Journal of Fish Biology (2004) **65,** 1080–1095
doi:10.1111/j.1095-8649.2004.00517.x, available online at http://www.blackwell-synergy.com

The physiological effects of social status in the cooperatively breeding cichlid *Neolamprologus pulcher*

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(Received 27 June 2003, Accepted 7 July 2004)

The physiological effects of social rank were examined in three different experiments with *Neolamprologus pulcher* a cooperatively breeding cichlid, endemic to Lake Tanganyika, East Africa. The effects of rank on physiology between pairs of dominant and subordinate size-matched fish (experiment 1) and among groups of four size-matched fish (experiment 2) were examined. A third experiment mimicked the natural social structure in the wild; pairs were observed with other group members including breeders. The effect of social position was investigated on growth rates, liver concentrations of adenosine triphospate (ATP), lipids, proteins, creatine phosphate (CrP), glucose and glycogen as well as plasma cortisol. In naturalistic group settings, dominants displayed higher levels of liver protein and plasma cortisol. In the absence of breeders, dominant individuals (of helper pairs) had higher liver glycogen levels and dominant fish (held in groups of four) grew most. These results support previous cooperatively breeding mammal studies and suggest that dominant individuals experience higher cortisol levels as well as higher growth rates.

Key words: dominance; energy; growth; helpers; *Neolamprologus pulcher*.

INTRODUCTION

Dominance hierarchies are most commonly seen in permanent or semi-permanent social groups (Drickamer *et al.*, 1996) and are believed to reduce the amount of severe aggression among members of a group (Krebs & Davies, 1997). While these social hierarchies may benefit group stability, many physiological costs and benefits have been associated with the social position of individuals. The conventional view is that social subordination is stressful. The physiological costs to subordinate animals have been described for many mammalian species and have also been demonstrated in fishes (Sloman & Armstrong, 2002). While physiological correlates of social rank have been studied in a small number of non-salmonid species of fishes (Fox *et al.*, 1997; Winberg *et al.*, 1997; Hofmann *et al.*, 1999; Hofmann & Fernald, 2001), no study to date has considered the physiological effects of social status in a cooperatively breeding species of fish with a long-term stable social hierarchy.

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Cooperative breeding typically increases the level of social complexity in a group and the mammalian literature suggests that the costs and benefits of social rank in cooperatively breeding species can be considerably different to those of non-cooperatively breeding species (Creel *et al.*, 1996, 1997; Creel, 2001; Creel & Sands, 2002). The aim of this study was to examine the physiological costs and benefits of social rank among group members in a cooperatively breeding species of fish with a long-term social hierarchy.

Physiological costs in fishes are most commonly associated with subordinance. Lower growth rates (Li & Brocksen, 1977; Fausch, 1984; Abbott & Dill, 1989; Sloman *et al.*, 2000), elevated basal plasma cortisol concentrations (Pottinger & Pickering, 1992; Fox *et al.*, 1997; Øverli *et al.*, 1999*a*; Sloman *et al.*, 2001*a*, *b*) and reduced resistance to disease (Peters *et al.*, 1988) are some examples of the physiological costs demonstrated in subordinate fishes. Elevation of plasma cortisol in subordinates is generally a chronic increase of basal cortisol levels, which can be costly, as it may lead to changes in metabolism and has implications for immune system function, growth and reproduction (Pickering & Pottinger, 1995; Abbott *et al.*, 2003). Physiological costs, however, have also been associated with dominance. Taborsky (1982) studied the African cichlid, *Lamprologus brichardi* Poll and suggests that aggressive acts may be more costly than submissive ones and in some circumstances dominance may be associated with a higher metabolic rate (Cutts *et al.*, 1998).

Social status can therefore incur physiological costs and, particularly in the mammalian literature, it has been demonstrated that these costs can vary with hierarchy stability. Studies on Kenyan baboons *Papio anubis* show that when dominance hierarchies are unstable (*i.e.* when the dominant baboons are being challenged by lower ranking males) the dominant baboons have higher cortisol concentrations in comparison to the subordinates (Drickamer *et al.*, 1996). The reverse, however, was true when dominance hierarchies were stable. In a recent meta-analysis study of cortisol levels in relation to ranks in primates, Abbott *et al.* (2003) found that subordinates will typically show higher cortisol levels when subject to high rates of physical or psychological stressors and decreased opportunities for social support.

