Dominant convict cichlids (*Amatitlania nigrofasciata*) grow faster than subordinates when fed an equal ration

Short title: Growth rate of dominant and subordinate cichlids

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Summary

Previous studies indicate that dominant fish grow faster than subordinate fish when fed equal rations. It is unclear, however, whether this growth differential is caused by intrinsic differences related to their propensity to become dominant, or by the extrinsic effect of the social stress experienced by subordinates. We first tested whether dominant convict cichlids (*Amatitlania nigrofasciata*) grew faster than subordinates when fed an equal amount of food. Second, we tested whether the growth advantage of dominants occurred when only visual interactions were allowed between pairs of fish. Third, we randomly assigned social status to the fish to rule out the possibility that intrinsic differences between fish were responsible for both the establishment of dominance and the growth differences. In three separate experiments, dominant fish grew faster than size-matched subordinate convict cichlids, but the growth advantage of dominants was higher when there were direct interactions between fish compared to only visual interactions. Our results provide strong support for the hypothesis that the slower growth rate of subordinate fish was due to the physiological costs of stress.

Keywords: social status, growth rate, stress, convict cichlids, *Amatitlania nigrofasciata*

Introduction

The growth rate of individual animals is affected by both extrinsic factors, such as food intake and ambient temperature (Weatherley & Gill, 1987; Scanes, 2003), and intrinsic factors, such as aggressiveness and metabolic rate (Metcalfe et al., 1992; Riebli et al., 2011). In cohorts of animals, growth rate differences result in growth depensation, the increase in the variance of body size within a cohort over time (Magnuson, 1962) in a wide variety of taxa (Łomnicki, 1988). Growth depensation is of particular interest for the aquaculture industry, in which the goal is to maximize growth rate of fish while minimizing differences in body size between individuals (Thorpe & Huntingford, 1992).

Growth depensation in fishes is thought to be primarily related to differences in food intake (Rubenstein, 1981; Koebele, 1985). For example, when food is presented in an economically defendable manner, dominants tend to monopolize a large share of the resource, leading to large growth rate differences within groups (Magnuson, 1962; Noël et al., 2005). However, food intake alone cannot explain growth depensation, because dominant fish grow faster than subordinates, even when fed the same amount of food (Abbott & Dill 1989; Earley et al., 2004). The lower growth rate of subordinate fish may be due to the physiological stress of being forced to interact with a dominant individual (Filby et al., 2010). Indeed, the presence of a conspecific increases the metabolic rate, decreases the food conversion efficiency and decreases the growth rate of a focal fish (Wirtz, 1975; Wirtz & Davenport, 1976; Earley et al., 2004; Millidine et al., 2009a). Hence, dominance status is a potentially important extrinsic factor affecting growth rate.

Because previous studies allowed size-matched fish to establish their dominance status at the beginning of feeding trials (Abbott & Dill, 1989; Earley et al., 2004), it is possible that intrinsic differences between fish might have been related to both the probability of becoming dominant and to growing faster. Fish that become dominant in laboratory conditions tend to have higher intrinsic rates of metabolism, the capacity for growth, and the ability to process meals (Metcalfe et al., 1992, 1995; Millidine et al., 2009a,b). Hence, the growth differential observed between dominants and subordinates may have been caused by any of these intrinsic differences rather than dominance status *per se.*

