eLife

Reviewed Preprint
v1 • April 1, 2026
Not revised

For correspondence:
lvizer@bu.edu

Competing interests: No competing interests declared

Funding: See page 21

Reviewing editor: Sonia Sen, Tata Institute for Genetics and Society, India

Plasticity Associated with Adoption of Social Roles in Clown Anemonefish

Lili F Vizer[1], Douglas Alvarado[1], Colleen Bove[1], Marcela Herrera[2], Annabel Hughes[1], Kian Thompson[1], Steven M Bogdanowicz[3], Vincent Laudet[2], Sarah W Davies[1], Peter M Buston[1]

[1]Department of Biology, Boston University, Boston, United States • [2]Marine Eco-Evo-Devo Unit, Okinawa Institute of Science and Technology, Onna, Japan • [3]Department of Ecology and Evolutionary Ecology, Cornell University, Ithaca, United States

eLife Assessment

Using the clownfish model, this study examines how growth, feeding, and agonistic behavior result in socially dominant or subordinate states in size- and age-matched individuals of the clownfish, Amphiprion percula. The authors complement this work with whole-body transcriptomics and find significant variation in genes and gene co-expression modules related to growth and satiety-related pathways, as well as ossification-related genes. They provide **solid** evidence that emerging dominants grow more, eat more, and behave more aggressively than subordinate or solitary individuals; these phenotypic differences are accompanied by distinct gene expression profiles, including variation in growth- and satiety-related pathways. The work is **valuable** in advancing our understanding of how the social environment regulates phenotypic change; however, claims regarding the mechanistic role of gene expression are only partially supported by the current analyses.

https://doi.org/10.7554/eLife.109871.1.sa4

Abstract

A central question in animal behavior is how individual phenotypes shape, and are shaped by, dominance hierarchies. Using clownfish (*Amphiprion percula*) as a model system, we investigated how individuals strategically modify their phenotype during hierarchy formation. We tested the overarching hypothesis that interactions among size- and age-matched rivals will lead to the emergence of social roles (dominant, subordinate, solitary), accompanied by divergence in four aspects of individual phenotypes: growth, feeding behavior, agonistic behaviors (aggression, submission), and gene expression. To test this, we created 30 replicates of juvenile clownfish, each comprising a size-matched pair housed together, and a solitary size-matched individual housed separately. Our results show that an individual's social context shapes its growth trajectory through coordinated changes in feeding behavior, agonistic behaviors, and gene expression. Individuals emerging as dominant within pairs grew twice as much compared to those emerging as subordinates, while subordinate and solitary individuals showed comparable growth. Initially, paired individuals consumed more food per capita than solitary individuals, but this difference declined as size hierarchies became established. Agonistic behaviors also decreased over time among paired individuals as size differences emerged. Transcriptomic analyses revealed upregulation of conserved vertebrate growth pathways and ossification related genes, and downregulation of satiety-associated genes, in dominant individuals compared to subordinates and solitaries. Here we link changes in gene expression with growth and feeding behavior regulation that underpin and reinforce social role differentiation during dominance hierarchy formation in clownfish. Our findings lay the foundation for a broader framework to explore the mechanisms underpinning strategic growth in social vertebrates.

Introduction

Social groups, in which some individuals forgo their own reproduction and help others to reproduce, are found in a wide variety of taxa (Rubenstein & Abbot, 2017). Within such social groups, dominance hierarchies in which individuals adopt particular social positions, are ubiquitous (Chase, 1982). Dominance hierarchies are determined by and subsequently determine interactions among group members (Milewski et al., 2022). Such hierarchies are commonly based on dominance and submission behaviors, where certain individuals of higher social rank often hold power over and are aggressive toward submissive individuals in lower social ranks (Tibbetts et al., 2022). While dominance hierarchies are often associated with asymmetric aggression toward subordinates, the structure, the overall number of members, and the number of rivals at any given social rank can vary within and among species (Drews, 1993). Regardless of the exact nature of the hierarchy, dominant individuals typically enjoy priority access to limited resources and can monopolize food, mates, and/or shelter, while subordinate members may have to wait their turn or accept less desirable resources (Chase, 1982).

Being in a dominant position confers advantages leading to higher fitness, so selection is expected to favor traits that support the acquisition of dominant positions. Social ranks, and thus the acquisition of a dominant position, at the establishment of dominance hierarchy is predominantly determined by costly (in terms of risk of injury, time, and energy invested) antagonistic interactions (Chase et al., 2002; Tibbetts et al., 2022; Vieira & Peixoto, 2013). During these interactions, phenotypic differences, such as fighting capacity, morphology, and physiology, will largely determine winners and losers (Tibbetts et al., 2022). Winners tend to be larger in size (López-Segoviano et al., 2018; Thornton et al., 2015; Wong et al., 2016), be more aggressive (Hobson et al., 2021), while losers tend to be smaller in size (Buston, 2003; Johnston et al., 2021), and submissive (Hobson et al., 2021; Tibbetts et al., 2022). Winners and losers may also differ in their feeding behavior (Huchard et al., 2016; Wong et al., 2008). These observations have generated a traditional view of dominance hierarchies where the phenotypes of individuals determine their social position.

Once the initial conflict over social position is resolved, selection will favor individuals who adjust their phenotypes in a way that maximizes the benefits in their new social position and minimizes the costs of potential future conflicts. Indeed, empirical data suggest that most hierarchies are somewhat stable over time (Gronenberg et al., 2008; Tibbetts et al., 2022), and this stability can be attributed to phenotypic changes as members take on fixed social roles leading to distinctly different phenotypes across social ranks. These distinct phenotypes are adjusted as individuals ascend or descend within the hierarchy, thus social rank influences phenotypes of individuals. For example, in naked mole-rats (*Heterocephalus glaber*), when the queen dies, non-breeding subordinates often engage in violent competition for her role. Individuals that acquire the dominant position will undergo rapid sexual maturation and growth, including elongation of the lumbar vertebrae, to become the new queen; other individuals keep their subordinate positions, and remain sexually immature and small (Johnston et al., 2021). This example highlights that adjustment of phenotypes to fit social roles is favored, playing a crucial role in the maintenance of dominance hierarchy stability. Nevertheless, processes where social rank drive the phenotype of members within a dominance hierarchy are less well understood and often overlooked, as it can be challenging to disentangle whether a phenotype of an individual is the cause or the consequence of their social rank without long-term investigations of marked individuals (Milewski et al., 2022).

Understanding the proximate mechanisms that facilitate phenotypic adjustments is key to disentangling whether phenotypes are the cause or consequence of social rank, yet relatively few studies have addressed this question. Research in social mammals, such as the naked mole rats (Dengler-Crish & Catania, 2007), and Damaraland mole rats (Johnston et al., 2021; Thorley et al., 2018) as well as in social fish (Maruska & Fernald, 2014; Renn et al., 2008), has shown that shifts in social rank are accompanied by complex changes in hormonal and neural pathways, which lead to the observed coordinated morphological shifts. In Damaraland and naked mole-rats,

ascension to queen social role involves changes in behavior in conjunction with rapid skeletal remodeling that occurs without the need of pregnancy (Dengler-Crish & Catania, 2007). Hormonal profiling further revealed that estrogens and androgens play nuanced, context-dependent roles in relation to an individual's social status (Johnston et al., 2021). In cichlids, transitions from subordinate to dominant males are characterized by immediate early gene activation in the brain, followed by changes in coloration, growth, and sexual maturation, alongside dynamic regulation of the stress axis (Alward et al., 2020; Culbert et al., 2018; Hofmann & Fernald, 2000; Maruska & Fernald, 2014; Renn et al., 2008). Despite these insights, our understanding of proximate mechanisms remains limited. Most studies, often conducted without relevant social context of an individual or in species entirely lacking dominance hierarchies, have examined single pathways to uncover variation in coloration (Salis et al., 2019, 2021), appetite regulation (Volkoff, 2019), behavior (Bender et al., 2006; Santema et al., 2013; Solomon-Lane et al., 2022), and growth (Lu et al., 2020; Zhou et al., 2024), leaving the broader molecular shifts associated with social role adoption unresolved. Gene expression profiling offers a powerful approach to uncover coordinated changes in gene expression across multiple pathways, providing a mechanistic link between the development of social role-specific phenotypes and their underlying proximate mechanisms.

The clown anemonefish, *Amphiprion percula*, provides an ideal system to investigate both how an individual's phenotype influences its social rank and how its social rank influences its phenotype during the formation and maintenance of dominance hierarchies. Its well-annotated genome (Lehmann et al., 2019) enables transcriptomic analyses of the molecular mechanisms underlying these processes. Clown anemonefish live in close association with sea anemones, with one social group per anemone. Each social group is composed of a breeding pair and 0-4 non-breeders. Within each group, there is a size-based dominance hierarchy: the female is largest (rank 1), the breeding male is second largest (rank 2), followed by non-breeders (rank 3-6) (Buston, 2003, 2004b; Buston & Cant, 2006). This size hierarchy represents a queue for breeding positions: when the female of the group dies, the breeding male changes sex and assumes the position vacated by the female, and in turn, the largest non-breeder ascends to the breeding male role by growing and becoming sexually mature (Buston, 2003, 2004a; Mitchell, 2005). Whenever a rank is vacated, the individual next in line ascends by growing and modifying its phenotype to fulfill the new social role, triggering a cascade where all lower-ranked individuals advance one rank up in a similar manner. While these aspects of clownfish social dynamics are well-understood, other phenotypic changes, physiological changes, and underlying changes in gene expression associated with the formation and maintenance of the social hierarchy of *A. percula* remain unexplored.

Here, our goal is to determine how phenotypes of size-matched and age-matched rivals change at the initiation of size-based dominance hierarchies in clownfish. In other words, how members adjust their phenotypes as they grow into their respective social roles as dominant and subordinates, and how their ultimate gene expression patterns reflect these phenotypes. Given that an individual's social role is dependent on its social position (dominant, subordinate, solitary), we tested the overarching hypothesis that interactions among size- and age-matched rivals will lead to the emergence of social roles accompanied by divergence in four aspects of individual phenotypes: growth, feeding behavior, agonistic behaviors, and gene expression (Fig. 1). We predicted that paired size-matched rivals will grow faster than size-matched solitary individuals, and that one of the pair will grow faster than the other, as they attempt to outgrow each other (Desrochers et al., 2020; Huchard et al., 2016; Reed et al., 2019). In parallel, we predicted that paired size-matched rivals will eat more than solitary individuals as they attempt to outgrow each other (Huchard et al., 2016). Within size-matched rival pairs, we also predicted that agonistic behaviors (aggression, submission) will decline over time, as size differences emerge (Wong et al., 2016). Finally, we predicted that individuals taking on dominant roles will show upregulation of genes associated with ossification, growth, and appetite stimulation, consistent with their enhanced growth trajectories (Lu et al., 2020; Volkoff, 2019; Zhou et al., 2024).

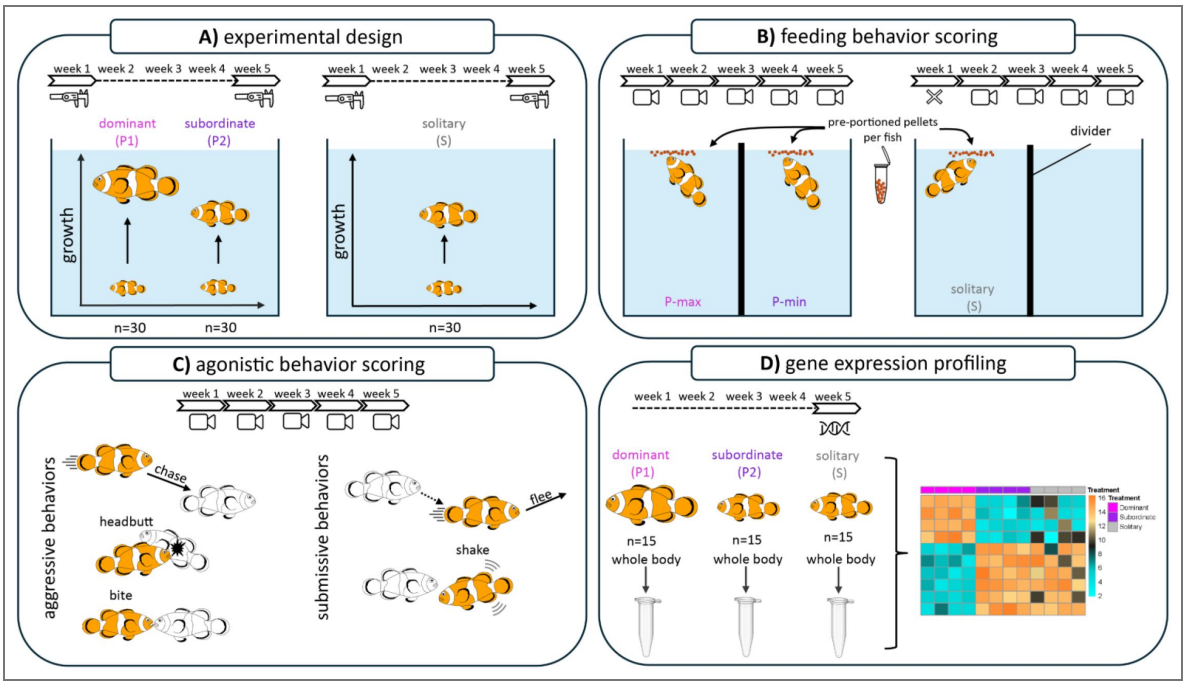


Figure 1. Overview of experimental design and methods

A) *Schematic of the experimental design*. Replicate groups of juvenile clownfish (*Amphiprion percula*) housed as size-matched pairs (n = 30 pairs) and size-matched solitary individuals (n = 30 individuals). Standard length (SL) was measured at the beginning and end of the five-week experiment, and individuals were denoted as dominant (P1), subordinate (P2), and solitary (S) at the end of the experiment based on their emergent social positions. **B)** *Feeding behavior scoring method for pairs and solitary fish*. During feeding, pairs were separated by a divider to prevent competition, divider was included in solitary tanks as a sham control, and individuals were provided with equal amounts of pre-portioned food daily. Feeding behavior was quantified weekly from video recordings by counting the number of bites taken by each fish over a 5-minute period starting from the first bite. Initially, paired fish were denoted as P-max and P-min based on the total number of bites taken, because individuals could not be reliably identified due to their similar size and lack of distinguishing markings. From week 3 onwards, bites taken were scored separately for dominant (P1) and subordinate (P2) fish, in pairs where fish could be reliably identified based on clear size differences. **C)** *Agonistic behavior scoring method for pairs*. Agonistic behaviors (aggression, submission) were quantified during a five-minute observation period from video recordings of paired interactions. Aggression was quantified by the number of chases, headbutts, and bites, while submissive behavior included flees and shakes. Initially, total aggression and submission were measured at the tank level, because fish were similar in size and lacked distinguishing markings. From week 3 onward, agonistic behaviors were scored separately for dominant (P1) and subordinate (P2) fish, in pairs where individuals could be reliably identified due to the emergence of clear size differences. In the accompanying illustrations, the focal fish is shown in orange, with its partner depicted in black and white. **D)** *Gene expression profiling for pairs and solitary fish*. At the end of the experiment (week 5), whole-body samples were collected from a subset of individuals [n = 15 per social role: dominant (P1), subordinate (P2), solitary (S)]. To maximize detection of gene expression patterns associated with social roles, replicates with the largest size differences between dominant and subordinate fish were selected for transcriptomic profiling. The resulting RNA-seq data were used to compare molecular signatures across emergent social roles. In all panels, the timeline at the top indicates when (week 1 – week 5) and how (calipers, videos, tissue samples) data was collected.