To examine the physiological costs and benefits of social status in a cooperative breeder, the African cichlid, *Neolamprologus pulcher* (Trewavas & Poll) was used. *Neolamprologus pulcher* (formally known as *Lamprologus pulcher*) lives in social groups in the southern basin of Lake Tanganyika and is a sub-species or sister species of *Neolamprologus brichardi* (Taborsky & Grantner, 1998). These social groups typically consist of two breeders (a male and female), and one to 14 helpers (five on average) (Balshine *et al.*, 2001) which share territorial responsibilities with the breeding pair and aid in direct brood care (Grantner & Taborsky, 1998; Taborsky & Grantner, 1998). Like other cooperatively breeding animals such as Florida scrub jays, dwarf mongooses and meerkats (Woolfenden & Fitzpatrick, 1990; Creel & Waser, 1994; Clutton-Brock *et al.*, 1999), helpers within the group are subordinate to the breeding pair but dominance hierarchies also form among helpers.

The physiological correlates of social status were explored by placing *N. pulcher* in three different experimental conditions. First, two size-matched individuals were placed together in a tank and allowed to form a short-term (48 h) dominance hierarchy. Second, four size-matched individuals were selected and allowed to set up a long-term stable dominance hierarchy. Finally, two individuals were examined within their natural social groups in the presence of a breeding pair thus closely mimicking their natural social environments. Individuals were first measured and then observed regularly during the experiments to ascertain rank assignment. Finally, individuals were killed and the levels of cortisol, adenosine triphosphate (ATP), creatine phosphate (CrP; alternatively known as phosphocreatine), protein, lipids and glucose were measured and related to their social status. In this study, the obvious physiological differences between breeders and helpers were ignored and instead the subtle differences between size-matched group members were focused on. These size-matched *N. pulcher* helpers naturally form a dominance hierarchy that in the wild represents a queue for breeding opportunities (Balshine-Earn *et al.*, 1998).

The concentrations of various chemical compounds in the liver were examined as it is a major storage organ in fishes. In particular, liver proteins, lipids, glycogen, glucose, ATP and CrP were measured. Glycogen is a storage compound in fishes and is probably the first storage compound to be broken down when an animal is faced with stress. ATP and CrP are both chemical compounds that contain a high-energy phosphate bond which can be broken to create energy. The combined amounts of cell ATP and phosphocreatine are known as the phosphagen energy system. These compounds are synthesized from proteins, carbohydrates and fats providing the vital link between these nutrients and energy production. Compared to mammals, glucose turnover in fishes is low and carbohydrates are not the primary fuel. Instead, teleosts rely primarily on protein and lipids for energy (Van der Boon *et al.*, 1991). Also examined was plasma cortisol which is involved in some aspects of intermediary metabolism, exerting a variety of effects on protein, carbohydrate and lipid metabolism (Van der Boon *et al.*, 1991). Based on previous studies of other teleosts, it was predicted that subordinate fish would display elevated plasma cortisol concentrations in conjunction with decreases in liver energy storage compounds.

MATERIALS AND METHODS

Three experiments were conducted between September 2001 and May 2002. The three experiments shared many methodological similarities. *Neolamprologus pulcher* (2·9–5·5 cm standard length, L_S) were used in all three experiments. All fish sampled in these experiments were F1s originating from wild caught breeders from Lake Tanganyika along the coastline of northern Zambia. Before each experiment, all fish of interest, focal fish, were measured (L_S to the nearest mm and body mass to the nearest 0·01 g) and marked (either by injection of non-toxic acrylic paint or by fin clips) and allowed a minimum of 24 h to recover from marking. No adverse effects of the marking procedure were seen and all fish recovered quickly. The tank size varied between experiments but all tanks contained *c.* 25 mm thick layer of coral sand as substratum, two plant pot halves as shelters, filters, a heater and a thermometer. The photoperiod was 14L : 10D and the water temperature was maintained at 25–27° C. The sides and back of the tanks were covered with blue adhesive casing, which minimized reflection and prevented interference between fish in neighbouring tanks. During the experiments fish were fed twice daily (morning, 0900–1100 hours and afternoon, 1600–1800 hours) and food was distributed randomly across the water surface of each tank. Each tank received a mixture of brine shrimp and blood worms three mornings per week and cichlid flake food for the remaining feeding times.

