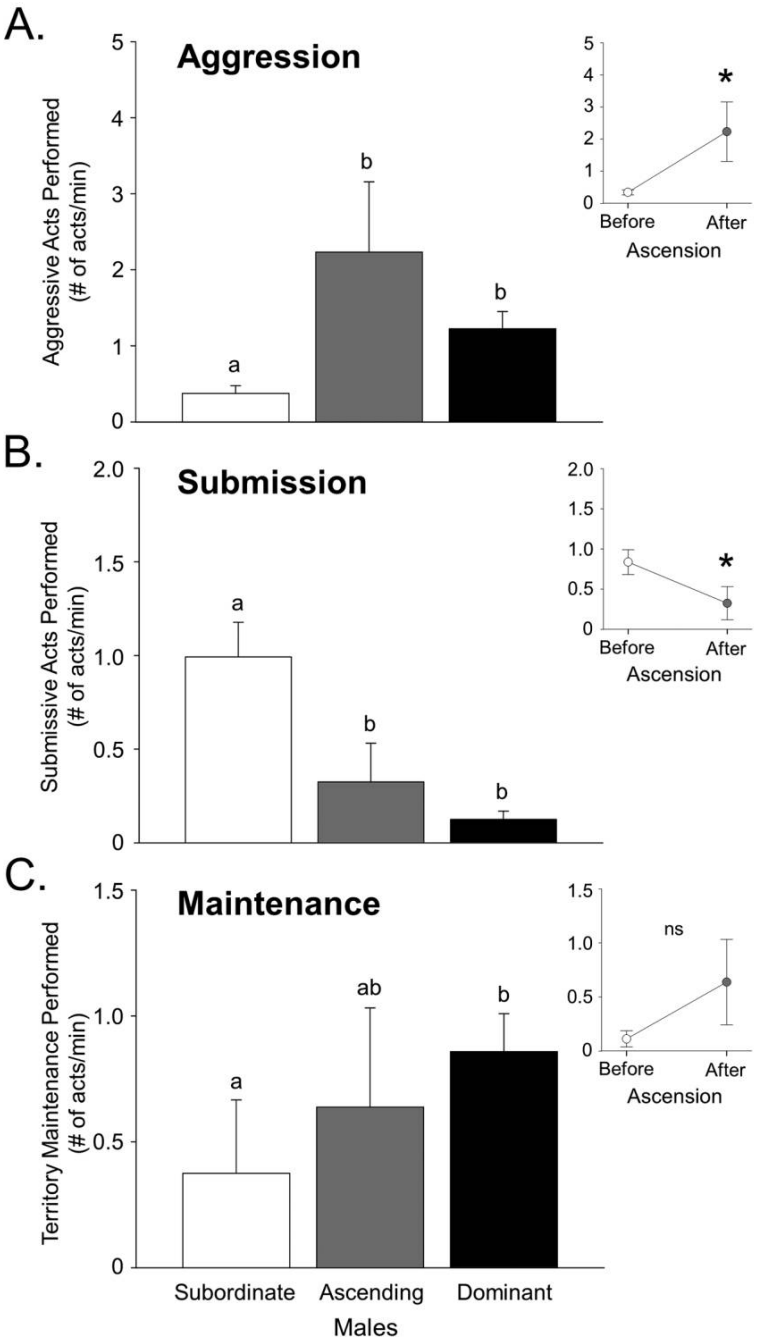

Figure 3. Rates of aggression (*A*), submission (*B*), and territory maintenance (*C*) performed by subordinate ($n = 12$), ascending ($n = 8$), and dominant ($n = 12$) male *Neolamprologus pulcher*. Treatment groups that share a letter are not significantly different from one another. The insets show the behavior of ascending males before and after ascension ($n = 8$). An asterisk indicates a significant difference as a result of ascension. Behaviors were recorded over 10 min and are expressed as the number of acts performed per minute. Values are means ± SEM.