To test these predictions, we compared the four phenotypic aspects of juveniles housed as size-matched pairs and size-matched solitary controls. At the end of the experiment, individual identities within pairs were verified using gene expression data or microsatellite genotyping (see Supplementary Materials), and the larger individual in each pair was denoted as pair rank 1 (P1), the smaller as pair rank 2 (P2), and solitary individuals as S (Fig. 1A). After excluding three replicates due to mortality, 27 replicates were used for downstream analysis (N = 54 paired fish, N = 27 solitary fish). Standard length measurements taken at the beginning and end of the experiment were used to calculate growth over five weeks (Fig. 1A). Feeding behavior (Fig. 1B), and agonistic behaviors (aggression, submission; Fig. 1C) were measured weekly over five weeks, and whole-body gene expression was profiled after week five in a subset of 15 replicates (n = 45 fish; 15 P1, 15 P2, 15 S; Fig. 1D) selected to represent distinct growth trajectories across social roles. Our results show that an individual's social context shapes its growth trajectory through coordinated changes in feeding behavior, agonistic behaviors, and gene expression: dominant individuals grow faster, eat more, exhibit more aggression, and upregulate genes linked to growth and ossification while suppressing satiety signals, compared to subordinate and solitary fish.

Results

Dominant individuals exhibit accelerated growth compared to subordinates and solitaries

To test the prediction that paired size-matched rivals will grow faster than size-matched solitary individuals, and one of the pair will grow faster than the other, as they attempt to outgrow each other, standard length (SL) was measured at the beginning and end of the five-week experiment (Fig. 1A). A generalized linear mixed model (GLMM) revealed a significant effect of time (DF = 1, F-value = 159.794, $p < 0.0001$), social position of fish (DF = 2, F-value = 31.030, $p < 0.0001$), and their interaction (DF = 2, F-value = 10.534, $p < 0.0001$) on fish size. At the beginning of the experiment (t1), post-hoc Tukey tests revealed that there was no difference in the size of P1, P2, or S (all $P > 0.05$; Fig. 2A), consistent with the initial size-matching design. At the end of the experiment (t5), P1 was significantly different from P2 and S ($p < 0.001$). Parameter estimates revealed that P1 individuals grew twice as much as P2 and S (Fig. 2A.1). The model, including the fixed effects (social position and time) and random effect (replicate ID), explained 65% of the variation in size ($R^2c = .650$, $R^2m = .527$; Fig. 2A).

Higher food intake in paired rivals during periods of intense conflict over size

To test the prediction that feeding behavior will be dependent on social position, and that paired size-matched rivals will eat more than solitary individuals as they attempt to outgrow each other, we quantified food intake. The number of bites of food taken by each fish following the first bite was scored from videos, across five weekly timepoints (Fig. 1B). Due to time constraints, no videos were recorded for solitary fish during week 1. All fish were provided equal quantities of food, and paired individuals were separated during feeding to prevent interference. A divider was also placed in solitary tanks during feeding as sham control. Given that P1 and P2 identities were not initially distinguishable in videos, individuals were classified based on feeding behavior within pairs as P-max (higher intake) or P-min (lower intake) while solitaries were denoted as S. The best-fit GLMM revealed a significant effect of time (experimental week; DF = 4, F-value = 27.6042, $p < 0.0001$), social position (P-max, P-min, S; DF = 2, F-value = 58.3621, $p < 0.0001$), and their interaction (DF = 7, F-value = 2.2745, $p = 0.0288$) on feeding behavior. Post-hoc pairwise contrasts revealed that both P-max and P-min ate more than S early in the establishment of the social hierarchy (weeks 1-3) (all $p < 0.01$); however, no significant difference was found between P-min and S during weeks 4 and 5 (both $p > 0.05$; Fig. 2B). Significantly higher food intake was found when comparing P-max and S during weeks 4 ($p < 0.01$), however, no significant difference was found by week 5 ($p > 0.05$). Within pairs, contrasts revealed significant differences between P-

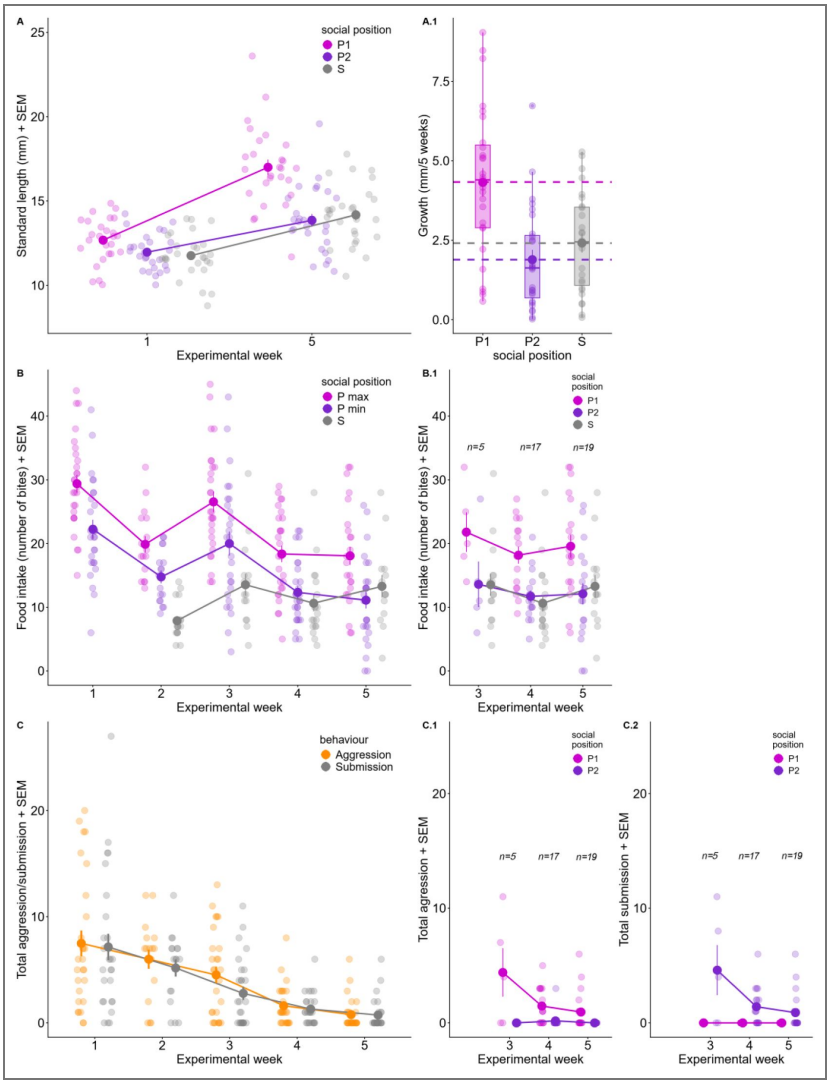


Figure 2.

A) Growth over time. Standard length (mm) of individuals of each social position (pair rank 1 = P1; pair rank 2 = P2; solitary = S) over time with mean and standard error of mean (SEM). **A.1)** Overall growth (mm) over 5 weeks of each social position are shown with mean (dashed lines) and SEM. **B) Feeding behavior over time.** Individual food intake, measured in number of bites of size-matched pairs (P-max and P-min) vs solitary (S) individuals over time with mean and SEM. **B.1)** Food intake for a subset of replicates in which dominant (P1), subordinate (P2), and solitary (S) individuals could be reliably identified due to the emergence of clear size differences (week 3, n = 5; week 4, n = 17; week 5, n = 19), shown over time with mean ± SEM. **C) Agonistic behavior of pairs over time.** Aggression (the total number of chases, bites, and headbutts) and submission (based on the total number of flees and shakes) within pairs over time with mean and SEM. **C.1)** Aggression and **C.2)** Submission for a subset of pairs in which dominant (P1) and subordinate (P2) individuals could be reliably identified based on clear size differences (week 3, n = 5; week 4, n = 17; week 5, n = 19), shown over time with mean and SEM. Data points shown jittered on all figures for visual aid, but collected at the same time.

max and P-min throughout the experiment (all $p < 0.05$), except week 2 ($p = 0.776$). The model, including the fixed effects (social position and time) and random effect (replicate ID), explained 55% of the variation in feeding behavior ($R^2c = .552$, $R^2m = .445$; Fig. 2B).

For a subset of replicates in which dominant (P1) and subordinate (P2) members could be distinguished due to the emergence of clear size differences (week 3, n=5; week 4, n=17; week 5, n=19; Fig. 2B.1), we performed a complementary analysis using known social roles (P1, P2, S rather than P-max, P-min, S) to test the prediction that feeding behavior will be dependent on social position. In this subset, the best-fit GLMM revealed a significant effect of social position (P1, P2, S; DF = 2, F-value = 29.706, $p < 0.0001$), but not time (weeks 3–5; $p=0.104$) on feeding behavior. Post-hoc comparisons showed that P1 consistently ate more food than both P2 and solitaries (all $p < 0.0001$) in these later weeks, while no significant differences were observed between P2 and solitaries ($p = 0.882$). The model, including fixed effect of social position and random effect of replicate ID, explained 63% of the variation in feeding behavior ($R^2c = .627$, $R^2m = .233$; Fig. 2B.1).

Agonistic behaviors (aggression, submission) decline as social roles stabilize

To test the prediction that in paired, size-matched rivals, agonistic behaviors will decline over time as size differences emerge, we scored aggression and submission from weekly videos (Fig. 1C). Aggression was quantified based on the sum of all bites, chases, and headbutts within pairs, while submission was quantified by the total numbers of flees and shakes (Wong et al., 2013, 2016). Because individuals within pairs could not be reliably distinguished on video, scores reflect total agonistic behavior per pair rather than per individual; however, aggression was typically only displayed by one individual while submission was typically only displayed by the other. Aggression and submission were not observed in solitary fish. The GLMM revealed that experimental week was a significant predictor of total aggression (DF = 4, F-value = 16.073, $p < 0.001$). Post-hoc pairwise comparisons showed significant differences in total aggression among most non-consecutive weeks (most $p < 0.05$), e.g., week 1 and 3, week 2 and 4, week 3 and 5 (Supplementary Materials Table 3). Aggression within pairs declined over time (Fig. 2C). The model, including the fixed effect (time) and random effect (replicate ID), explained 42% of the variation in total aggression ($R^2c = .417$, $R^2m = .306$; Fig. 2C). Furthermore, the results showed experimental week was a significant predictor of total submission (DF = 4, F-value = 18.055, $p < 0.001$). As with aggression, Tukey's post-hoc comparison showed significant differences in total submission among most non-consecutive week comparisons (most $p < 0.05$; Supplementary Materials Table 3). The model, including the fixed effect (time) and random effect (replicate ID), explained 47% of the variation in total submission ($R^2c = .476$, $R^2m = .308$; Fig. 2C).

For a subset of pairs in which dominant (P1) and subordinate (P2) members could be reliably identified due to the emergence of clear size differences (week 3, n=5; week 4, n=17; week 5, n=19; Fig. 2C.1 & 2C.2), we examined how aggression and submission varied with known social roles (P1, P2). Using these distinctions, the best-fit GLMM showed that time (weeks 3–5; DF = 2, F-value = 3.883, $p = 0.02$), social position (P1, P2; DF = 1, F-value = 43.478, $p < 0.0001$), and their interaction (DF = 2, F-value = 7.455, $p = 0.001$) significantly predicted total aggression. Post-hoc contrasts revealed a decrease in aggression over time for P1 individuals, with significant differences between weeks 3–4 ($p = 0.0009$) and 3–5 ($p = 0.0001$), but not 4–5 because aggression in both weeks was close to zero ($p = 0.477$). No significant changes occurred for P2 fish whose aggression in all weeks was close to zero ($p > 0.05$). This model explained 52% of variation in total aggression ($R^2c = 0.522$, $R^2m = 0.311$; Fig. 2C.2). Similarly, the best-fit GLMM for total submission showed significant effects of time (weeks 3–5; DF = 2, F-value = 4.675, $p = 0.012$), social position (DF = 1, F-value = 41.071, $p < 0.0001$), and their interaction (DF = 2, F-value = 7.374, $p < 0.001$). Post-hoc contrasts indicated decreased submission over time for P2 individuals, with significant differences between weeks 3–4 ($p = 0.0004$) and 3–5 ($p < 0.0001$), but not 4–5 because submission in both weeks

was close to zero (p = 0.526). No significant differences were found for P1 individuals whose submission was always close to zero (p > 0.1). This model explained 48% of variation in total submission (R^2c = 0.486, R^2m = 0.332; Fig. 2C.3).

Social roles shape global gene expression patterns

To test the prediction that whole-body gene expression patterns will vary with social roles once clear social positions have emerged (P1, P2 and S), we performed gene expression profiling on 45 individual fish (15 samples per social position; Fig. 1D). TagSeq libraries (Meyer et al., 2011) were sequenced on NovaSeq 6000 (single end, 100 bp), yielding an average 8.2 million raw reads per sample (Supplementary Materials Table 1). After quality filtering, reads were mapped to the *A. percula* genome (Lehmann et al., 2019), using STAR (Dobin et al., 2013), and gene-level counts were generated with *featureCounts*. Genes with a mean count <10 were excluded from downstream analysis. Differential gene expression analysis was performed using DESeq2 (Love et al., 2014), with the model ~ social position + genotype (clutch ID), and genes were considered differentially expressed at an FDR adjusted p < 0.05. To assess global gene expression differences across social positions, count data were rlog-transformed and used for principal component analysis (PCA) based on Euclidean distances using the vegan package (Dixon, 2003). PCA revealed similar overall patterns between P2 and S individuals, with P1 individuals exhibiting more distinct clustering (Fig. 3A). We found a significant effect of social position ($p_{social\_position}$ =0.003; Fig. 3A) and no effect of genetic background (clutch ID) on overall gene expression profiles. Pairwise contrasts across social position further confirmed no differentially expressed genes (DEGs) between P2 and S individuals (Fig. 3B; Supplementary Materials Fig. 3). A total of 96 DEGs were significantly upregulated in P2 individuals, and 390 DEGs were significantly upregulated in S individuals, when compared to P1. Eighty-four of these upregulated DEGs were shared between P2 and S (Supplementary Materials Table 4). A total of 37 DEGs were significantly downregulated in P2 individuals, and 130 DEGs in S individuals, when compared to P1 individuals. A total of 25 downregulated DEGs were shared between P2 and S individuals (Fig. 3B; Supplementary Materials Table 4).

Gene Co-expression Networks Link Expression Patterns to Phenotypic Traits

To test the prediction that global gene expression patterns will be correlated with observed phenotypic changes in growth and feeding behavior while accounting for the individual's social position, a weighted gene co-expression network analysis (WGCNA; Langfelder & Horvath, 2008) was conducted. A total of 9 WGCNA modules were found (Supplementary Materials Fig. 4; only 8 modules are shown, as the 9th contained over 10,000 genes with no clear eigengene expression pattern and was therefore excluded). Of the remaining modules, four (brown, blue, red, black) showed significant associations with the phenotypes of interest. These modules were further explored using GO enrichment with Fisher's exact test (Supplementary Materials Fig. 5).

Growth related genes are upregulated in dominant individuals

The brown module identified via WGCNA showed strong positive correlations with body size (Supplementary Materials Fig. 4). Subsequent GO enrichment analysis revealed enrichment for biological process (BP) and molecular function (MF) GO terms associated with development, ossification, morphogenesis, and cytoskeletal regulation (Fig. 4). From the genes associated with these GO terms, a total of 38 genes were significantly upregulated in P1 individuals compared to P2 and S fish (FDR p ≤ 0.05; Fig. 5A). These included extracellular matrix and bone development genes (*col1a2, col6a1, mmp14b, plod2, loxa*), signaling factors (*bmp4, tgfb1a, tgfb3, fstl1b*), and regulators of muscle and cytoskeletal function (*myh7ba, tpm1, actn2b, mstna, smo*, among others; Fig. 5B).