EXPERIMENT 1: MATCHED PAIRS

In this experiment, six pairs of size-matched helpers (target pairs, *n* = 12) were selected from pre-established groups of *N. pulcher* living in 190 l tanks. The pre-established groups consisted of a breeding pair and an average of seven helpers (range: six to eight). All pairs were sexually mature and three pairs were sex matched, three were not. Target pairs were weighed, measured and marked as described above, removed from their groups and maintained as a pair without breeders or other group members in 38 l tanks for a 48 h period. The difference in size between pairs was small (mean ± S.E. difference in body mass = 0·14 ± 0·04 g, range 0·00–0·46 g and mean ± S.E. difference in L_S = 0·1 ± 0·1 cm, range 0·0–0·6 cm). Each pair was placed in a different 38 l tank, which differed from the 190 l tanks only in size. After 2 days, an observational watch was performed on the fish in these 38 l tanks and a dominance ranking between the pair was determined based on behaviour, colour and movement. The fish were then killed.

EXPERIMENT 2: FOUR MATCHED FISH

In this experiment, 40 sexually immature *N. pulcher* helpers were selected from a stock tank (*c.* 150 fish), weighed, marked and measured as above. Fish were then placed in 38 l tanks in groups of four (*n* = 10 groups; size-matched for mass and L_S). Each individual was observed five times for 10 min (totalling 50 min per fish overall) over the course of 57 days. Following the completion of observations on all fish in each tank, a dominance ranking (from one indicating most dominant to four indicating least dominant) was determined. The mean difference in body size among fish in a tank at the beginning of the experiment was small (mean ± S.E. body mass difference = 0·11 ± 0·04 g, range 0·02–0·40 g and mean ± S.E. L_S difference = 0·2 ± 0·1 cm, range 0·0–0·6 cm).

EXPERIMENT 3: MATCHED PAIRS IN NATURAL GROUPS

Twelve pairs of size-matched helpers (*n* = 24) were selected from pre-established groups of *N. pulcher* living in 190 l tanks. Again, the pre-established groups consisted of a breeding pair and an average of eight helpers (range: six to nine). All target pairs were briefly removed from their groups and weighed, marked and measured as detailed above. Throughout the experiment, each marked helper was observed three times for 10 min (totalling 30 min per fish overall) over the course of 21 days. Following each of these observation sessions, a dominance ranking was determined. The mean difference between the pairs at the beginning of the experiment was small (mean ± S.E. body mass difference = 0·29 ± 0·08 g, range 0·01–1·12 g and mean ± S.E. L_S difference = 0·16 ± 0·04 cm, range 0·0–0·5 g). Groups ranged from four to eight individuals with six as the median group size.

RANK ASSIGNMENT

As mentioned above, dominance rank of target fish was assigned using a combination of behavioural interactions among fish, fish position in the tank and fish colouration. In each experiment, the fish were observed during several 10 min observations. The exact number of focal watches varied between experiments. To habituate the fish to the observers, observers sat for 10 min prior to recording any behaviours at a distance of 1·5 m from the tank. During the observations made on each fish, dominant and submissive behaviours were observed and scored (Table I), and fish position and colouration noted. Dominance emerged quickly (it was clear by the end of one observation period). The first component of the dominance rank assignment was based on behavioural interactions between fish. Aggressive and submissive acts were classified into four groups: aggressive acts given, aggressive acts received, submissive acts given and submissive acts

TABLE I. List of aggressive and submissive behaviours observed. These behaviours follow the ethograms of Coeckelberghs (1974) and Kalas (1975)

Behaviour	Description
Swim	Slow locomotion using the pectoral fins
Still	No locomotion or movement
Aggressive behaviours	
Chase	Focal fish quickly darts towards another fish
Bite	Focal fish bites another fish
Ram	Focal fish makes contact with another fish using its head or mouth, but no obvious bite is taken (jaws remain closed)
Mouth-fighting	Focal fish and another fish lock jaws and push against one another
Pseudo mouth-fighting	Back and forth movement occurs (as if about to mouth fight), but no physical contact is established
Head shake	Fish thrashes its head from left to right repeatedly
Puffed throat	Fish ‘flares out’ its operculum and lower jaw cavity. Often associated with a posture where the head is pointed downwards
Submissive behaviours	
Submissive posture	The head of the focal fish is directed upwards and tail is downwards
Submissive display	Focal fish is positioned with a submissive posture accompanied by a quivering tail (and sometimes the entire body quivers)
Flee	Focal fish quickly swims away from another fish
Bitten	Focal fish is bitten by another fish