In this study, we found that ascending males had half the liver glycogen stores of subordinates 72 h after ascending to a dominant position, supporting the hypothesis that subordinates stockpile energy reserves in preparation for ascension. Periods of ascension are often associated with elevated glucocorticoid production (Huffman et al. 2015; Culbert et al. 2018), and the catabolic actions of glucocorticoids likely aid in the rapid mobilization of these energy reserves (Mommsen et al. 1999). In rainbow trout (*Oncorhynchus mykiss*), social subordination is associated with elevated cortisol production, which, at least in part, results in increased

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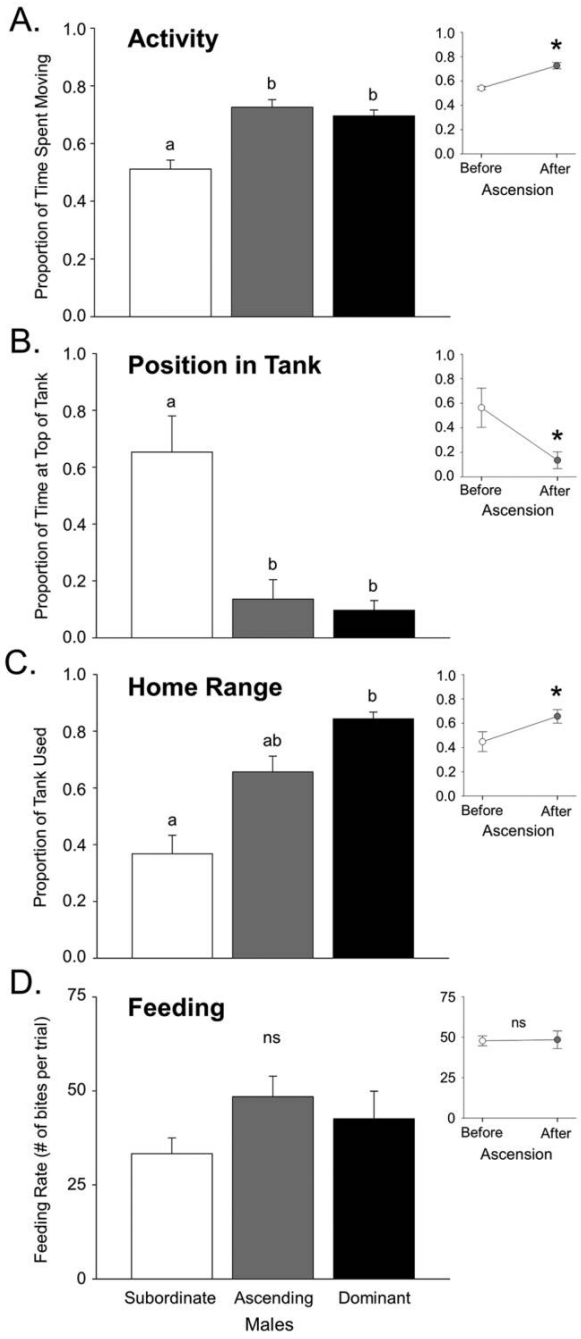

Figure 4. Activity levels (*A*), position in tank (*B*), size of home range (*C*), and feeding rates (*D*) of subordinate ($n = 12$), ascending ($n = 8$), and dominant ($n = 12$) male *Neolamprologus pulcher*. Treatment groups that share a letter are not significantly different from one another. The insets show the behavior of ascending males before and after ascension ($n = 8$). An asterisk indicates a significant difference as a result of ascension. Activity, position in tank, and home range were recorded over 10 min. Feeding rates are reported as the number of feeding attempts performed over a period of 5 min, or until all food was consumed. Values are means ± SEM.

rates of hepatic gluconeogenesis (Gilmour et al. 2012) and beta-oxidation (Kostyniuk et al. 2018), along with depletion of energy reserves, including liver glycogen (Gilmour et al. 2012; Culbert and Gilmour 2016). A similar process may occur during ascension, where increased cortisol production (Culbert et al. 2018) stimulates the mobilization of energy reserves, providing ascenders with the fuel necessary to establish dominance.