Figure 3. Overall gene expression profiles showed no significant differences between P2 and S individuals

A) Principal Component Analysis (PCA) of all differentially expressed genes (DEGs) across social positions, with PERMANOVA results indicating a significant effect of social position. **B)** Venn diagram illustrating DEGs from pairwise comparisons of social positions (FDR-adjusted p-value of 0.05). In the pairwise comparisons, P2 and S were compared to P1 individuals. Orange upward arrows and corresponding numbers represent significantly upregulated DEGs, while blue downward arrows and numbers indicate downregulated DEGs (Supplementary Materials Table 4).

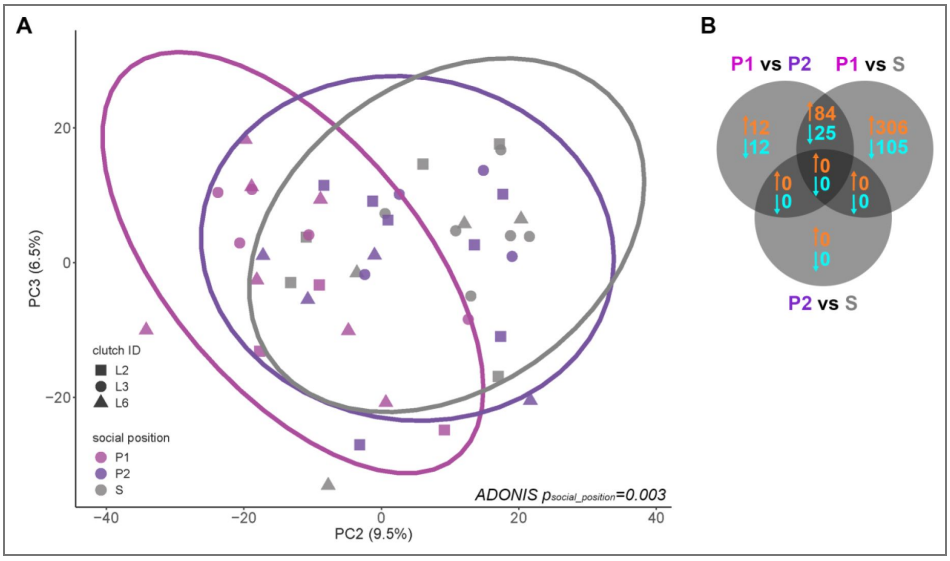
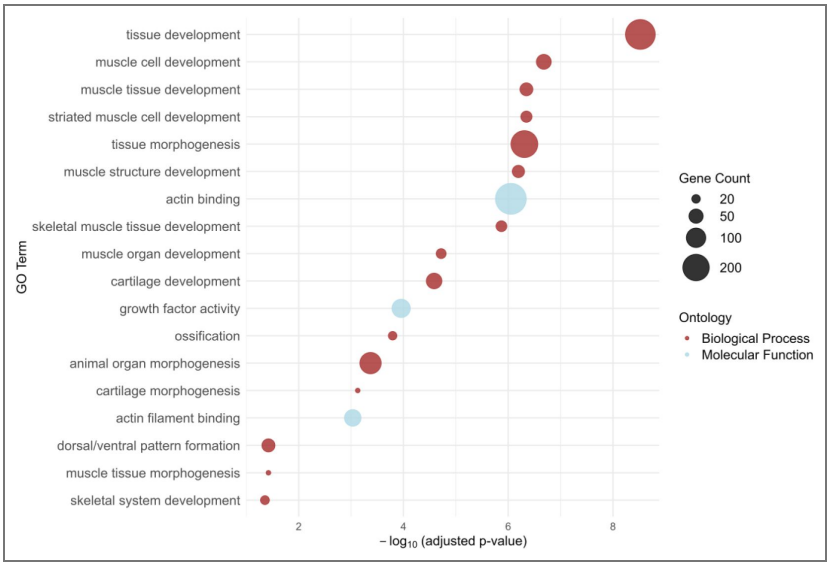


Figure 4. Gene ontology (GO) enrichment of the brown module associated with growth and ossification

An exhaustive list of GO enrichment results for genes assigned to the brown WGCNA module (Fig. 5). Bubble size reflects numbers of genes associated with each term, color indicates GO category. Terms are ordered by adjusted p-value significance on the x-axis (–$\log_{10}$ scale), with more significant terms on the right.



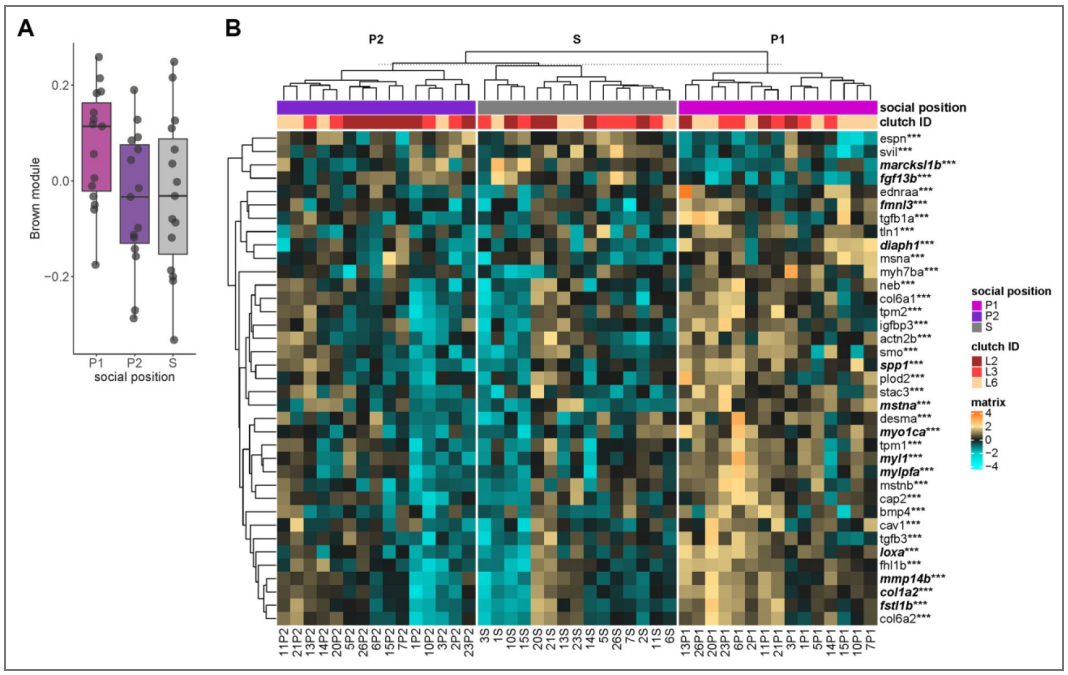


Figure 5. P1 individuals showed upregulation of genes associated with growth and ossification

A) Average eigen gene values across social positions for the brown module. **B)** Heatmap of significant DEGs (FDR p-value ≤ 0.05) of growth and ossification genes in the brown Biological Process (BP) and Molecular Function (MF) GO terms across the three social positions. Genes that are significantly differentially expressed between individuals of P1 vs P2 are denoted with an asterisk (*), those between P1 vs S with a double asterisk (**), and three asterisk (***) represents both comparisons. Genes shown in ***bold and italics*** indicate significance at an FDR adjusted p-value < 0.1.

Dominant social role is associated with suppression of satiety signals and altered metabolic pathway activity

While the red WGCNA module was positively associated with appetite-related gene expression, we found no strong enrichment of appetite and metabolism related GO terms (Supplementary Materials Fig. 5). To further examine differences in appetite and metabolism across social roles, we analyzed genes known to play a role in these processes identified in the sister species *Amphiprion ocellaris* (Herrera et al., 2025). High-confidence *A. percula* orthologs were identified using a reciprocal BLAST+ approach (Camacho et al., 2009) (Supplementary Table 2) and matched to our whole-body expression dataset. We found a set of genes controlling appetite (*ghrl, insra, irs2a, irs2b, adycap1b, cckb*) to be significantly downregulated in P1 individuals while P2 and S show upregulation of these genes (FDR $p \leq 0.01$; Fig. 6). Similarly, genes that control energy metabolism via TCA cycle (*mdh1aa, suclg1, suclg2, sdha, ogdhb, ogdhl*), and glycolysis (*gapdhb, aldob, tpi1a, eno2b*) were also significantly downregulated in P1 individuals, while P2 and S individuals showed upregulation of these genes (Fig. 6).

Discussion

Here we show that in paired, size-matched rivals, individual growth trajectories are associated with coordinated changes in feeding behavior, agonistic behaviors, and underlying gene expression, leading to the emergence of clear social roles. We found that the individual ultimately emerging as P1 (dominant) grew significantly more compared to individuals taking on the P2 (subordinate) role. Moreover, S (solitary) individuals showed similar growth to P2 fish (Fig. 2A). To facilitate this differential growth dependent on social position, we have shown that early in the experiment both paired individuals, P-max and P-min, ate more per capita compared to S individuals (Fig. 2B). Later in the experiment, as size differences within pairs emerged, P1 ate more than either P2 or S (Fig. 2B.1). In conjunction with increased food intake in pairs, we have also shown that agonistic behaviors within pairs was significantly higher at the beginning of the experiment compared to the end of the experiment, once size differences were established (Fig. 2C). Furthermore, aggression was displayed by the dominant member of pairs while submissive behaviors were shown by subordinate fish (Fig. 2C.1 & 2C.2). These findings show that coordinated changes in growth, feeding behavior, and agonistic behaviors reinforce phenotypes that match each individual's social role. Interestingly, in 25% of the 27 replicates, the initially smaller fish ultimately emerged as the dominant member, demonstrating that winners and losers were determined during early social interactions rather than being predetermined by initial size differences. These behavioral and morphological shifts were mirrored at the molecular level. P1 individuals showed significantly different gene expression profiles compared to P2 and S individuals with P1 individuals upregulating genes linked to ossification (e.g., *collagen, myoglobin, bmp, and sost*) relative to P2 and S fish (Fig. 5). Likewise, genes that promote satiety signals (e.g., *cholecystokinin (CCK), adycap1b*) were downregulated in P1 relative to P2 and S, consistent with their heightened appetite, suggesting appetite suppression in P2 and S individuals (Fig. 6). Additionally, metabolic pathway genes involved in the TCA cycle (*ogdhl, ogdhb, sdha, mdh1aa*) and glycolysis (*tpi1a, eno2b, gapdhb, aldob*) were more active in P2 and S fish (Fig. 6), suggesting different energy utilization strategies.

Our findings on growth across social positions are broadly consistent with previous research, showing that size-matched rivals exhibit divergent growth trajectories based on their ultimate social rank. P1 individuals grew significantly more than P2 (mean ± SD: 4.32 ± 2.34 mm vs. 1.89 ± 1.60 mm), consistent with earlier studies in clownfish (Desrochers et al., 2020; Reed et al., 2019) and other social vertebrates such as meerkats (Huchard et al., 2016). Desrochers et al. (2020) also observed that P1 individuals grew 5.0 mm on average, compared to 3.1 mm for P2s and 1.6 mm for solitary fish, in a similar time frame. Consistent with previous findings, our P1 individuals exhibited significantly greater growth (4.32 ± 2.34 mm) than P2 individuals (1.89 ± 1.60 mm) or S individuals (2.41 ± 1.55 mm). In contrast to previous findings, we did not detect a difference between P2 (1.89 ± 1.60 mm) and S individuals (2.41 ± 1.55 mm). This contrast is likely

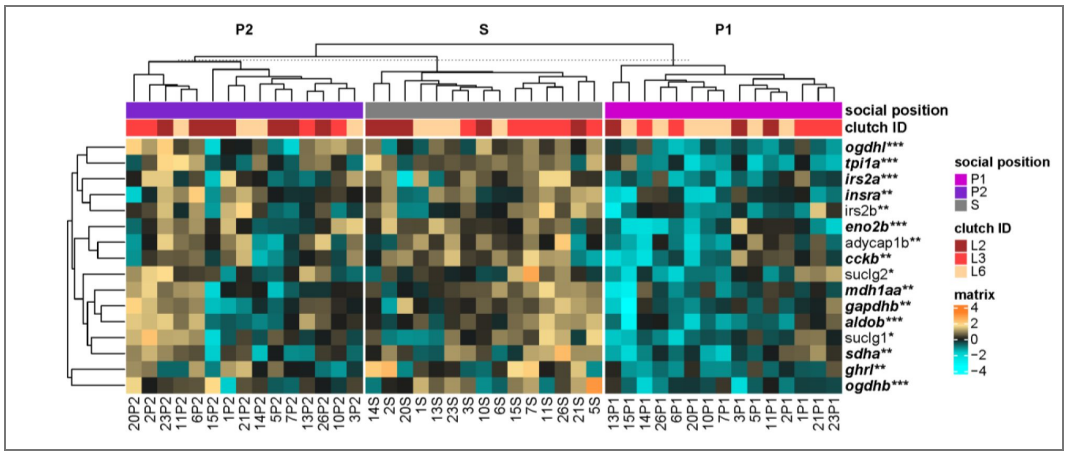


Figure 6. P1 individuals showed downregulation of appetite suppression genes

Heatmap of significantly differentially expressed genes (DEGs) (FDR p-value ≤ 0.01) of appetite, TCA cycle and glycolysis pathways across the three social positions indicating genetic background of individuals (clutch ID). Genes that are significantly differentially expressed between individuals of P1 vs P2 are denoted with an asterisk (*), those between P1 vs S with a double asterisk (**), and three asterisk (***) represents both comparisons. Genes shown in ***bold and italics*** indicate significance at an FDR adjusted p-value < 0.1.

due to improved experimental conditions in our study, particularly by the inclusion of host anemones, which seemed to improve the growth and condition of solitary individuals relative to previous experiments (Desrochers et al., 2020; Reed et al., 2019; Rueger et al., 2022).

Furthermore, this is amongst the first studies where the adjustment of feeding behavior across social positions has been quantified in clownfish. Despite equal food availability provided to all individuals, we show that feeding behavior differed significantly across social ranks, with individuals adjusting their food intake in accordance with their social roles. Similar socially mediated regulation of food intake has been reported in Kalahari meerkats (Huchard et al., 2016) and emerald coral goby (Wong et al., 2008). Meerkat size-matched rivals closely monitor each other's food intake and modulate their own in attempt to match growth and body size (Huchard et al., 2016), while emerald coral goby subordinates have been shown to "diet" as they near their immediate dominant's size, to avoid conflict (Wong et al., 2008). Our findings suggest a comparable mechanism in clownfish, wherein feeding behavior is fine-tuned to emerging social roles. Additionally, here we quantified aggression and submission within pairs during the establishment of the dominance hierarchy, revealing peak agonistic interactions during the first two weeks of the experiment. This is consistent with other evidence showing that conflict intensity increases as size differences between individuals decrease, with the highest level of conflict observed between size-matched rivals (Bender et al., 2005; Wong et al., 2016). Within pairs, we show that conflict over size is resolved by taking on differing social roles via the adjustment of growth trajectories, feeding behavior and agonistic behaviors. Indeed week 3 onward, when pair members could be reliably identified, dominant individuals consistently consumed more food and displayed aggressive behaviors, while subordinates ate less and exhibited submissive behaviors, clearly reflecting the emergence of two distinct social roles. While we cannot be certain, it is highly likely that these differences were present during the earlier weeks as well. Likely, winners and losers emerge soon after their initial interactions (Milewski et al., 2022; Wong et al., 2016), so that members of the social group can adjust their social role while progressing into their social position.