received. An aggressive act by one animal did not necessarily result in a submissive act by another animal and *vice versa*. Second, dominant fish moved freely around the tank and spent the most time in the 'good quality' areas (*e.g.* underneath the plant pot halves, which served as shelters), while subordinates stayed relatively still and were constrained to a small section of the tank. Third, dominants generally had brighter operculum marks and such interactions between social status and colour have been documented previously in other species, including cichlids (Magurran, 1986). Dominance was assigned by combining the scores from each of these indicators of dominance. For example, the fish giving the most aggressive displays was given a score of one, the most colourful fish was given a score of one and the fish that moved around the largest area of the tank was given a score of one. The operculum marks and movement scores were used because some pairs did not display distinct differences in the rates of aggressive displays given.

Separate dominance ranking was calculated after each observation session. Dominance ranks remained quite stable between observation periods. For example, in experiment 2, only 22 switches occurred out of 300 possible rank position switches between observation periods. One tank exhibited switches between fish in ranks one and two while all other switches occurred between fish in ranks three and four. Such stable dominance hierarchies have been seen in other fish species (Metcalfe, 1986) both in an experimental situation (Sloman *et al.*, 2000) and in their natural habitat (Bachman, 1984). To analyse the results, the final rank on the last observation session was used as the definitive one.

Animals did not appear to be in distress during the course of the experiments. In a few circumstances, a subordinate animal was immediately excluded from the experiment when it began to receive too much aggression from a dominant animal. Only three fish (all dominance ranking of four; experiment 2) needed to be removed from the study due to repeated aggression. There were no apparent behavioural effects (changes in aggression or rank order) following these removals.

Immediately following the final behavioural observation of each tank, fish were euthanized quickly by injecting a lethal dose of benzocaine ($0{\cdot}5\,\mathrm{mg\,ml^{-1}}$) into the tank. As the baseline differences in plasma cortisol related to rank were being examined, not in response to the stress of being chased and caught by the experimenters, the fish needed to be sampled quickly. All fish lost equilibrium and stopped breathing within 1 min of benzocaine addition, thus ensuring the animal endured minimal distress and that physiological samples could be taken as quickly as possible. All fish were then netted, weighed, measured (L_S and L_T), and a blood sample was taken by caudal severance. The procedure, from the time the fish were disturbed by the researchers approaching the tank to the completion of blood sampling of all fish in a tank, took <3 min to ensure that cortisol levels were not elevated during sampling. Blood was collected in heparinized capillary tubes, centrifuged at 13 000*g* for 2 min and the plasma removed, placed in capped 1·5 ml polypropylene (Eppendorf®) tubes and frozen in liquid nitrogen. Fish were then dissected and, where possible, the sex of the fish determined. Livers were removed and placed in pre-weighed Eppendorf® tubes and frozen in liquid nitrogen. All samples obtained were stored at −80° C for later analysis of ATP, CrP, glycogen, glucose, lactate, lipid and protein content in the carcass and liver tissues and cortisol concentrations in the blood plasma. The physiological variables chosen reflect those most commonly associated with stress-induced changes in physiology (Pickering & Pottinger, 1995) and have also previously been associated with social stress (Ejike & Schreck, 1980; Peters *et al.*, 1980; Fox *et al.*, 1997; Sloman *et al.*, 2000).

PHYSIOLOGICAL ANALYSES

Fish liver tissues were homogenized under liquid nitrogen. ATP, CrP and glycogen contents were determined according to the method of Bergmeyer (1983). Protein was analysed using the method of Bradford (1976) and total lipid content by the sulphophosphovanillin method (Barnes & Blackstock, 1973). Glucose and lactate concentrations were measured using Sigma diagnostic kits and cortisol was analysed by radioimmunoassay (ICN Pharmaceuticals Costa mesa, CA, U.S.A.; Gamperl *et al.*, 1994).

STATISTICAL ANALYSES

The specific growth rate (G, % change in mass per day) was calculated from $G = 100(\ln M_2 - \ln M_1)(t_2 - t_1)^{-1}$, where M_2 = mass at the end of the experiment (g), M_1 = mass at beginning (g) and $t_2 - t_1$ = duration of experiment (days). To examine whether the physiology of the fish varied among ranks, paired *t*-tests were performed between pairs of fish in experiments 1 and 3, and a one-way ANOVA followed by a Bonferroni *post hoc* test was performed on the data collected in experiment 2. All data were tested for normality prior to statistical analysis using the Kolmogorov-Smirnov test and any variables that were not normally distributed were ln transformed before statistical analysis. Two-tailed tests were used throughout. Variations in sample sizes for physiological variables were due to the loss of a few samples during analysis.