The goal of our study was to test for the extrinsic effect of dominance status on growth rate while controlling for intrinsic factors that might be related to the propensity to become dominant. Because we were unaware of any test of Abbott & Dill's (1989) study, our first objective was to replicate their finding that dominants grow faster than subordinates when fed an equal ration. Convict cichlids (*Amatitlania nigrofasciata*) are an ideal species for this objective because they readily establish dominance relationships in laboratory conditions (Keeley & Grant, 1993; Koops & Grant, 1993), and subordinate individuals experience social stress that negatively affects growth (Praw & Grant, 1999; Earley et al., 2004). We then extended their study in two important ways. First, we tested whether the observed growth rate difference between dominants and subordinates persisted when the two fish were only in visual contact. Second, we randomly assigned dominance status by pairing focal fish with larger or smaller conspecifics, respectively, to control for any intrinsic differences that might be correlated with the establishment of dominance. Specifically, we tested the following predictions: dominants grow faster than subordinate convict cichlids when fed equal rations and (1) allowed to interact freely, (2) allowed to interact only visually; and (3) dominance status was randomly assigned. Furthermore, we expected the growth advantage of dominants: (4) to be greater when direct interactions were possible compared to only visual interactions; and (5) to be similar whether or not dominance status was randomly assigned, if the growth advantage was due to the social stress experienced by subordinate fish, but (6) to be greater when dominance status was determined by the animals themselves rather than randomly, if the growth advantage was due to intrinsic differences between fish.

Methods

Subjects

The test fish, likely *A. nigrofasciata* (sensu Schmitter-Soto 2007), originated from the laboratory stock at Concordia University. We used juveniles to maximize growth rate and to minimize any reproductive behavior. Fish were held in 110-l stock tanks on a 12:12 light:dark cycle with the lights on at 7am. Experimental tanks, measuring 40.6 x 20.3 x 26cm (l x w x h), were filled with natural-coloured gravel to a depth of 2cm and dechlorinated tap water, which was maintained from 25-27ºC. An air stone in each tank provided aeration. Three sides of each experimental tank were covered with opaque plastic to prevent fish from viewing the adjacent experimental tanks; the front was left uncovered to facilitate observations.

Experiment 1: physical interactions between fish

All 22 fish (mean = 0.339 g; see below) came from a single brood in a single stock tank, so that all fish were of a similar age and social experience. Fish were weighed to the nearest 0.001g on an electronic balance and held individually in a holding tank, identical to the experimental tanks, until a size-matched individual (<8% difference in weight) was found. To facilitate individual recognition, each fish of the 11 pairs was given a caudal fin clip, either the top or bottom corner of the fin, which was determined randomly. By the end of the 7-day trial, the clipped fin had almost entirely regrown in many cases. The pair of fish was transferred to an experimental tank within minutes of each other to avoid any prior-residency effects.

Each pair of fish was monitored frequently on the day of introduction to the tank (day 1) for signs of the establishment of dominance. An individual was defined as being dominant following three or more chases within a 3-min period, without retaliation by the other fish. The latency to establish dominance varied widely among pairs, from just a few minutes after introduction to the tank to several hours (see Koops & Grant, 1993). However, dominance status was always established on day 1, and was consistent for the duration of the trial. The dominant fish typically swam freely around the tank, whereas the subordinate fish remained in a corner of the tank near the substrate.

Fish were fed once per day beginning on day 2 for seven days, sufficient time to detect growth in juvenile convict cichlids (Praw & Grant 1999; Breau & Grant 2002). A central, removable, opaque divider was inserted in the tank to separate the two fish, and prevent visual contact during feeding. The fish were fed one pellet (Vigor #4, Corey Feed Mills) at a time with a plastic medicine dropper, in an alternating fashion until one fish stopped eating. The uneaten pellets were removed from the tank and the number of pellets eaten by each fish was recorded. The divider was removed after the daily feeding to allow the fish to interact. If one fish ate one fewer pellet on a given day, it would be fed first the next day to ensure that an equal number of pellets were consumed by both members of the pair over the trial. On average, each member of a pair ate 79 pellets, with different pairs eating between 39 and 111pellets over the 7-day feeing trial. The fish were weighed on day 9, 24 hours after the last feeding, to allow for the digestion of food.