In vertebrates, growth is regulated by four major signaling pathways: the Growth Hormone (GH)/IGF pathway, the insulin/PI3K-AKT pathway, the mTOR pathway, and the Hippo pathway (Fuentes et al., 2013; Glass, 2003). Together, these pathways coordinate cellular processes that promote overall growth and ossification. Their downstream effects are reflected in several functional gene categories, including those involved in ossification, bone matrix formation and structure, muscle development and contraction, and other structural regulators (Valenti et al., 2020). P1 individuals exhibited significantly greater growth than P2 and S fish, which was accompanied by coordinated upregulation of genes involved in skeletal and muscular development. This growth phenotype appears to be regulated via conserved vertebrate hormonal pathways. We found genes associated with the GH/IGF axis, including *igsbp3*, a proliferation-promoting member of the IGF-binding protein family (Zheng et al., 2024), and *mstna* and *mstnb*, negative regulators of muscle growth (Lee & McPherron, 2001), upregulated in P1 individuals, consistent growth-promoting state. For the insulin/ PI3K-AKT pathway we showed significant upregulation of insulin receptor genes (e.g., *insra, irs2a, irs2b*) and several structural and cytoskeletal genes known to be downstream targets of PI3K-AKT signaling (*cap2, tln1, actn2b*) (Glass, 2010). The mTOR pathway, which drives protein synthesis and cellular hypertrophy (Schiaffino et al., 2013), was also found to be activated as P1 individuals showed upregulation for muscle contractile genes (*myh7ba, myl1, mylpfa, tpm1, tpm2*) and cytoskeletal regulators (*diaph1, fmnl3*) (Gunning et al., 2008). Additionally, increased expression of *smo*, a Hedgehog pathway gene that interacts with Hippo signaling, points to active control of tissue growth boundaries (Kodaka & Hata, 2015). These four pathways appear to stimulate structural and matrix-related genes: P1 fish upregulated bone matrix collagens (*col1a2, col6a1, col6a2*), matrix remodeling enzymes (*mmp14b, plod2, loxa*), and morphogenetic regulators (*bmp4, tgfb1a, tgfb3, fstl1b*), all contributing to bone growth and extracellular matrix turnover (Gistelinck et al., 2016; van Boxtel et al., 2011). A key mineralization gene, *ssp1*, was also upregulated in P1 fish, supporting the activation of osteogenic pathways (Windhausen et al., 2015). In contrast, P2 and S individuals showed consistent downregulation of these genes and pathways, aligning with their

reduced growth. These results provide a broad framework for understanding the molecular mechanisms of strategic growth in other social vertebrates. Pathways underlying skeletal and muscular morphogenesis are highly conserved between mammals and fish (Howe et al., 2013; Valenti et al., 2020), suggesting that similar molecular mechanisms may underlie skeletal remodeling during rank transitions of meerkats and mole-rats. Nonetheless, some differences in these pathways are expected, particularly capacity for bone regeneration, which is present in fish but absent in mammals (Pfefferli & Jaźwińska, 2015).

In our study, P1 individuals showed an increased food intake compared to P2 individuals. To explore molecular drivers of this pattern we examined GE profiles and found downregulation of genes linked to appetite regulation and metabolic pathways in P1 individuals. As time since last meal can strongly alter GE profiles, all fish were fed ~14 hours before euthanasia; however, residual pellets may have remained, making the exact time of last ingestion uncertain. To assess whether differences in appetite-related gene expression could underlie the distinct feeding behaviors associated with dominant and subordinate roles, we explored appetite suppressor genes (e.g.: cholecystokinin *(CCK),* cocaine- and amphetamine-regulated transcript *(CART),* leptin *(leptin),* peptide YY *(PYY), and* oxytocin *(OXT)*) that usually rise after feeding, as well as appetite stimulators (e.g.: ghrelin *(ghrl),* agouti-related neuropeptide *(AGRP),* neuropeptide Y *(NPY),* and hypocretin neuropeptide precursor *(HCRT*)), that typically increase during fasting to promote feeding behavior (Volkoff, 2019). We found two appetite suppressor genes, *CCK* and *adycap1b*, to be significantly downregulated in P1 individuals. *CCK* functions as a short-term satiety signal, increasing after feeding and decreasing during fasting in goldfish (Thavanathan & Volkoff, 2006). Similarly, *adycap1b*, a homolog of *PACAP2*, has been shown to suppress food intake in zebrafish (Nakamachi et al., 2019). Of the appetite stimulator genes, we observed downregulation of *ghrl* in P1 individuals. Ghrelin is traditionally classified as an appetite stimulator, typically increasing during fasting to promote feeding (Assan et al., 2021; Soengas, 2021). However, in teleosts, ghrelin can also act as a short-term satiety signal (Volkoff, 2019). The observed concurrent downregulation of *CCK*, *adycap1b* and *ghrl* of P1 individuals may point towards a reduced need to suppress feeding, providing a potential mechanistic explanation for higher food intake in dominant fish, which reinforces their social role. A similar pattern has been observed in domesticated chickens, where rapid growth is associated with increased appetite which is facilitated via the decreased expression of *CCK* leading to an altered satiety signal (Dunn et al., 2013). Molecular signals indicative of appetite suppression were likely even more pronounced earlier in the experiment (e.g., at week 2), when we showed increased food intake coinciding with intense conflict over size.

Another way of thinking about these results, since all of the gene expression measures are relative, is to take the perspective of P2 individuals: P2 individuals exhibited lower food intake and upregulation of appetite-related genes associated with satiety. Upregulation of appetite suppressor genes (*CCK and adycap1b*) may reflect a physiological drive to suppress appetite. This pattern is consistent with the observed subordinate strategy of reduced food intake and hence growth, providing a molecular basis for social position driven behavioral restraint. Indeed, at the molecular level this reduced food intake might parallel the “dieting” behavior observed in emerald coral goby subordinates, a strategy thought to limit growth and reduce conflict with immediate dominants (Wong et al., 2008). However, this behavioral strategy in the context of dominance hierarchies has not been demonstrated at the molecular level. *Ghrl* was also significantly upregulated in P2 individuals, despite their lower food intake. In rainbow trout, it has been shown that ghrelin-treated individuals exhibited reduced appetite ultimately leading to a ghrelin-induced decrease in growth (Jönsson et al., 2010). Interestingly, increased ghrelin expression was also shown to be associated with increased locomotion both in goldfish and zebrafish (Matsuda et al., 2006; Navarro-Guillén et al., 2019). Given that P2 fish tend to be subjected to aggressive behaviors in the form of chases, increased *ghrl* levels may support the need for increased movement.

From a metabolic perspective, P1 individuals also exhibited downregulation of genes involved with aerobic metabolism (TCA cycle; *ogdhl, ogdhb, sdha, mdh1aa* genes) and anaerobic glycolysis (*tpi1a, eno2b, gapdhb, aldob* genes). This pattern is somewhat contradictory to previous findings (Lu et al., 2020; Roux et al., 2023; Yin et al., 2023), and unexpected given the increased growth observed in P1 individuals, a process that is energetically costly and dependent on ATP production. Some parallels can be drawn with freshwater cavefish, where fast-growing individuals also showed downregulation of some key glycolysis genes (*aldob, eno2*) compared to slow-growing counterparts (Yin et al., 2023), however, they found other glycolysis genes (e.g.:*agl, pfkm, gapdh, bpgm*) to be upregulated in fast-growing fish suggesting a more nuanced regulation. It is possible P1 individuals rely on glycolysis under conditions of high food availability to facilitate growth, but we failed to detect such signals because they are likely tissue specific and we used a whole-body RNA-seq approach. P1 individuals also showed downregulated insulin receptor genes (*insra, irs2a, irs2b*), which regulate glucose uptake and energy balance post-feeding (Sheridan, 2021). This could indicate that P1 individuals shift toward fatty-acid β-oxidation metabolism which yields substantially more ATP than glycolysis (Darvey, 1998) and could support increased growth, however, we did not find any upregulated β-oxidation related DEGs. The appetite and metabolic profiles of P1 individuals characterized by the downregulation of appetite suppressors along with suppressed expression of both aerobic TCA cycle and anaerobic glycolytic genes, is somewhat contradictory. It is likely that P1 individuals are meeting their energy demands efficiently via increased food intake and allocating energy towards growth.

In contrast, P2 fish showed significant upregulation of both aerobic TCA cycle genes and anaerobic glycolysis genes, as well as upregulation of insulin receptor genes. The pronounced upregulation of appetite-suppressing genes, alongside the elevated ghrelin expression, suggests that P2 individuals may be experiencing an energy deficit, likely due to reduced food intake and increased locomotory activity. Correspondingly, while it is somewhat contradictory, the upregulation of both aerobic (TCA cycle) and anaerobic (glycolysis) metabolic genes may suggest a physiological response to meet energy demands during “dieting” behaviors. Finally, S individuals maintained a stable appetite but showed an analogous gene expression profile of appetite and metabolic genes to P2 individuals. This may be linked to the stress of social isolation, which in larval zebrafish has been shown to alter feeding behavior and suppress food intake (Wee et al., 2022). Although many well-known appetite-regulating genes and metabolic pathway genes were not differentially expressed, the overall expression patterns suggest that individuals modulate their molecular phenotypes to reinforce their social roles as dominant, subordinate, and solitary members.

A striking finding of our study was the similarity in growth trajectories, feeding behavior, and overall gene expression profiles between subordinate (P2) and solitary (S) individuals. Although solitary individuals had the opportunity to assume the dominant (P1) role in their tanks, they instead adopted subordinate-like characteristics, indicating that social context, not opportunity alone, drives social role expression. Similar patterns have been anecdotally observed in the wild, where juvenile clownfish remained small for extended periods (over four months) following the loss of a dominant partner, only initiating changes in social role towards dominant characteristics upon the arrival of a new group member (personal observations). It’s almost as if these fish are remaining dormant, until their future social position becomes clear. Comparable results have been reported in Damaraland mole rats, where subordinate helpers and solitaries were indistinguishable both on a morphological and on a gene expression level (Johnston et al., 2021) and therefore were treated as one group of non-breeders for downstream analysis. This approach, however, overlooks the differing social contexts these individuals experience. Other studies in cichlids (Hesse & Thünken, 2014), zebrafish (Wee et al., 2022), and mice (Benfato et al., 2022) have shown that social isolation often results in decreased growth, increased corticoid levels, altered appetite, and behavioral changes. However, none of these studies considered changes associated with social isolation in the ecological context of social behavior and dominance hierarchies. Our results suggest that the presence of a social partner is essential for the establishment of social roles, likely because progression through the social hierarchy involves

irreversible transitions in social roles (Buston, 2003, 2004b), a pattern also true for Damaraland mole rats (Johnston et al., 2021). Consequently, social role differentiation appears to require mutual recognition and reinforcement within a group context.

We have used clownfish (*Amphiprion percula*) as a model system to investigate the role of phenotypic plasticity during the establishment of dominance hierarchy. We showed that an individual's social context shapes its growth trajectory through coordinated changes in behaviors and underlying gene expression, with dominant members strategically upregulating their growth, while subordinates and solitaries strategically downregulating their growth. This study lays the foundation for a broader framework to explore the mechanisms underpinning strategic growth in social vertebrates. Future research should focus on characterizing periods of strategic upregulation and downregulation of growth dependent on social context, with particular emphasis on gene expression signatures. In our current study, individuals within pairs were ultimately progressing towards rank 1 dominant female and rank 2 subordinate male roles, and thus individuals showed early gene expression signatures of sexual maturation. In other species known to exhibit strategic growth, such as cichlids (Maruska & Fernald, 2014; Renn et al., 2008), meerkats (Huchard et al., 2016; Russell et al., 2004), and mole rats (Dengler-Crish & Catania, 2007; Johnston et al., 2021; Thorley et al., 2018) strategic upregulation of growth cannot be disentangled from sexual maturation, due to the nature of their dominance hierarchy. However, clownfish offers an ideal system to disentangle growth-related transcriptional profiles from those associated with sexual maturation, as subordinate individuals of rank 3-6 exhibit strategic growth, without signs of sexual maturation (Buston, 2003; Buston & Clutton-Brock, 2022; Reed et al., 2019).

While whole-body transcriptomics revealed overarching expression trends, it cannot resolve the fine-scale, tissue-specific gene expression changes (Lu et al., 2020; Yin et al., 2023) critical to understanding social role differentiation, such as strategic growth. To disentangle gene expression signatures associated with the strategic up- and downregulation of growth, future studies should include sampling points aligned with the onset of these physiological shifts. Moreover, to understand the metabolic and hormonal pathways involved, tissue specific transcriptomics will be essential. Sampling multiple tissues across key time points would allow to disentangle fine-scale regulatory processes underlying strategic growth. These include appetite regulation (orexigenic and anorexigenic signaling), metabolic pathways (e.g., glycolysis, lactic fermentation, TCA cycle, fatty acid β-oxidation), growth-regulating pathways (GH/IGF, insulin/PI3K-AKT, mTOR, and Hippo), and potential molecular responses to social stress. Additionally, while laboratory studies can provide controlled environments, complementary field-based studies will be crucial to validate laboratory results. Once candidate hormonal or molecular pathways are identified, their functional roles could be experimentally confirmed through targeted hormonal or CRISPR manipulation (Mitchell et al., 2021). Together, these approaches provide a powerful framework for uncovering the dynamic, context-dependent mechanisms that regulate strategic growth and drive the establishment and maintenance of dominance hierarchies in social vertebrates.

Methods

Adult Broodstock

We conducted these experiments at Boston University (Boston, MA, USA) during the summer of 2022. All fish used in these experiments were reared from broodstock that were wild-caught as non-breeders (less than 30 mm in standard length) in Papua New Guinea and supplied by Quality Marine and Sea Dwelling Creatures. The removal of non-breeders is considered a sustainable practice because they do not directly influence the survival, growth, or reproduction of the breeders (Buston, 2004a). Laboratory adult broodstock were housed as sixty pairs each in their own 120 L tanks with sand bottom and *Entacmaea quadricolor* anemone habitat on rocks over a 15×15 cm ceramic tile. A detailed description of broodstock housing conditions can be found elsewhere (Pacaro et al., 2023; Schlatter et al., 2022).

Experimental Juveniles

To obtain experimental juveniles, egg clutches of adult broodstock were monitored daily until day 8, when they were moved to closed saltwater larval rearing tanks (20 L). To stimulate hatching, egg clutches were gently aerated using an air stone placed directly below the clutch. Multiple egg clutches hatched simultaneously, and the larvae were reared to 30 days of age in their respective tanks. During rearing, a daily water change of 10 L was conducted, and abiotic conditions of pH 8.1 ± 0.1, temperature 26 ± 1°C and salinity 35 ± 2 ppt were maintained. Hatched larvae were fed rotifers (*Brachionus rotundiformis*) (15 rotifers ml^{-1}) in water tinted with *Nannochloropsis* algae paste (Rotigreen Nanno, Reed Mariculture, USA) between days 0-8. From day 4 until 14, decapsulated *Artemia salina* nauplii (3 Artemia ml^{-1}) were fed. From days 4 to 8, both rotifers and Artemia were fed simultaneously to allow the larvae to gradually adjust to the transition between food sources. Once the fish reached 14 days of age, TDO-B1 Chroma BOOST Granule (Reef Nutrition) fish pellets were fed in addition to Artemia. Food pellets are composed of 49% crude protein, 13% crude fat, 9.7% carbohydrates. Juvenile fish were reared to 30 days of age, after which individuals were photographed, measured, and moved to experimental housings.

Experimental Housing

Experimental fish were housed in 3.5 L breeder boxes with a sea anemone (*Entacmaea quadricolor)*, with a mean anemone surface area of 16.263 cm^2 ± 1.739. Breeder boxes were submerged in 120 L tanks, which were part of a re-circulating saltwater aquarium system. Flow through each 120 L tank was approximately 9 L h^{-1} ± 0.5 L. Abiotic conditions were monitored regularly and maintained as constant as possible. pH (8.35 ± 0.45), temperature (26.7 ± 1.2°C) and salinity (34.5 ± 1.5 ppt) were monitored daily by GHL profilux system and ammonia (0 ppm), nitrite (0 ppm), and nitrate (0 ppm) were monitored weekly (API test kits, Mars Fishcare, North America). Lighting was provided by two T5 bulbs per tank—one actinic blue and one aquablue special (ATI North America)—delivering approximately 50 PAR. The light cycle was maintained at 12 hours light and 12 hours dark (12 h L: 12 h D). The life support system included a biomedia bed for biological filtration and a UV sterilizer for disinfection.