RESULTS

EXPERIMENT 1

Liver glycogen concentration was significantly higher in dominant fish than in their paired subordinates ($P = 0{\cdot}02$). Liver protein, however, was significantly higher in subordinates ($P = 0{\cdot}03$). Due to the loss of some samples during analysis of ATP and CrP, there were insuffcent data to make comparisons. There were no other statistical differences in physiological variables (Table II) and there was no effect of social status on growth (Fig. 1). There were no significant differences in the numbers of aggressive and submissive acts given [Fig. 2(a), (b)] but dominants received significantly more submissive acts [Fig. 2(c)].

EXPERIMENT 2

Growth rates differed statistically in this experiment among ranks of fish. Dominant fish (rank 1) had significantly higher G than the other three ranks of fish (Fig. 1; $P < 0{\cdot}001$). Dominant fish also had significantly higher liver protein contents than more subordinate ranks of fish but no other differences in physiology were noted (Table III).

In this experiment there were significant differences among the ranks of fish in both aggressive and submissive acts given [Fig. 2(a), $P = 0{\cdot}002$; Fig. 2(b),

TABLE II. Physiological variables measured in the liver of dominant and subordinate fish housed as size-matched pairs without breeders for 2 days (experiment 1). Plasma cortisol values are also presented. Data are given as means ± S.E.

Variable	Dominants	Subordinates	*t*	*P*
Glucose (μmol g^{-1})	13·60 ± 3·66	19·26 ± 2·92	1·612	0·168
Lipid (mg g^{-1})	0·06 ± 0·01	0·07 ± 0·008	1·623	0·180
Lactate (μmol g^{-1})	5·62 ± 1·73	5·15 ± 0·83	0·246	0·815
Glycogen (μmol g^{-1})	101·78 ± 22·87	31·46 ± 4·33	3·372	0·02*
Protein (mg g^{-1})	24·65 ± 2·92	34·67 ± 3·16	3·133	0·026*
Plasma cortisol (ng ml^{-1})	18·55 ± 1·49	17·75 ± 5·21	0·518	0·627

*, Significant.

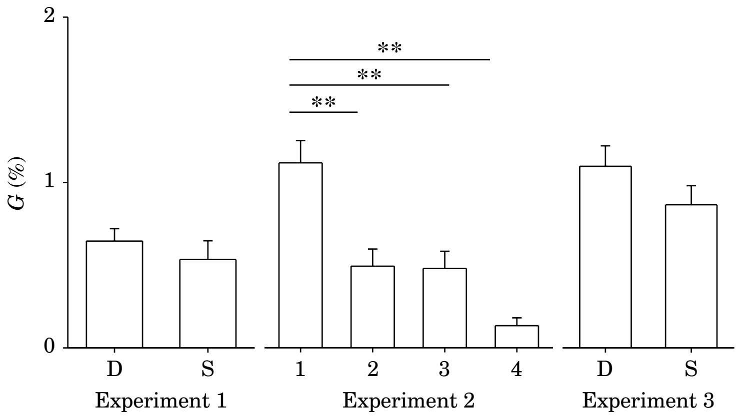

FIG. 1. Specific growth rates (as per cent change in mass per day) of dominant (D) and subordinate (S) fish (experiments 1 and 3) and ranks 1–4 (1, most dominant; 4, least dominant) (experiment 2). Paired *t*-tests were used in experiments 1 and 3 ($t = 0{\cdot}980$, $P = 0{\cdot}350$ and $t = 1{\cdot}703$, $P = 0{\cdot}187$ respectively) and a one-way ANOVA in experiment 2 ($F_{3,26} = 13{\cdot}711$, $P < 0{\cdot}001$). *Post hoc* (Bonferroni) significance levels **, $P < 0{\cdot}001$. Lines above the bars denote significance where a statistical significance occurs between the bars at which the line starts and ends. Data are means ± S.E.

$P = 0{\cdot}000$ respectively). Dominant individuals performed significantly more aggressive acts and significantly less submissive acts than more subordinate ranks of fish. There were also significant differences among ranks in the amount of submission received. Dominant fish (rank 1) received significantly more submission than other ranks of fish [Fig. 2(c); $P = 0{\cdot}005$].