During periods of high metabolic demand, many vertebrates will increase their food intake and/or enhance their capacity to absorb nutrients to offset energetic costs (Christiansen et al. 1992; Dykstra and Karasov 1992; Speakman and McQueenie 1996; Hammond and Kristan 2000). However, we did not observe any changes in food intake during ascension. This observation is consistent with recent findings for wild Kalahari meerkats (*Suricata suricatta*), in which food intake was not adjusted during periods of social ascension (Huchard et al. 2016). In the wild, *N. pulcher* forage in the water column above their territories on ephemeral patches of zooplankton, with feeding occurring in large aggregations consisting of members of many groups (Balshine et al. 2001). As such, socialization (on their territories) and feeding (above their territories) usually occur separately. When food availability was experimentally reduced, rates of helping behaviors (territory maintenance/defense and broodcare) decreased because fish had to spend more time foraging (Bruintjes et al. 2010). Therefore, it is possible that ascending males were unable to increase their foraging rates because they were too busy securing their newly acquired social rank through social interactions.

Social interactions are important for group-living animals (Taborsky and Oliveira 2012), but the nature and frequency of specific types of interactions typically vary with social rank (Milinski and Parker 1991; Stockley and Bro-Jørgensen 2011; Clutton-Brock and Huchard 2013). We observed that ascending males became more aggressive and less submissive while continuing to perform high levels of territory maintenance—characteristics associated with dominance in *N. pulcher* (Wong and Balshine 2011*a*; Taborsky 2016). Grantner and Taborsky (1998) observed that aggressive, submissive, and territory maintenance behaviors in *N. pulcher* resulted in 3.9-, 3.3-, and 6.1-fold increases in routine metabolic rate, respectively. To assess how energy consumption owing to the performance of social behaviors changed during ascension, the rates at which behaviors of each class were performed in this study were multiplied by the relative metabolic cost associated with each behavioral class determined by Granter and Taborsky (1998). Based on these calculations, we estimate that males spent almost three times as much energy on the performance of social behaviors after ascension (before ascension: 4.79 ± 0.77; after ascension: 13.64 ± 3.93). Additionally, ascending males also became more active and defended a larger home range, further increasing their energy expenditure. Despite spending more energy on locomotion and the performance of costly social behaviors, ascenders managed to achieve high growth rates.

Across animal taxa, body size is one of the most reliable predictors of competitive ability (Rabeni 1985; Haley et al. 1994; Schuett 1997; Reddon et al. 2011), and ascending males in this

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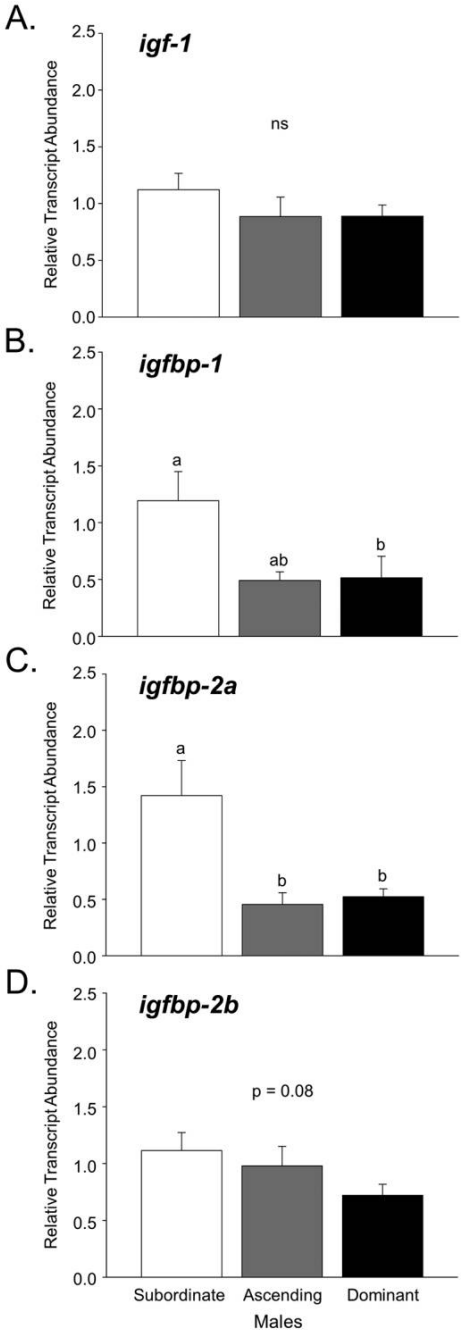