Experimental Set-Up

We tested the overarching hypothesis that interactions among size- and age-matched rivals will lead to the emergence of social roles accompanied by divergence in four aspects of individual phenotypes. A total of 90 juveniles, 30 from each of three clutches, were used. Using these juveniles, we created 30 replicates with an unrelated size-matched pair housed in one breeder box (n = 30 pairs; n = 60 individuals) and a solitary individual housed in another breeder box (n = 30 individuals). At the end of the experiment, individual identities within pairs were verified using either gene expression data or microsatellite genotyping (see Supplementary Materials). The larger individual in each pair was classified as pair rank 1 (P1), the smaller as pair rank 2 (P2), and solitary individuals were denoted as S.

Body Size

To measure juvenile size, fish were photographed in a Petri dish marked with grid paper under a dissecting microscope. These photos were used to measure standard length (SL) of juveniles to 0.01 mm using ImageJ (Schneider et al., 2012). Once measured, we size-matched pairs and solitary individuals as closely as possible: average size difference between individuals within these trios was 0.66 ± 0.21mm, which is 0.08% of their average standard length.

Feeding Behavior and Agonistic Behaviors

To measure juvenile behavior, we used cameras (Olympus Stylus Tough TG-Tracker Action camera) to record each pair and each solitary for 15 minutes, once per week, for five weeks. Note that there were no video recordings made of tanks with solitaries during the first week due to time constraints during experimental setup.

To measure feeding behavior, we introduced a divider to separate paired individuals prior to feeding, which prevented them from interacting with each other, and we introduced a divider to solitary individuals as a sham control. Then each fish was fed an identical portion of food. We scored feeding behavior — total number of bites of food taken — for 5 minutes after the first bite of food was taken, in both paired and solitary individuals. During the first two experimental weeks, P1 and P2 could not be distinguished in videos because of their similar sizes and lack of distinguishing markings. Therefore, we identified the individual who consumed more food in a given video as P-max and the one who consumed less as P-min, and we maintained this designation throughout the experiment unless members of a pair could be reliably distinguished. From week 3 onward, members of a subset of pairs could be reliably distinguished due to the emergence of clear size differences (week 3, n=5 pairs; week 4, n=17 pairs; week 5, n=19 pairs), allowing us to score food intake separately for dominant (P1) and subordinate (P2) fish.

To measure aggression and submission in paired individuals, we scored agonistic behaviors for 5 minutes after 1 minute of acclimatization (no divider present). We scored aggression based on the sum of all bites, chases, and headbutts between pairs (Wong et al., 2013, 2016). Flees and shakes between individuals were recorded to measure submissiveness between pairs (Wong et al., 2013, 2016). Because P1 and P2 could not be distinguished in videos during the first two experimental weeks, we quantified aggression as a total aggression score and submission as a total submission score for the pair, and we maintained this assignment throughout the experiment unless members of a pair could be reliably distinguished. However, aggression was typically displayed by only one member of the pair. From week 3 onward, members of a subset of pairs could be reliably distinguished due to the emergence of clear size differences (week 3, n = 5 pairs; week 4, n = 17 pairs; week 5, n = 19 pairs), allowing us to score aggression and submission separately for dominant (P1) and subordinate (P2) fish. Aggression and submission were not scored for solitary individuals.

RNA Isolation and Sequencing

Following the experiment described above, all fish were preserved for gene expression profiling. At the time of euthanasia, all fish had not been fed for over 14 hours, ensuring consistency for downstream analysis. Before preservation, all fish (n=90) were individually cold-anesthetized according to protocols previously used in the lab (Maytin et al., 2018) after which whole fish tissue samples were immediately preserved in 200 proof ethanol. Following preservation, 15 replicates were chosen for subsequent gene expression profiling based on preliminary results indicating that growth varied consistently with social position. To maximize our ability to detect underlying gene expression patterns contributing to the expected growth signature (P1 > P2 = S), 15 replicates that exhibited the greatest size difference between P1 and P2 were selected for gene expression profiling. We also controlled, to the degree possible, for genetic effects by choosing an equal number of fish from each clutch across social positions (P1, P2, S). Fish from these 15 replicates (n=45 samples) were individually homogenized to allow equal RNA extraction from all tissues.

Tissue samples were cut into smaller pieces with a razor blade and RNA was isolated using an established phenol-chloroform DNA/RNA isolation protocol, with the modification of not adding RNAse (Davies et al., 2013). Samples were eluted in 35μL of molecular biology grade water and DNAsed using RNA Clean & Concentrator-5 kit (Zymo Research, no. R1015) according to manufacturer's protocol. RNA concentrations were determined using a DeNovix DS-11+ Spectrophotometer (DeNovix Inc., Wilmington, DE, USA) and RNA integrity was visualized on a 1% agarose gel. Next, all samples were individually normalized to 100ng/25μL and sent to Genomic Sequencing and Analysis Facility at the University of Austin, Texas, where TagSeq libraries (Meyer et al., 2011) were prepared and sequenced (single end 100bp) on a NovaSeq 6000. RNA sequencing and downstream analyses were conducted separately for each of the 45 samples (15 P1, 15 P2, and 15 S).

Raw Sequence Processing and Read Mapping

A total of 366 million raw reads were generated with average raw read counts of 8.2 million (range: 5.4 to 11.3 million per sample; Supplementary Materials Table 1). Raw reads were trimmed with *Fastx_toolkit* (Hannon, 2010), which removed 5'-Illumina leader sequences and poly(A)+ tails. Sequences <20bp in length with <90% of bases having quality cutoff scores <20 were also trimmed. In addition, because degenerate bases were incorporated during cDNA synthesis, PCR duplicates were removed from all libraries. After quality filtering, an average of 3.2 million reads per sample remained (ranging from 2.3 to 4.4 million per sample; Supplementary Materials Table 1), which were then mapped to the *Amphiprion percula* genome (Lehmann et al., 2019) using *STAR* (Dobin et al., 2013). Mapped bam files were used to generate per gene read counts across samples using *featureCounts*. The read count file was then imported into R version 4.3.1 (R Core Team, 2021) and any gene with a mean raw count <10 was removed from all downstream analysis. To correctly identify individuals within pairs and ensure they are correctly assigned to clutches, we called single-nucleotide polymorphisms (SNPs) from TagSeq data (see Supplementary Materials), prior to downstream analysis.

Statistical analysis

From the total of 30 replicates, 3 replicates lost fish throughout the five-week experiment. All three losses were within pairs. These three replicates were removed from all downstream analyses, leaving a total of 27 replicates (N=54 paired individuals; N=27 solitary individuals). To test our four hypotheses, we ran four general linear mixed models (GLMM) using the *LmerTest* package (Kuznetsova et al., 2017) in R. We considered size as the dependent variable, with social position of individual (P1, P2, or S), time (t1 or t5), and their interaction as the independent variables. We considered feeding behavior as the dependent variable, with social position of individual (P-max, P-min, or S), time (t1, t2, t3, t4, or t5), and their interaction as the independent variables. In addition, for the subset of replicates where dominant (P1) and subordinate (P2) individuals could be reliably distinguished (weeks 3–5), we ran a complementary GLMM using known social roles (P1, P2, S), time (t1, t2, t3, t4, or t5), and their interaction as the independent variables. Furthermore, we considered agonistic behavior (aggression or submission) between individuals within pairs as the dependent variables, with time (t1, t2, t3, t4, or t5) as the independent variable. For the subset of pairs where P1 and P2 could be distinguished (weeks 3–5), we also ran complementary GLMMs using known social roles (P1, P2) as the independent variables. To account for the lack of independence among individuals from the same replicate, all our models included replicate ID as a random effect. Model assumptions were assessed by visual inspection of residuals and fitted values; residuals were approximately normally distributed and showed no evidence of heteroscedasticity, thus no data transformations were required. In all cases, the best fit model was determined by carrying out a backward model selection (*step* function in *LmerTest* package (Kuznetsova et al., 2017). Post hoc pairwise contrasts using *emmeans* (Searle et al., 1980) were used to determine where differences exist between categories.

Global Gene Expression Patterns

All gene expression (GE) analyses were carried out in R (version 4.3.1). To test for differentially expressed genes (DEGs) across the social positions (P1, P2, and S) DESeq2 (Love et al., 2014) with the model: design = ~ genotype + social position was run. Count data were normalized and independent pairwise contrasts across 3 social positions were computed. Genes identified as differentially expressed were corrected for multiple testing using false discovery rate (FDR: adjusted $p < 0.05$). Annotated DEGs of up- and down-regulated genes for all three pairwise comparisons were visualized using *ComplexHeatmaps* (Gu et al., 2016) and volcano plots. To test for overall GE patterns between social positions and genotypes, data were rlog-normalized and the effect of genotype and social position were compared through a Principal Component Analysis (PCA) using Euclidean distances of all genes with the vegan package (Dixon, 2003). To test for overall gene expression differences, a PERMANOVA was performed using the *adonis2* function within the vegan package (Dixon, 2003).

Gene Co-expression Networks Link Expression Patterns to Phenotypic Traits

To test whether gene expression patterns are correlated with observed phenotypic changes in growth and appetite across social positions, a Weighted Gene Correlation Network Analysis (WGCNA) was conducted (Langfelder & Horvath, 2008). A signed network was constructed using a soft-threshold power of 6 with both the network and Topological Overlap Matrix (TOM) set to signed. Modules were identified with a minimum module size of 600, and module merging threshold was set to 0. Modules of interest in WGCNA were further explored with gene ontology (GO) enrichment analysis using Fisher's Exact Tests (DEG presence/absence) to identify sets of genes in GO divisions of Biological Processes (BP), Molecular Functions (MF), and Cellular Components (CC) (Wright et al., 2015). Genes associated with specific GO terms were explored and significant DEGs (FDR: $p \leq 0.05$) were extracted and visualized with *ComplexHeatmaps* (Gu et al., 2016).

To further investigate GE differences related to growth, appetite regulation, and metabolism across social positions, we examined the expression patterns of relevant genes. Specifically, we focused on genes associated with these pathways previously identified in the sister species, *Amphiprion ocellaris* (Herrera et al., 2025). Genes identified in *A. ocellaris* were used to retrieve *A. percula* orthologs, using a reciprocal BLAST search (BLAST+ version 2.11.0) approach (Camacho et al., 2009). Both BLAST searches were performed with an e-value threshold of 0.0001 to ensure high ortholog confidence. Only gene pairs that were the best match in both blast directions were considered as orthologs for downstream analysis. A complete list of retrieved gene IDs of *A. percula* were then filtered against the whole-body GE dataset (Supplementary Materials Table 2; appetite and metabolic genes shown). Of these, significant DEGs (FDR $p \leq 0.01$) were visualized using *ComplexHeatmaps* (Gu et al., 2016) while taking into account genotype and social position.

Data availability

Raw FASTQ files for all gene expression samples are publicly accessible through the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1280023. All data, along with the code used for analyses and figure generation, are available on GitHub at: *https://github.com/lillentyu/A-percula-Adaption-Of-Social-Roles*. All data and corresponding analyses are also available at Mendeley Data: *https://data.mendeley.com*. This repository is currently a draft and will be made publicly accessible upon the final publication of the manuscript.

Acknowledgements

We are particularly grateful to Karen Warkentin, Shannon Mary Fisher, Paige Becker, and Zoé Chamot for their constructive feedback on the manuscript. We also thank Natalia Karadimitriou for her invaluable assistance during larval rearing and experimental setup.

Additional information

Funding

Funding support was provided by an NSF grant to P.M.B. (NSF award number: 2333286), and Economakis Summer Research Fellowship as well as Warren McLeod Summer Research Fellowship awarded by the Boston University Marine Program to L.F.V.

Ethics

All applicable national and/or institutional guidelines for the care and use of animals were followed. All procedures with live animals were reviewed and approved by the Boson University IACUC (protocol number PROTO201800525).

Authors' contributions

L.F.V.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, visualization, writing—original draft, writing—review and editing; D.A.: data curation, investigation, methodology, writing—review and editing; C.B.: methodology, writing—review and editing; M.H: methodology, writing—review and editing; A.H.: methodology, writing—review and editing; K.T.: methodology, writing—review and editing; S.M.B.: data curation, methodology, writing—review and editing; V.L.: methodology; supervision, writing—review and editing; S.W.D.: methodology; visualization, supervision, writing—review and editing; P.M.B.: conceptualization, funding acquisition, investigation, methodology, project administration, supervision, writing—review and editing.

Declaration of AI use

The artificial intelligence tool ChatGPT (OpenAI, version GPT-4) was used to support R script development, particularly for data management, analysis, and visualization. Prompts included requests such as: "Identify errors or issues in the following code based on this data frame structure: [description of code and data], and suggest possible alternatives or solutions," and "Explain and resolve this error message: [error message and associated code]." ChatGPT assisted by identifying and explaining coding issues, offering corrected code, and suggesting alternative approaches where appropriate. All suggestions provided by ChatGPT were reviewed by an expert before implementation.

Funding

Funder	Grant reference number	Author
National Science Foundation (NSF)	2333286	Pete Buston

Author ORCID iDs

Lili F Vizer: https://orcid.org/0009-0009-7302-6491
Colleen Bove: https://orcid.org/0000-0003-0340-1371
Marcela Herrera: https://orcid.org/0000-0001-6021-3989
Annabel Hughes: https://orcid.org/0009-0004-3833-9971
Steven M Bogdanowicz: https://orcid.org/0000-0002-9269-1181
Vincent Laudet: https://orcid.org/0000-0003-4022-4175
Sarah W Davies: https://orcid.org/0000-0002-1620-2278
Peter M Buston: https://orcid.org/0000-0001-5513-8259

Additional files

Supplementary file 1

References

Alward Beau A., Laud Vibhav A., Skalnik Christopher J., York Ryan A., Juntti Scott A., Fernald Russell D (2020) Modular genetic control of social status in a cichlid fish. *Proceedings of the National Academy of Sciences of the United States of America* **117**:28167-28174

Assan Daniel, Mustapha Umar Farouk, Chen Huapu, Li Zhiyuan, Peng Yuhao, Li Guangli (2021) The roles of neuropeptide Y (Npy) and peptide YY (Pyy) in teleost food intake: A mini review. *Life* **11**:547

Bender Nicole, Hamilton Ian M., Heg Dik (2005) Size differences within a dominance hierarchy influence conflict and help in a cooperatively breeding cichlid. *Behaviour* **142**:1591-1613

Bender Nicole, Heg Dik, Hamilton Ian M., Bachar Zina, Taborsky Michael, Oliveira Rui F (2006) The relationship between social status, behaviour, growth and steroids in male helpers and breeders of a cooperatively breeding cichlid. *Hormones and Behavior* **50**:173-182

Benfato, Dias Izabelle, Quintanilha, Carolina Ana, Silvares, Henrique, Salles Jessica, Souza, Alves Melyssa, Rosário, *et al.* (2022) Effects of long-term social isolation on central, behavioural and metabolic parameters in middle-aged mice. *Behavioural Brain Research* **417**:113630

Buston Peter M (2003) Size and growth modification in clownfish. *Nature* **424**:145-146

Buston Peter M (2004a) Does the presence of non-breeders enhance the fitness of breeders? An experimental analysis in the clown anemonefish Amphiprion percula. *Behavioral Ecology and Sociobiology* **57**:23-31

Buston Peter M (2004b) Territory inheritance in clownfish. *Proceedings of the Royal Society B: Biological Sciences* **271**:252-254

Buston Peter M., Cant Michael A (2006) A new perspective on size hierarchies in nature: Patterns, causes, and consequences. *Oecologia* **149**:362-372

Buston Peter M., Clutton-Brock Tim (2022) Strategic growth in social vertebrates. *Trends in Ecology & Evolution* **37**:694-705

Camacho Christiam, Coulouris George, Avagyan Vahram, Ma Ning, Papadopoulos Jason, Bealer Kevin, Madden Thomas L (2009) BLAST+: architecture and applications. *BMC Bioinformatics* **10**:421