Experiment 2: visual interactions only

The protocol for this experiment was identical to Experiment 1, except for the following changes. After the establishment of dominance on day 1, a central, clear divider was used to separate the two fish for the remainder of the trial. The fish could see each other and often interacted through the divider. To ensure that both halves of the tank were identical, we placed a heater and an airstone in each half. We also switched the type of food used from trout pellets to frozen brine shrimp, after noticing that the fish occasionally took up to 1 minute to handle a pellet in Experiment 1. An opaque divider was placed beside the clear divider to prevent visual interactions during the daily feeding, as in Experiment 1. On average, each member of a pair ate 52 shrimp (range = 30-82) over the 7-day trial. Ten pairs of fish were tested (mean = 0.261 g; see below).

Experiment 3: dominance status randomly assigned

The protocol was identical to Experiment 1 with one major difference. Instead of allowing the fish in a size-matched pair to establish dominance, the dominant role was randomly assigned to one fish by placing it in a tank with a fish 25-50% smaller in weight. The other fish in the size-matched pair was assigned the subordinate role by placing it in a separate tank with a fish 25-

50% larger. As in Experiment 1, the two fish in the same tank established their social status on day 1, typically within 1 hour of being placed in the tank. Opaque barriers were used to ensure that each fish in the same tank ate approximately the same amount of food; the smaller fish typically ate less than the dominant because of size constraints. The size-matched dominant and subordinate fish were in separate tanks and did not see each other during the trial, but were fed the same number of food items over the 7-day trial. Because the two fish within a tank differed considerably in size, we added an artificial plant to each tank to provide a hiding place for the smaller fish. On average each member of the size-matched pair ate 69 shrimp (range = 55-95) during the 7-day trial. Ten pairs of fish were tested (mean = 0.487 g; see below).

Results

In Experiment 1, with physical interactions between fish, the dominant (mean ± SD = 0.343 ± 0.078) was initially larger than the subordinate fish (0.335 ± 0.068) in 9 of 11 pairs (0.011 g ± 0.010; paired *t*-test, $t_{10} = 1.83$, $p = 0.099$; all tests are 2-tailed). After 7 days of feeding , dominants gained more weight than subordinate fish in 10 of 11 pairs (Figure 1; paired *t*-test, $t_{10} = 4.51$, $p = 0.002$).

In Experiment 2, with only visual interactions between fish, dominants (0.263 ± 0.048) were initially larger than subordinate fish (0.259 ± 0.047) in 6 of 10 pairs (0.004 ± 0.011; paired *t*-test, $t_9 = 1.278$, $p = 0.234$). After 7 days of feeding, dominants gained more weight than subordinate fish in 9 of 10 trials (Figure 1; paired *t*-test, $t_9 = 2.912$, $p = 0.020$).

In Experiment 3, with no interactions between the size-matched fish, all focal fish adopted the social status to which they were assigned. Because status was randomly assigned, dominants (0.482g ± 0.077) were initially larger than subordinate fish (0.491g ± 0.082) in only 4 of 10 pairs (-0.009 ±0.027; paired *t*-test, t_9 = -1.117, p = 0.294). After 7 days of feeding while interacting with either a larger or smaller fish, dominants gained more weight than subordinate fish in 9 of 10 trials (Figure 1; paired *t*-test, t_9 = 3.708, p = 0.0066).

The relative growth rate of the dominant compared to the subordinate fish differed between the three experiments (Figure 1; one-way ANOVA: $F_{2,28}$ = 3.67, p = 0.038). As predicted, dominants gained relatively more weight than subordinates in Experiment 1 and 3, when the two fish could interact throughout the 7-day trial, than in Experiment 2, where the two fish were separated by a clear divider (planned contrast for unequal variances: t_{28} = 3.406, DF adjusted for unequal variances = 24, p = 0.002). However, the growth advantage experienced by the dominant fish did not differ between experiment 1 and 3 (planned contrast for equal variances: t_{28} = 0.61, p = 0.545).