Figure 5. Relative transcript abundance of *igf-1* (*A*), *igfbp-1* (*B*), *igfbp-2a* (*C*), and *igfbp-2b* (*D*) in the livers of subordinate ($n = 12$), ascending ($n = 8$), and dominant ($n = 12$) male *Neolamprologus pulcher*. Treatment groups that share a letter are not significantly different from one another; in *D*, the *P* value is reported. Values are means ± SEM.

study tripled their somatic growth rates after dominant removal. These findings are consistent with previous studies of growth during social ascension in both mammals (Dengler-Crish and Catania 2007; Huchard et al. 2016; Thorley et al. 2018) and fishes (Hofmann et al. 1999; Buston 2003; Bergmüller et al. 2005). Interestingly, this increase in somatic growth occurred in the absence of changes in gonadal investment. Fitzpatrick et al. (2008) observed that by 7 d after dominant removal, ascending male *N. pulcher* had greater gonadal investment (by 66%) compared to subordinates, and ascenders with the largest gonads displayed the lowest growth rates. These authors therefore suggested a trade-off between somatic growth and gonadal investment during periods of social ascension. Our results indicate that the direction of this trade-off varies as a function of time after dominant removal. Early investment in somatic growth (as observed in this study) may aid in securing the dominant position within a group, followed by a later period of increased gonadal investment (Fitzpatrick et al. 2008) that would be necessary to enhance reproductive capacity once dominance is secured.

Although IGF-1 is an important regulator of growth and development—stimulating growth and cellular proliferation (Froesch et al. 1985; Wood et al. 2005; Duan et al. 2010)—increased growth during ascension did not appear to be mediated by changes in production of IGF-1, because no differences in hepatic *igf-1* transcript levels were detected. This observation contrasts with the situation in dyadic hierarchies of Nile tilapia (*Oreochromis niloticus*), where differences in growth between dominants and subordinates mirrored differences in IGF-1 production (Vera Cruz and Brown 2007). Instead, elevated hepatic transcript levels of *igfbp-1* and *2a* in subordinates suggest an important role of IGFBPs in the social suppression of growth. IGFBP-1 and 2 bind circulating IGF-1, preventing it from binding to IGF receptors and hence suppressing its growth-stimulating actions (Shimizu and Dickhoff 2017). During ascension, *igfbp* transcript levels fell in ascenders, likely relieving the suppression of IGF-1 activity by IGFBPs, allowing ascenders to increase their growth rates. Not only does this mechanism explain increased somatic growth during periods of ascension but also it is, to our knowledge, the first time IGFBPs have been implicated in the social regulation of growth.

In conclusion, social ascension is an energetically costly event for which subordinates must prepare through the accumulation of energy reserves. These reserves appear to be rapidly utilized during ascension, fueling increased activity, the performance of costly social behaviors, and rapid growth. Our study also implicated IGFBPs as key regulators of growth during ascension, providing a novel mechanism for the social regulation of growth.

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