Chase Ivan D (1982) Dynamics of Hierarchy Formation: The Sequential Development of Dominance Relationships. *Behaviour* **80**:218-240

Chase Ivan D., Tovey Craig, Spangler-Martin Debra, Manfredonia Michael (2002) Individual differences versus social dynamics in the formation of animal dominance hierarchies. *Proceedings of the National Academy of Sciences of the United States of America* **99**:5744-5749

Culbert Brett M., Gilmour Kathleen M., Balshine Sigal (2018) Stress axis regulation during social ascension in a group-living cichlid fish. *Hormones and Behavior* **103**:121-128

Darvey Ivan G (1998) How does the ratio of ATP yield from the complete oxidation of palmitic acid to that of glucose compare with the relative energy contents of fat and carbohydrate?. *Biochemical Education* **26**:22-23

Davies Sarah W., Rahman Muneeb, Meyer Eli, Green Elizabeth A., Buschiazzo Emmanual, Medina Mónica, Matz Mikhail V (2013) Novel polymorphic microsatellite markers for population genetics of the endangered Caribbean star coral, Montastraea faveolata. *Marine Biodiversity: A Journal of the Senckenberg Research Institute / Senckenberg Forschungsinstitut Und Naturmudeum* **43**:167-172

Dengler-Crish Christine M., Catania Kenneth C (2007) Phenotypic plasticity in female naked mole-rats after removal from reproductive suppression. *The Journal of Experimental Biology* **210**:4351-4358

Desrochers Leah, Branconi Rebecca, Schlatter E., Dent Brianne, Buston Peter (2020) Sensory cues underlying competitive growth in the clown anemonefish (Amphiprion percula). *Behavioural Processes* **181**:104276

Dixon Philip (2003) VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science: Official Organ of the International Association for Vegetation Science* **14**:927-930

Dobin Alexander, Davis Carrie A., Schlesinger Felix, Drenkow Jorg, Zaleski Chris, Jha Sonali, Batut Philippe, Chaisson Mark, Gingeras Thomas R (2013) STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**:15-21

Drews Carlos (1993) The Concept and Definition of Dominance in Animal Behaviour. *Behaviour* **125**:283-313

Dunn Ian C., Meddle Simone L., Wilson Peter W., Wardle Chloe A., Law Andy S., Bishop Valerie R., Hindar Camilla, Robertson Graeme W., Burt Dave W., Ellison Stephanie J. H., *et al.* (2013) Decreased expression of the satiety signal receptor CCKAR is responsible for increased growth and body weight during the domestication of chickens. *American Journal of Physiology: Endocrinology and Metabolism* **304**:E909-21

Fuentes Eduardo N., Valdés Juan Antonio, Molina Alfredo, Björnsson Björn Thrandur (2013) Regulation of skeletal muscle growth in fish by the growth hormone – Insulin-like growth factor system. *General and Comparative Endocrinology* **192**:136-148

Gistelinck Charlotte, Witten Paul Eckhard, Huysseune Ann, Symoens Sofie, Malfait Fransiska, Larionova Daria, Simoens Pascal, Dierick Manuel, Van Hoorebeke Luc, De Paepe Anne, *et al.* (2016) Loss of type I collagen telopeptide lysyl hydroxylation causes musculoskeletal abnormalities in a zebrafish model of Bruck syndrome. *Journal of Bone and Mineral Research: The Official Journal of the American Society for Bone and Mineral Research* **31**:1930-1942

Glass David J (2003) Molecular mechanisms modulating muscle mass. *Trends in Molecular Medicine* **9**:344-350

Glass David J (2010) PI3 kinase regulation of skeletal muscle hypertrophy and atrophy. *Current Topics in Microbiology and Immunology* **346**:267-278

Gronenberg Wulfia, Ash Lesley E., Tibbetts Elizabeth A (2008) Correlation between facial pattern recognition and brain composition in paper wasps. *Brain, Behavior and Evolution* **71**:1-14

Gu Zuguang, Eils Roland, Schlesner Matthias (2016) Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* **32**:2847-2849

Gunning Peter, O'Neill Geraldine, Hardeman Edna (2008) Tropomyosin-based regulation of the actin cytoskeleton in time and space. *Physiological Reviews* **88**:1-35

Hannon G. J. (2010) FASTX-toolkit. http://hannonlab.cshl.edu/fastx_toolkit

Herrera Marcela, Vianello Stefano, Mitchell Laurie, Chamot Zoé, Lorin-Nebel Catherine, Bonnelye Edith, Roux Natacha, Besseau Laurence, Gibert Yann, Laudet Vincent (2025) From genes to pathways: A curated gene approach to accurate pathway reconstruction in teleost fish transcriptomics. *Journal of Experimental Zoology: Part B, Molecular and Developmental Evolution* https://doi.org/10.1002/jez.b.23299

Hesse Saskia, Thünken Timo (2014) Growth and social behavior in a cichlid fish are affected by social rearing environment and kinship. *The Science of Nature* **101**:273-283

Hobson Elizabeth A., Mønster Dan, DeDeo Simon (2021) Aggression heuristics underlie animal dominance hierarchies and provide evidence of group-level social information. *Proceedings of the National Academy of Sciences of the United States of America* **118** https://doi.org/10.1073/pnas.2022912118

Hofmann H. A., Fernald R. D (2000) Social status controls somatostatin neuron size and growth. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* **20**:4740-4744

Howe Kerstin, Clark Matthew D., Torroja Carlos F., Torrance James, Berthelot Camille, Muffato Matthieu, Collins John E., Humphray Sean, McLaren Karen, Matthews Lucy, *et al.* (2013) The zebrafish reference genome sequence and its relationship to the human genome. *Nature* **496**:498-503

Huchard Elise, English Sinead, Bell Matt B. V., Thavarajah Nathan, Clutton-Brock Tim (2016) Competitive growth in a cooperative mammal. *Nature* **533**:532-534

Johnston Rachel A., Vullioud Philippe, Thorley Jack, Kirveslahti Henry, Shen Leyao, Mukherjee Sayan, Karner Courtney M., Clutton-Brock Tim, Tung Jenny (2021) Morphological and genomic shifts in mole-rat 'queens' increase fecundity but reduce skeletal integrity. *eLife* **10**:e65760

Jönsson Elisabeth, Kaiya Hiroyuki, Björnsson Björn Thrandur (2010) Ghrelin decreases food intake in juvenile rainbow trout (Oncorhynchus mykiss) through the central anorexigenic corticotropin-releasing factor system. *General and Comparative Endocrinology* **166**:39-46

Kodaka Manami, Hata Yutaka (2015) The mammalian Hippo pathway: regulation and function of YAP1 and TAZ. *Cellular and Molecular Life Sciences: CMLS* **72**:285-306

Kuznetsova Alexandra, Brockhoff Per B., Christensen Rune H. B (2017) lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software* **82**:1-26

Langfelder Peter, Horvath Steve (2008) WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics* **9**:559

Lee S. J., McPherron A. C (2001) Regulation of myostatin activity and muscle growth. *Proceedings of the National Academy of Sciences of the United States of America* **98**:9306-9311

Lehmann Robert, Lightfoot Damien J., Schunter Celia, Michell Craig T., Ohyanagi Hajime, Mineta Katsuhiko, Foret Sylvain, Berumen Michael L., Miller David J., Aranda Manuel, *et al.* (2019) Finding Nemo's Genes: A chromosome-scale reference assembly of the genome of the orange clownfish Amphiprion percula. *Molecular Ecology Resources* **19**:570-585

López-Segoviano Gabriel, Bribiesca Rafael, Arizmendi María Del Coro (2018) The role of size and dominance in the feeding behaviour of coexisting hummingbirds. *The Ibis* **160**:283-292

Love Michael I., Huber Wolfgang, Anders Simon (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* **15**:1-21

Lu Xue, Chen Hui-Min, Qian Xue-Qiao, Gui Jian-Fang (2020) Transcriptome analysis of grass carp (Ctenopharyngodon idella) between fast- and slow-growing fish. *Comparative Biochemistry and Physiology: Part D, Genomics & Proteomics* **35**:100688

Maruska Karen P., Fernald Russell D (2014) *Social Regulation of Gene Expression in the African Cichlid Fish Astatotilapia burtoni* Oxford University Press.

Matsuda Kouhei, Miura Tohru, Kaiya Hiroyuki, Maruyama Keisuke, Uchiyama Minoru, Kangawa Kenji, Shioda Seiji (2006) Stimulatory effect of n-octanoylated ghrelin on locomotor activity in the goldfish, Carassius auratus. *Peptides* **27**:1335-1340

Maytin Alexander K., Davies Sarah W., Smith Gabriella E., Mullen Sean P., Buston Peter M (2018) De novo transcriptome assembly of the clown anemonefish (Amphiprion percula): A new resource to study the evolution of fish color. *Frontiers in Marine Science* **5**:1-11

Meyer E., Aglyamova G. V., Matz M. V (2011) Profiling gene expression responses of coral larvae (Acropora millepora) to elevated temperature and settlement inducers using a novel RNA-Seq procedure. *Molecular Ecology* **20**:3599-3616

Milewski T. M., Lee W., Champagne F. A., Curley J. P (2022) Behavioural and physiological plasticity in social hierarchies. *Philosophical Transactions of the Royal Society of London: Series B, Biological Sciences* **377**:20200443

Mitchell Jeremy (2005) Queue selection and switching by false clown anemonefish, Amphiprion ocellaris. *Animal Behaviour* **69**:643-652

Mitchell Laurie J., Tettamanti Valerio, Rhodes Justin S., Marshall N. Justin, Cheney Karen L., Cortesi Fabio (2021) CRISPR/Cas9-mediated generation of biallelic F0 anemonefish (Amphiprion ocellaris) mutants. *PloS One* **16**:e0261331

Nakamachi Tomoya, Tanigawa Ayano, Konno Norifumi, Shioda Seiji, Matsuda Kouhei (2019) Expression patterns of PACAP and PAC1R genes and anorexigenic action of PACAP1 and PACAP2 in zebrafish. *Frontiers in Endocrinology* **10**:227

Navarro-Guillén Carmen, Dias Jorge, Rocha Filipa, Castanheira M. F., Martins Catarina I. M., Laizé Vincent, Gavaia Paulo J., Engrola Sofia (2019) Does a ghrelin stimulus during zebrafish embryonic stage modulate its performance on the long-term?. *Comparative Biochemistry and Physiology: Part A, Molecular & Integrative Physiology* **228**:1-8

Pacaro Madison, Barbasch Tina, Rogers Michaela, Chavez Juan, Buston Peter, Majoris John (2023) Nocturnal parental care and the role of parents in hatching their eggs in the clown anemonefish. *Ethology: Formerly Zeitschrift Fur Tierpsychologie* https://doi.org/10.1111/eth.13384

Pfefferli Catherine, Jaźwińska Anna (2015) The art of fin regeneration in zebrafish. *Regeneration* **2**:72-83

Reed Cymone, Branconi Rebecca, Majoris John, Johnson Cara, Buston Peter M (2019) Competitive growth in a social fish. *Biology Letters* **15** https://doi.org/10.1098/rsbl.2018.0737

Renn Susan C. P., Aubin-Horth Nadia, Hofmann Hans A (2008) Fish and chips: functional genomics of social plasticity in an African cichlid fish. *The Journal of Experimental Biology* **211**:3041-3056

Roux Natacha, Miura Saori, Dussenne Mélanie, Tara Yuki, Lee Shu-Hua, de Bernard Simon, Reynaud Mathieu, Salis Pauline, Barua Agneesh, Boulahtouf Abdelhay, *et al.* (2023) The multi-level regulation of clownfish metamorphosis by thyroid hormones. *Cell Reports* **42**:112661

Rubenstein Dustin R., Abbot Patrick (2017) *Comparative Social Evolution* Cambridge University Press.

Rueger Theresa, Bhardwaj Anjali Kristina, Turner Emily, Barbasch Tina Adria, Trumble Isabela, Dent Brianne, Buston Peter Michael (2022) Vertebrate growth plasticity in response to variation in a mutualistic interaction. *Scientific Reports* **12**:11238

Russell Andrew F., Carlson Anne A., McIlrath Grant M., Jordan Neil R., Clutton-Brock Tim (2004) Adaptive size modification by dominant female meerkats. *Evolution; International Journal of Organic Evolution* **58**:1600-1607

Salis Pauline, Lorin Thibault, Lewis Victor, Rey Carine, Marcionetti Anna, Escande Marie-Line, Roux Natacha, Besseau Laurence, Salamin Nicolas, Sémon Marie, *et al.* (2019) Developmental and comparative transcriptomic identification of iridophore contribution to white barring in clownfish. *Pigment Cell & Melanoma Research* **32**:391-402

Salis Pauline, Roux Natacha, Huang Delai, Marcionetti Anna, Mouginot Pierick, Reynaud Mathieu, Salles Océane, Salamin Nicolas, Pujol Benoit, Parichy David M., *et al.* (2021) Thyroid hormones regulate the formation and environmental plasticity of white bars in clownfishes. *Proceedings of the National Academy of Sciences of the United States of America* **118** https://doi.org/10.1073/pnas.2101634118

Santema Peter, Teitel Zachary, Manser Marta, Bennett Nigel, Clutton-Brock Tim (2013) Effects of cortisol administration on cooperative behavior in meerkat helpers. *Behavioral Ecology: Official Journal of the International Society for Behavioral Ecology* **24**:1122-1127

Schiaffino Stefano, Dyar Kenneth A., Ciciliot Stefano, Blaauw Bert, Sandri Marco (2013) Mechanisms regulating skeletal muscle growth and atrophy. *The FEBS Journal* **280**:4294-4314

Schlatter E., Klawon Caitlynn, Webb Colleen, Buston Peter (2022) Heritability of dispersal-related larval traits in the clown anemonefish Amphiprion percula. *Ecology and Evolution* **12**:e9541

Schneider Caroline A., Rasband Wayne S., Eliceiri Kevin W (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **9**:671-675

Searle S. R., Speed F. M., Milliken G. A (1980) Population Marginal Means in the Linear Model: An Alternative to Least Squares Means. *The American Statistician* **34**:216-221

Sheridan Mark A (2021) Coordinate regulation of feeding, metabolism, and growth: Perspectives from studies in fish. *General and Comparative Endocrinology* **312**:113873

Soengas José L (2021) Integration of nutrient sensing in fish hypothalamus. *Frontiers in Neuroscience* **15**:653928

Solomon-Lane Tessa K., Butler Rebecca M., Hofmann Hans A (2022) Vasopressin mediates nonapeptide and glucocorticoid signaling and social dynamics in juvenile dominance hierarchies of a highly social cichlid fish. *Hormones and Behavior* **145**:105238

Thavanathan Rajiv, Volkoff Hélène (2006) Effects of amylin on feeding of goldfish: interactions with CCK. *Regulatory Peptides* **133**:90-96

Thorley Jack, Katlein Nathan, Goddard Katy, Zöttl Markus, Clutton-Brock Tim (2018) Reproduction triggers adaptive increases in body size in female mole-rats. *Proceedings*. *Biological Sciences* **285**:20180897

Thornton B., Knowlton J., Kuntz W (2015) Interspecific competition and social hierarchies in frugivorous neotropical birds of Costa Rica. *Journal of Young Investigators* **29**:1-6

Tibbetts Elizabeth A., Pardo-Sanchez Juanita, Weise Chloe (2022) The establishment and maintenance of dominance hierarchies. *Philosophical Transactions of the Royal Society of London: Series B, Biological Sciences* **377**:20200450

Valenti Maria Teresa, Marchetto Giulia, Mottes Monica, Dalle Carbonare Luca (2020) Zebrafish: A suitable tool for the study of cell signaling in bone. *Cells* **9**:1911

van Boxtel Antonius L., Gansner John M., Hakvoort Henk W. J., Snell Heather, Legler Juliette, Gitlin Jonathan D. (2011) Lysyl oxidase-like 3b is critical for cartilage maturation during zebrafish craniofacial development. *Matrix Biology: Journal of the International Society for Matrix Biology* **30**:178-187