Discussion

Our results provided strong support for Abbott & Dill's (1989) findings for juvenile steelhead trout (*Oncorhynchus mykiss*). Dominants gained 107, 57, and 105% more weight than subordinate convict cichlids in the three experiments, respectively, compared to an 11.5% difference for steelhead trout. The greater growth differential in our study was even more striking, considering that our trials lasted only 7 days, compared to the 34 days of Abbott & Dill (1989). If physiological stress is responsible for the slower growth of the subordinate fish, then these results suggest that the dominance relationships in cichlids were more intense than in steelhead (e.g. Sloman et al., 2000). A potential method to quantify differences in stress levels of our pairs of fish would be to measure the cortisol levels released by individual fish when held in small aquaria (Wong et al., 2008).

Experiment 2 indicated that the lingering effects of the establishment of dominance plus the continuing visual interactions between fish were sufficient to cause dominants to grow faster than subordinates. These findings are consistent with previous findings indicating that the mere sight of a larger or dominant conspecific is sufficient to increase the metabolic rate of the smaller fish (Wirtz & Davenport, 1976; Millidine et al., 2000a; Sloman et al., 2000). Not surprisingly, the growth differential was greater when direct interactions between fish occurred compared to when only visual interactions were permitted. The results of Experiment 3 were consistent with the hypothesis that the lower growth rate of subordinate individuals was due to the extrinsic effect of social status (e.g. Earley et al. 2004; Filby et al., 2010), rather than intrinsic differences between the fish (e.g. McGhee & Travis, 2010). Comparisons of the growth differential between experiments need to be interpreted with caution, however, because of the different food types used in our three Experiments.

Our results may have implications for the animal husbandry and aquaculture industries. If direct interactions with, or the sight of, dominants causes stress in subordinates, then adding structure to the rearing environment might reduce the frequency of aggressive interactions (Carfagnini et al., 2009; Barley & Coleman, 2010). Furthermore, the addition of structure tends to make aggressive behaviour less economical as a competitive strategy (Höjesjö et al., 2004), and reduces the variance in food intake within groups (Basquill & Grant, 1998). The negative effects of the dominant individual on subordinates in the group can also be diluted by increasing the group size or density (e.g. Kim & Grant, 2007).

The convict cichlid is an interesting model species for studying the interactions between body size, dominance status and growth rate. As in previous studies (Keeley & Grant, 1993; Earley et al. 2004), dominance status was related to body size, even within size-matched pairs; dominant fish were larger in 16 of 21 pairs in Experiments 1 and 2 (Sign Test, $p = 0.027$). In competitive feeding experiments, food intake is the best predictor of growth rate (Praw & Grant, 1999), which leads to growth depensation, particularly when food is economically defendable (Noël et al., 2005). In addition to our findings, which indicate a stress cost to being subordinate, circumstantial evidence suggests a cost of aggression and of being dominant. Mean growth rate in groups of convict cichlids decreases with increasing rates of aggression (Noël et al., 2005), and the growth rate of dominants decreases with the number of intruders on its territory (Praw & Grant, 1999).

While numerous studies on fishes focus on the physiological costs of being subordinate (see Gilmour et al., 2005), fewer studies have investigated the costs of being dominant (but see Noakes & Leatherland, 1977; Riebli et al. 2011). By contrast, the physiological costs of being both dominant and subordinate have been investigated more in the mammalian literature (e.g. Sands & Creel, 2004). Future studies should focus on the costs of dominance in fishes, and on the differential costs of social status in more natural circumstances (e.g. Sloman et al., 2008).