Vieira Marcos C., Peixoto Paulo E. C (2013) Winners and losers: a meta-analysis of functional determinants of fighting ability in arthropod contests. *Functional Ecology* **27**:305-313

Volkoff Helene (2019) Fish as models for understanding the vertebrate endocrine regulation of feeding and weight. *Molecular and Cellular Endocrinology* **497**:110437

Wee Caroline L., Song Erin, Nikitchenko Maxim, Herrera Kristian J., Wong Sandy, Engert Florian, Kunes Samuel (2022) Social isolation modulates appetite and avoidance behavior via a common oxytocinergic circuit in larval zebrafish. *Nature Communications* **13**:2573

Windhausen Thomas, Squifflet Steeve, Renn Jörg, Muller Marc (2015) BMP signaling regulates bone morphogenesis in zebrafish through promoting osteoblast function as assessed by their nitric oxide production. *Molecules* **20**:7586-7601

Wong Marian, Medina A., Uppaluri C., Arnold S., Seymour J. R., Buston P. M (2013) Consistent behavioural traits and behavioural syndromes in pairs of the false clown anemonefish Amphiprion ocellaris. *Journal of Fish Biology* **83**:207-213

Wong Marian, Uppaluri C., Medina A., Seymour J., Buston Peter M (2016) The four elements of within-group conflict in animal societies: an experimental test using the clown anemonefish, Amphiprion percula. *Behavioral Ecology and Sociobiology* **70**:1467-1475

Wong Marian Y. L., Munday Philip L., Buston Peter M., Jones Geoffrey P (2008) Fasting or feasting in a fish social hierarchy. *Current Biology: CB* **18**:R372-R373

Wright Rachel M., Aglyamova Galina V., Meyer Eli, Matz Mikhail V (2015) Gene expression associated with white syndromes in a reef building coral, Acropora hyacinthus. *BMC Genomics* **16**:1-12

Yin Yanhui, Zhang Yuanwei, Hua Zexiang, Wu Anli, Pan Xiaofu, Yang Junxing, Wang Xiaoai (2023) Muscle transcriptome analysis provides new insights into the growth gap between fast- and slow-growing Sinocyclocheilus grahami. *Frontiers in Genetics* **14**:1217952

Zheng Jichang, Zhang Wenjing, Xu Yongjiang, Cui Aijun, Jiang Yan, Wang Bin (2024) Insulin-like growth factor binding protein-3 (igfbp-3) and igfbp-5 in yellowtail kingfish (Seriola lalandi): molecular characterization and expression levels under different nutritional status and stocking density. *Fish Physiology and Biochemistry* **50**:1621-1633

Zhou Bo, Ling Leyan, Wang Bin, Yang Fei, Hou Mengdan, Liu Fan, Li Yu, Luo Hui, He Wenping, Ye Hua (2024) Hepatopancreas transcriptome analysis of Spinibarbus sinensis to reveal different growth-related genes. *Genes* **15**:949

Lili Vizer (2025) Raw FASTQ gene expression data for clownfish social roles. NCBI BioProject. ID PRJNA1280023 https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1280023

Peer reviews

Reviewer #1 (Public review):

Summary:

Overall, this is an interesting and well-written manuscript on a fascinating question in a "charismatic" model system.

Strengths:

1) The Introduction is concise, though it might be helpful to the non-specialist reader to learn a bit more about what is known about the social control of somatic growth across diverse species (including humans), which would help to make this work more generally interesting.

(2) The experiment is well-designed.

(3) The data collected are comprehensive.

(4) The complementary analysis of both feeding and aggression/submission data with and without known social roles is a neat idea and compelling!

Weaknesses:

(1) I was surprised that the HPA/stress axis was not considered here at all. Wouldn't we expect that subordinates have increased stress axis activation, which in turn could inhibit their growth and aggressive behavior?

(2) To what extent are growth, food intake, agonistic behavior, and/or gene expression patterns coordinated across P1 vs P2 pairs? The lack of such an analysis seems like a missed opportunity.

(3) What was the rationale for using whole bodies for the transcriptome analysis? Given the hypotheses, the forebrain or hypothalamus and certain other organ systems (e.g., liver, gonads, skin, etc.) would have been obvious candidate tissues here. I realize that cost is always a consideration, but maybe a focus on the fore-/midbrain could have been prioritized.

(4) Given the preceding point, why was a fold-change threshold used for assessing DEGs (supplementary Figure 3)? There is no biological justification to ever use a fold-change threshold, especially in bulk RNA-seq analysis. This is particularly true here, where whole bodies were used for RNA-seq analysis, which is a bit unusual. Relatively small cell populations (such as hypothalamic neurons that regulate growth or food intake) may show substantial gene expression variation across social types, yet will be masked by the masses of other cells in the whole body sample. However, gene expression may still vary significantly, albeit the fold-difference may be small. I therefore suggest a reanalysis that omits any fold-change threshold.

(5) Why is the analysis of color (hue, saturation) buried in the supplementary materials? Based on the hypotheses that motivated the study, color seems just as relevant as food intake, growth, and agonistic behavior, so even if the results are negative, they should be presented in the main paper.

(6) The Discussion is sometimes difficult to follow. The authors may want to consider including a conceptual graphic that integrates the different aspects of growth and satiety regulation, etc., into a work-in-progress model of sorts, which would also facilitate clearer hypotheses for future research.

https://doi.org/10.7554/eLife.109871.1.sa3

Reviewer #2 (Public review):

In this manuscript, the authors test growth, behavior, and gene expression in pairs of clownfish as they establish social dominance hierarchies, examining patterns of gene expression in these pairs after dominance has been established. The authors show solid evidence that emerging dominant clownfish show increased growth, aggression, and food consumption compared to their submissive or solitary counterparts, eventually adopting distinct gene expression profiles.

Major Comments:

(1) The Introduction is comprehensive, but it could be condensed. Likewise, the discussion could be condensed. There is considerable redundancy between the methods, the results, and the legend in Figure 1. The authors should consolidate and remove the redundancy.

(2) For Figure 3, the authors are showing PC2 and PC3; why is PC1 not shown? There is so much overlap between the three groups in PC2 vs PC3; it seems unlikely that researchers

could conclusively identify any individual as belonging to a group based on the expression profile. The ovals shown do not capture all the points within each of the groups, and particularly the grey S oval seems misaligned with the datapoints shown.

(3) The authors indicate that the 15 replicates exhibiting the greatest size difference between P1 and P2 were selected for gene profiling. Does this mean that each of the P1 and P2 were pairs with each other? Have the authors tried examining the gene expression patterns in a paired manner? E.g., for the pairs that showed the greatest size differences, do they also show the greatest differences in gene expression? Do the P1s show the most extreme differences from P2s that also show the most extreme P2 differences? Perhaps lines on Figure 3A connecting datapoints from the P1 and P2 pairs would be informative.

(4) For the specific target pathways that are up- and downregulated in the different backgrounds, I recommend that the authors include boxplots (or heatmaps) showing the actual expression values for these targets. Figure 6 shows a heatmap for appetite-related genes, and it would be great to see a similar graph for the metabolism and glycolysis genes; it would also be informative to see similar graphs for hormonal and sexual maturation pathways as well.

(5) Particularly given that there is a relatively small number of genes enriched in the different rank conditions, I did not understand the need to do the WGCNA module analysis. I thought that an analysis of GO terms across the dataset would have been more meaningful than the GO term analysis shown in Figure 4, which considers only genes assigned to the "brown WGCNA module". This should be simplified or clarified.

(6) The authors say that they have identified coordinated changes in behaviors and the "underlying gene expression, leading to the emergence" of social roles. This is a little bit misleading, since the gene expression analysis occurred well after the behavioral and phenotypic differences emerged. Presumably, the hormonal and genetic shifts that actually caused the behavioral and phenotypic difference occurred during the weeks during which the experiment was underway, and earlier capture of the transcriptome would presumably reveal different patterns, and ones that would be considered more causative. The authors acknowledge this in 434-435, but it could be emphasized further.

(7) The authors have measured a number of differences between the different dominance classes of fish. All these differences were measured relative to the other classes, but in my view, the Solitary group was the closest to a baseline control. So I'm not sure that it is fair to say that "P2 and S individuals showed consistent downregulation of these genes and pathways" (line 401). I encourage the authors to emphasize the differences in gene expression from the "perspective" of the P1 individuals compared to the baseline of P2 and S individuals. Line 474 says that "P2 fish showed significant upregulation" of a number of pathways. It should be very clear what that is compared to (compared to P1, presumably?)

(8) Along the same lines, the authors say in line 514 that subordinates and solitaries strategically downregulate their growth. I'm not convinced that this is the case: I would consider this growth trajectory to be the default and the baseline. I would interpret that under certain social conditions, a P1 dominant pattern of growth, behavior, and gene expression is allowed to emerge.

https://doi.org/10.7554/eLife.109871.1.sa2

Reviewer #3 (Public review):

Summary:

The authors tested the hypothesis that interactions among size- and age-matched rivals will lead to the emergence of social roles, accompanied by divergence in four aspects of individual phenotypes: growth, feeding behavior, fighting behaviors, and gene expression in clownfish.

Strengths:

The data on growth, feeding rate, and fighting behaviors support the authors' claims.

Weaknesses:

Gene analysis conducted in this study is not sufficient to clarify how the relevant genes actually regulate growth and behavior.

The information obtained from whole-body gene expression analysis is very limited. Various gene expression is associated with the regulation of fighting behaviors, food intake, growth, and metabolism, and these genes are regulated differently across tissues, even within a single individual. Gene expression analysis should be performed separately for each tissue.

Clownfish undergo sex change depending on social status and body size, as the authors mention in the manuscript. Numerous gene expressions are affected by sex change. It is unclear how this issue was addressed.

https://doi.org/10.7554/eLife.109871.1.sa1

Author Response:

> ***Public Reviews:***
>
> ***Reviewer #1 (Public review):***
>
> *Summary:*
>
> *Overall, this is an interesting and well-written manuscript on a fascinating question in a"charismatic" model system.*
>
> *Strengths:*
>
> *(1) The Introduction is concise, though it might be helpful to the non-specialist reader to learn a bit more about what is known about the social control of somatic growth across diverse species (including humans), which would help to make this work more generally interesting.*
>
> *(2) The experiment is well-designed.*
>
> *(3) The data collected are comprehensive.*
>
> *(4) The complementary analysis of both feeding and aggression/submission data with and without known social roles is a neat idea and compelling!*

Thank you for the positive feedback!

Here, we investigate phenotypic plasticity associated with the adoption of social roles in the clown anemonefish, with strategic growth being just one aspect of that plasticity. Strategic growth, also known as social control of growth, is a fascinating form of adaptive phenotypic plasticity, whereby individuals modify their growth and size in response to fine-scale changes in social conditions (Buston & Clutton-Brock, 2022). In cooperative breeding systems with high reproductive skew, particularly fishes and mammals (possibly including humans), individuals have been shown to i) increase growth/size on the acquisition of dominant status

(Dengler-Crish & Catania, 2007; Johnston et al., 2021; Thorley et al., 2018; Van Schaik & Van Hooff, 1996; Walker & McCormick, 2009), ii) increase growth/size when paired with size matched reproductive rivals (Huchard et al., 2016; Reed et al., 2019; this study), and iii) decrease growth/size to avoid conflict (Buston, 2003; Heg et al., 2004; Wong et al., 2007). While strategic growth is fascinating and clearly occurring in this study, we show coordinated changes of multiple aspects of the phenotype as fish adopt social roles. Therefore, we deliberately framed the Introduction broadly to avoid biasing the reader toward viewing growth as the sole or main driver.

> *Weaknesses:*
>
> *(1) I was surprised that the HPA/stress axis was not considered here at all. Wouldn't we expect that subordinates have increased stress axis activation, which in turn could inhibit their growth and aggressive behavior?*

We also expected to see the HPA/stress axis activated in subordinates, which is why we carried out a targeted exploration of genes known to play a role in this axis. We did not find any genes that were significantly differentially expressed. We believe that there could be two explanations for this. First, from a methodological perspective, it could be due to our use of a whole-body RNA-seq, which may have masked this signal. Alternatively, the stress axis might play a more complex role than just acting as a simple on/off switch for reduced growth. Its activation may peak when competition over size is at its highest (during week one) or, conversely, it may peak later and help maintain reduced growth once hierarchies are firmly established (particularly after the dominant individual reaches its maximum size). To understand the role of the stress axis, future studies should observe how its activation varies over time. We acknowledge that the absence of a stress-axis signal and its potential explanations were not clearly discussed in the original manuscript, in the revised version, we will address this issue.

> *(2) To what extent are growth, food intake, agonistic behavior, and/or gene expression patterns coordinated across P1 vs P2 pairs? The lack of such an analysis seems like a missed opportunity.*

We had a similar thought. Specifically, we were interested in testing the hypothesis that the final size ratio of pairs, which is indicative of the amount of conflict remaining, would predict gene expression. We examined gene expression within pairs to test for coordinated changes and repeated the analysis, accounting for the pair size ratio. In both cases, we found no clear or consistent pattern within pairs. We will consider including these figures in the Supplementary Materials document.

> *(3) What was the rationale for using whole bodies for the transcriptome analysis? Given the hypotheses, the forebrain or hypothalamus and certain other organ systems (e.g.,liver, gonads, skin, etc.) would have been obvious candidate tissues here. I realize that cost is always a consideration, but maybe a focus on the fore-/midbrain could have been prioritized.*

We decided to use whole-body samples for this initial transcriptomic analysis to capture a broad view of gene-expression differences while keeping sequencing costs and sample requirements manageable. We agree with the reviewer that future work should explore specific tissues sampled from individuals at multiple time points to disentangle transcriptomic differences across tissue types.

> *(4) Given the preceding point, why was a fold-change threshold used for assessing DEGs (supplementary Figure 3)? There is no biological justification to ever use a fold-change threshold, especially in bulk RNA-seq analysis. This is particularly true here, where wholebodies were used for RNA-seq analysis, which is a bit unusual. Relatively small cell*

> *populations (such as hypothalamic neurons that regulate growth or food intake) may show substantial gene expression variation across social types, yet will be masked by the masses of other cells in the whole body sample. However, gene expression may still vary significantly, albeit the fold-difference may be small. I therefore suggest a reanalysis that omits any fold-change threshold.*

We thank the reviewer for this important point, and agree that an arbitrary fold-change cutoff is inappropriate/unnecessary. It should be noted that this fold-change cut-off was only used in this single figure, and all other analyses used p-values from the entire dataset. We will remove the fold-change threshold cutoff and correct Supplementary Figure 3, and any corresponding text.

> *(5) Why is the analysis of color (hue, saturation) buried in the supplementary materials? Based on the hypotheses that motivated the study, color seems just as relevant as food intake, growth, and agonistic behavior, so even if the results are negative, they should be presented in the main paper.*

We agree that color can be an important social signal, so we included color measurements in our experimental design. However, after careful consideration of the color results, we decided that our experimental timing and husbandry changes introduced multiple confounding factors, preventing us from drawing confident conclusions. Specifically, our fish were ≈1 month old at the transfer from larval to experimental tanks and had already begun to deepen their orange hue, before our experiment. (In the wild, they would settle at two weeks of age, prior to the deepening of the orange hue). Once individuals attain a certain hue, it seems that color development can be halted, but not reversed. The transfer also involved changes in lighting, tank background, and diet, factors known to strongly affect coloration. Our results show a uniform shift in orange hue and saturation across social groups, suggesting that these confounding factors might have dominated changes in hue.

For transparency, we report the color data in the Supplementary Materials, but we caution against drawing any strong conclusions. In the revised manuscript, we will recommend that future work include a targeted experiment to robustly test for the effect of the adoption of social roles on coloration or the effect of coloration on the adoption of social roles.