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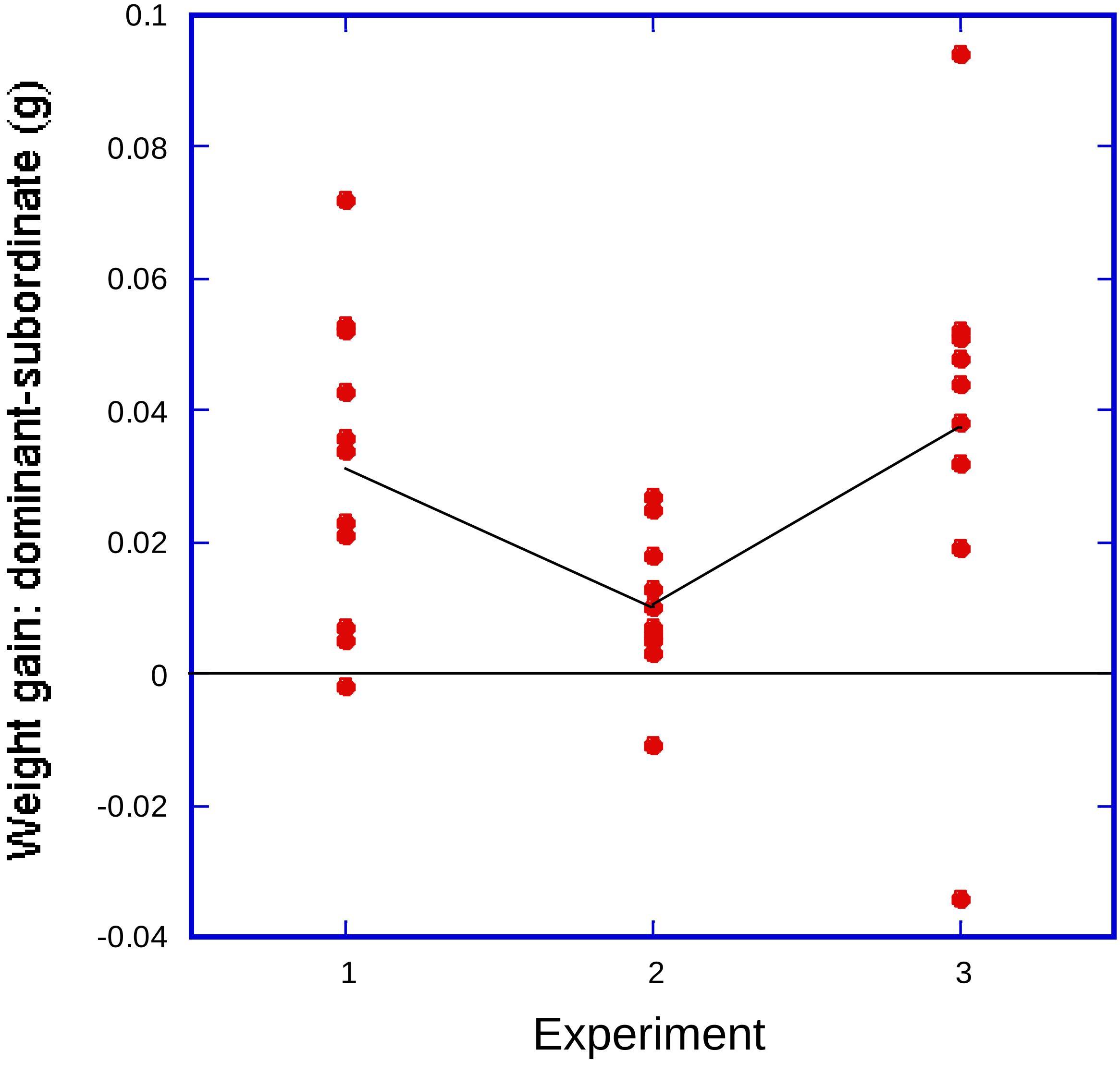
Legend for figure

Figure 1. Difference in weight gain between pairs of dominant and subordinate convict cichlids when fed equal rations in three experiments: (1) when allowed to interact freely (n = 11); (2) when allowed to interact only visually (n = 10); and (3) when the dominant and subordinate status were randomly assigned (n = 10).


Weight gain: dominant-subordinate (g)
0.1
0.08
0.06
0.04
0.02
0
-0.02
-0.04
1
2
3
Experiment