> *(6) The Discussion is sometimes difficult to follow. The authors may want to consider including a conceptual graphic that integrates the different aspects of growth and satiety regulation, etc., into a work-in-progress model of sorts, which would also facilitate clearer hypotheses for future research.*

Thank you for flagging that parts of the Discussion are a bit difficult to follow. In the revised manuscript, we will work to improve readability of the Discussion. We also appreciate the suggestion of including a conceptual schematic. We will consider whether adding such a graphic will add value to this manuscript or future manuscripts.

> ***Reviewer #2 (Public review):***
>
> *In this manuscript, the authors test growth, behavior, and gene expression in pairs of clownfish as they establish social dominance hierarchies, examining patterns of gene expression in these pairs after dominance has been established. The authors show solid evidence that emerging dominant clownfish show increased growth, aggression, and food consumption compared to their submissive or solitary counterparts, eventually adopting distinct gene expression profiles.*
>
> *Major Comments:*
>
> *(1) The Introduction is comprehensive, but it could be condensed. Likewise, the discussion could be condensed. There is considerable redundancy between the methods, the*

> *results,and the legend in Figure 1. The authors should consolidate and remove the redundancy.*

Thank you for flagging that parts of the manuscript could be condensed, we will work on this as we revise the manuscript.

> *(2) For Figure 3, the authors are showing PC2 and PC3; why is PC1 not shown? There is so much overlap between the three groups in PC2 vs PC3; it seems unlikely that researchers could conclusively identify any individual as belonging to a group based on the expression profile. The ovals shown do not capture all the points within each of the groups, and particularly the grey S oval seems misaligned with the datapoints shown.*

We understand the concern raised by the reviewer about the overlap among points in the PCA. We have explored PC1-PC3 and found that PC2 and PC3 showed the clearest, statistically significant clustering by social position, while PC1 did not capture any variation due to social position. We have explored whether other factors might be masking differences, such as genetic relatedness, tank effects, total read count per sample, and found that none of these factors explained sample clustering. Regarding the ellipses shown around the points, they were not intended to capture all points, but rather they show the estimated 95% multivariate t-distribution for that given social group. We will make sure this is clearly explained in the figure legend, and Methods section. In addition, in the revised version, we will show PC1 and PC2, and PC1 and PC3, in the Supplements for transparency.

> *(3) The authors indicate that the 15 replicates exhibiting the greatest size difference between P1 and P2 were selected for gene profiling. Does this mean that each of the P1and P2 were pairs with each other? Have the authors tried examining the gene expression patterns in a paired manner? E.g., for the pairs that showed the greatest size differences,do they also show the greatest differences in gene expression? Do the P1s show the most extreme differences from P2s that also show the most extreme P2 differences? Perhaps lines on Figure 3A connecting datapoints from the P1 and P2 pairs would be informative.*

Yes, “15 replicates exhibiting the greatest size difference between P1 and P2 were selected for gene profiling” refers to pairs of P1 and P2, we will make sure this is clearly stated in the revised Methods. Yes, we have explored gene expression data considering the size difference between pairs, and found that it showed no clear differences in gene expression patterns (see earlier response to Reviewer #1). We will consider including these figures in the Supplementary Materials document, as well as adding a version of Figure 3A that clearly shows information on pairs, as suggested by the reviewer.

> *(4) For the specific target pathways that are up- and downregulated in the different backgrounds, I recommend that the authors include boxplots (or heatmaps) showing the actual expression values for these targets. Figure 6 shows a heatmap for appetite-related genes, and it would be great to see a similar graph for the metabolism and glycolytic genes; it would also be informative to see similar graphs for hormonal and sexual maturation pathways as well.*

We have explored genes across a broad set of metabolic pathways (glycolysis, TCA cycle, lactic fermentation, PDH complex, cholesterol biosynthesis, fatty-acid synthesis, and beta-oxidation) and show all metabolic genes that showed significant differential expression between P1, P2, and S in Figure 6. Overall, very few metabolism-associated genes were significantly differentially expressed, which is why we decided to combine appetite-regulation and metabolism-associated genes into a single figure (Figure 6). In the revised version, we will ensure that Figure 6 clearly shows the gene sets associated with appetite and metabolism.

We also examined hormonal pathways (glucocorticoid and thyroid signaling), but did not find genes in these pathways that were significantly differentially expressed. Finally, we would like to clarify that our samples consist of two-month-old juvenile individuals that are sexually immature —under ideal conditions, clown anemonefish can mature in one to two years, but they can also remain sexually immature for a decade or more (Buston & García, 2007) — which is why we did not observe distinct molecular signatures of sexual maturation. We recognize that the sentence at line 520 may be misleading, as we did not identify any gene expression signature that we could confidently associate with signs of sexual maturation. We will make sure that these are clearly stated in the revised version of the manuscript.

> *(5) Particularly given that there is a relatively small number of genes enriched in the different rank conditions, I did not understand the need to do the WGCNA module analysis. I thought that an analysis of GO terms across the dataset would have been more meaningful than the GO term analysis shown in Figure 4, which considers only genes assigned to the "brown WGCNA module". This should be simplified or clarified.*

To clarify, GO enrichment analysis does not establish correlations with traits, it only describes which functions or pathways are over-represented in a given gene set. That is why we began by using WGCNA to define gene sets (modules) that are correlated to phenotypes. Our primary rationale for WGCNA was to identify modules of co-expressed genes that show significant statistical correlation with the phenotypes of interest (social role: P1, P2, S; growth; and food intake). Pairwise differential expression analysis (Figure 3B) identified a few hundred significantly differentially expressed genes, but those tests treat genes independently and are not able to help us link coordinated changes of co-expressed genes to phenotypes of interest. Because WGCNA is blind to traits, it first identifies groups of co-expressed genes, which can help resolve gene expression patterns.

We therefore ran WGCNA on the rlog-transformed dataset to identify modules of co-expressed genes that show significant correlation with phenotypes of interests. For every module that showed such a correlation, we performed GO enrichment and carefully evaluated the resulting GO enrichment trees (see Supplementary Figs. 4–5). The brown module was highlighted in the main text because it was one of the modules with a significant correlation to growth, and its associated GO enrichment showed clear growth-related signals that were not identified in the pairwise differential expression analysis results.

> *(6) The authors say that they have identified coordinated changes in behaviors and the"underlying gene expression, leading to the emergence" of social roles. This is a little bit misleading, since the gene expression analysis occurred well after the behavioral and phenotypic differences emerged. Presumably, the hormonal and genetic shifts that actually caused the behavioral and phenotypic difference occurred during the weeks during which the experiment was underway, and earlier capture of the transcriptome would presumably reveal different patterns, and ones that would be considered more causative.The authors acknowledge this in 434-435, but it could be emphasized further.*

We appreciate the reviewer raising this point. In the updated version of the manuscript, we will revise wording to convey that food intake, agonistic behavior, size and growth, and gene expression are all changing continuously, in response to each other and in response to social feedback. An underappreciated aspect of this system (and likely many other systems) is that phenotype (including transcriptome) influences the outcome of social interactions, and the outcome of social interactions influences the phenotype (including the transcriptome). Earlier capture of the transcriptome would reveal different levels of gene expression, reflecting the state of the system at that moment in time.

> *(7) The authors have measured a number of differences between the different dominance classes of fish. All these differences were measured relative to the other classes, but in my*

> *view, the Solitary group was the closest to a baseline control. So I'm not sure that it is fair to say that "P2 and S individuals showed consistent downregulation of these genes and pathways" (line 401). I encourage the authors to emphasize the differences in gene expression from the "perspective" of the P1 individuals compared to the baseline of P2and S individuals. Line 474 says that "P2 fish showed significant upregulation" of a number of pathways. It should be very clear what that is compared to (compared to P1, presumably?)*

We agree with the reviewer that solitary individuals are the most intuitive baseline. Indeed, the experimental design included solitary fish because we expected they would serve as a useful control. Without social restraint, we anticipated they would show unrestricted growth, feeding, behavior, and associated gene-expression patterns, similar to dominants.

We initially ran analyses using solitaries as the baseline, but after examining the results, which showed subordinate-like characteristics for the solitary individuals, we concluded that solitary individuals are not an ecologically appropriate control for this context. Removing juveniles from a social context and housing them in isolation may be stressful and can affect physiology and behavior in ways that do not reflect a natural baseline. From a life-history standpoint, solitary living is not the typical state for *A. percula*.

For these reasons, we reanalysed the dataset using the dominant (P1) as the reference to enable more ecologically meaningful comparisons (this choice was somewhat arbitrary, subordinates could also have been used as the reference). Given that gene expression is relative, we interpret results from both the dominant (P1) and subordinate (P2) perspectives in the Discussion to provide a complete view. We will clarify wording throughout the manuscript to make it clear that everything is relative (e.g., revising Line 474).

> *(8) Along the same lines, the authors say in line 514 that subordinates and solitaries strategically downregulate their growth. I'm not convinced that this is the case: I would consider this growth trajectory to be the default and the baseline. I would interpret that under certain social conditions, a P1 dominant pattern of growth, behavior, and gene expression is allowed to emerge.*

We respectfully disagree with the idea that a single baseline/reference growth trajectory exists for any individual of this species. Growth of individuals is entirely social context-dependent: neither fast nor slow growth represents an inherent baseline. When two size-matched juveniles meet and compete to establish dominance, accelerated growth is the expected trajectory. By contrast, juveniles joining an existing hierarchy are expected to exhibit reduced growth, which minimizes conflict and facilitates their social integration. Unlike species that show non socially mediated growth trajectories, clown anemonefish do not have a context-independent growth rate, rather, individuals constantly readjust their growth according to their immediate social environment.

Therefore, growth trajectories must be considered from the perspective of all group members, because they emerge from interactions among individuals rather than reflecting an intrinsic baseline. In this study, we were interested in the establishment of dominance hierarchy and how individuals adjust their phenotypes during this process. By experimentally pairing size-matched rivals, both individuals are initially expected to pursue the dominant trajectory, and thus neither individual represents a default state. Instead, the outcome reflects a social decision, after which both individuals reinforce their emerging social roles through coordinated changes.

> ***Reviewer #3 (Public review):***
>
> *Summary:*

> *The authors tested the hypothesis that interactions among size- and age-matched rivals will lead to the emergence of social roles, accompanied by divergence in four aspects of individual phenotypes: growth, feeding behavior, fighting behaviors, and gene expression in clownfish.*
>
> *Strengths:*
>
> *The data on growth, feeding rate, and fighting behaviors support the authors' claims.*

Thank you for the positive feedback!

> *Weaknesses:*
>
> *Gene analysis conducted in this study is not sufficient to clarify how the relevant genes actually regulate growth and behavior.*
>
> *The information obtained from whole-body gene expression analysis is very limited.Various gene expression is associated with the regulation of fighting behaviors, food intake, growth, and metabolism, and these genes are regulated differently across tissues,even within a single individual. Gene expression analysis should be performed separately for each tissue.*

We understand the reviewer's concern about whole-body transcriptomes and agree that tissue-specific sampling would provide greater resolution of the mechanisms linking gene expression to growth, agonistic behaviors, and food intake. For this initial study, however, we deliberately chose whole-body samples to capture a broad, unbiased view of gene expression differences while keeping sequencing costs and sample requirements manageable. We explicitly acknowledge the resulting interpretational limits in the Discussion (lines 464; 529–533), and suggest in the last paragraph that the patterns reported here should be used to build on in future studies exploring targeted, tissue-specific hypotheses.

> *Clownfish undergo sex change depending on social status and body size, as the authors mention in the manuscript. Numerous gene expressions are affected by sex change. It is unclear how this issue was addressed.*

We thank the reviewer for raising this point. Sex change and sexual maturation can indeed drive major transcriptional shifts in clown anemonefish, but our experiment did not encompass such a life-history transition. All individuals in this experiment were juveniles (≈1 month old at the start, ≈2 months old at the end) and were sexually immature at these ages. Clown anemonefish reach sexual maturation around one to two years under ideal conditions, can delay sexual maturation for years under normal conditions (Buston & García, 2007), and sex change in the genus Amphiprion is known to take over ~5 months (Moyer & Nakazono, 1978). Accordingly, individuals in this study were not sexually mature, and sex change was not biologically plausible over the five-week experimental period of our study. We recognize that the sentence at line 520 may be misleading, as we did not identify any gene expression signature that we could confidently associate with signs of sexual maturation. We will make sure that it is clearly stated that the fish in this study were sexually immature in the revised version.

References:

Buston, P. (2003). Forcible eviction and prevention of recruitment in the clown anemonefish. Behavioral Ecology, 14(4), 576–582. *https://doi.org/10.1093/beheco/arg036*

Buston, P. M., & García, M. B. (2007). An extraordinary life span estimate for the clown anemonefish Amphiprion percula. Journal of Fish Biology, 70(6), 1710–1719. *https://doi.org/10.1111/j.1095-8649.2007.01445.x*

Buston, P., & Clutton-Brock, Tim. (2022). Strategic growth in social vertebrates (WITH REVIEWER COMMENTS). Trends in Ecology & Evolution, 37(8), 694–705. *https://doi.org/10.1016/j.tree.2022.03.010*

Dengler-Crish, C. M., & Catania, K. C. (2007). Phenotypic plasticity in female naked mole-rats after removal from reproductive suppression. THE JOURNAL OF EXPERIMENTAL BIOLOGY.

Heg, D, Bender, N, & Hamilton, I. (2004). Strategic growth decisions in helper cichlids. Proceedings of the Royal Society of London. Series B: Biological Sciences, 271(suppl_6). *https://doi.org/10.1098/rsbl.2004.0232*

Huchard, E, English, S, Bell, M B. V., Thavarajah, N, & Clutton-Brock, T. (2016). Competitive growth in a cooperative mammal. Nature, 533(7604), 532–534. *https://doi.org/10.1038/nature17986*

Johnston, R A., Vullioud, P, Thorley, J, Kirveslahti, H., Shen, L., Mukherjee, S., Karner, C. M., Clutton-Brock, T, & Tung, J (2021). Morphological and genomic shifts in mole-rat 'queens' increase fecundity but reduce skeletal integrity. eLife, 10, e65760. *https://doi.org/10.7554/eLife.65760*

Moyer, J. T., & Nakazono, A. (1978). Protandrous Hermaphroditism in Six Species of the Anemonefish Genus Amphiprion in Japan (No. 2). The Ichthyological Society of Japan. *https://doi.org/10.11369/jji1950.25.101*

Reed, C., Branconi, R., Majoris, J., Johnson, C., & Buston, P. (2019). Competitive growth in a social fish. Biology Letters, 15(2), 20180737. *https://doi.org/10.1098/rsbl.2018.0737*

Thorley, J, Katlein, N, Goddard, K, Zöttl, M, & Clutton-Brock, T. (2018). Reproduction triggers adaptive increases in body size in female mole-rats. Proceedings of the Royal Society B: Biological Sciences, 285(1880), 20180897. *https://doi.org/10.1098/rspb.2018.0897*

Van Schaik, C P., & Van Hooff, J A. R. A. M. (1996). Toward an understanding of the orangutan's social system. In Linda F. Marchant, Toshisada Nishida, & William C. McGrew (Eds.), Great Ape Societies (pp. 3–15). Cambridge University Press. *https://doi.org/10.1017/CBO9780511752414.003*

Walker, S P. W., & McCormick, M I. (2009). Sexual selection explains sex-specific growth plasticity and positive allometry for sexual size dimorphism in a reef fish. Proceedings of the Royal Society B: Biological Sciences, 276(1671), 3335–3343. *https://doi.org/10.1098/rspb.2009.0767*

Wong, M. Y. L., Buston, P. M., Munday, Philip L., & Jones, Geoffrey P. (2007). The threat of punishment enforces peaceful cooperation and stabilizes queues in a coral-reef fish. Proceedings of the Royal Society B: Biological Sciences, 274(1613), 1093–1099. *https://doi.org/10.1098/rspb.2006.0284*

https://doi.org/10.7554/eLife.109871.1.sa0