EXPERIMENT 3

Dominant fish had significantly higher plasma cortisol concentrations when compared with subordinates ($P = 0{\cdot}045$). Growth rates did not differ between subordinates and dominants in this experiment (Fig. 1; $P = 0{\cdot}35$). Liver protein content, however, was found to be higher in dominants ($P = 0{\cdot}039$). No other differences in physiology variables were found (Table IV). No statistical significances were observed in the numbers of aggressive and submissive acts given or received (Fig. 2).

DISCUSSION

The present study is the first to investigate the physiological effects of social status in a cooperatively breeding fish. In 'natural' settings (experiment 3), dominant fish showed higher levels of cortisol compared to subordinates, supporting previous studies on cooperatively breeding birds and mammals. In less 'natural' settings, dominants had more liver glycogen (experiment 1, isolated pairs) or grew faster and had higher levels of liver protein (experiment 2, four isolated size-matched fish), supporting the previous results on effects of social rank on physiology in other fishes (Abbott & Dill, 1989; Sloman *et al.*, 2000, 2002, Sloman & Armstrong, 2002).

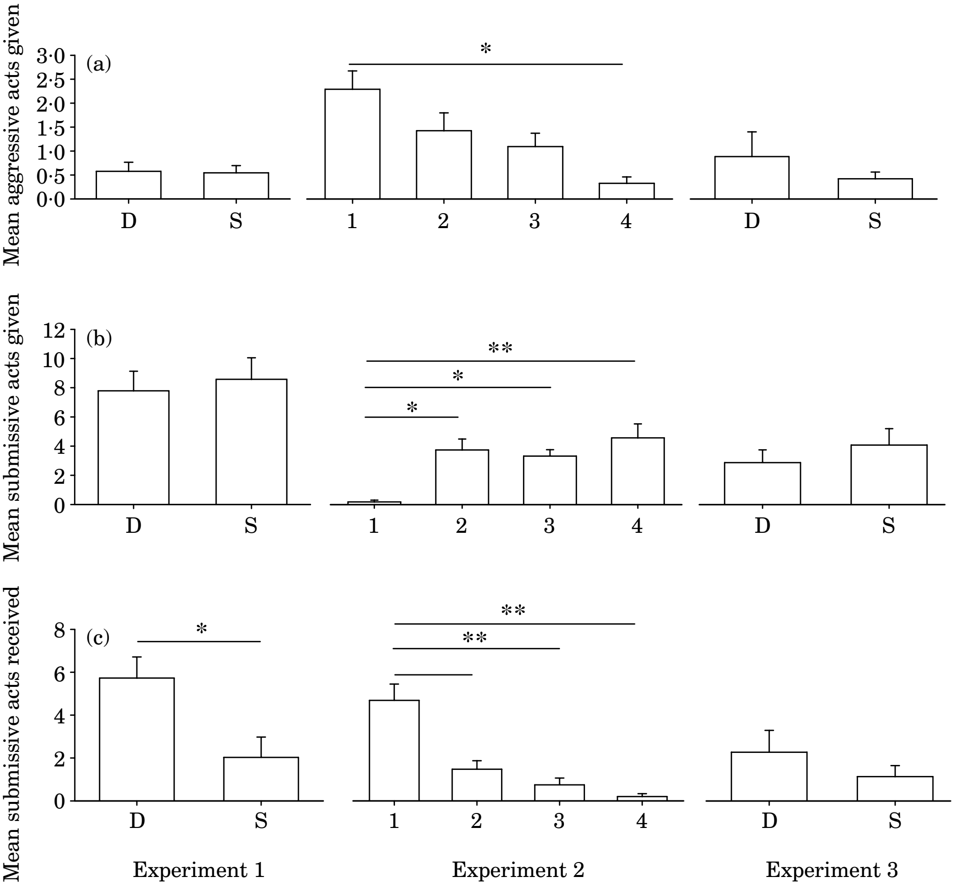

FIG. 2. (a) Mean aggressive and (b) submissive acts given and (c) submissive acts received during each behavioural observation period by dominant (D) and subordinate (S) fish (experiments 1 and 3) and ranks 1–4 (1, most dominant; 4, least dominant) (experiment 2). Paired *t*-tests were used in experiments 1 and 3; *, a significant difference. A one-way ANOVA was used in experiment 2 where *post hoc* (Bonferroni), $^{*}P < 0{\cdot}01$; $^{**}P < 0{\cdot}001$. Lines above the bars reflect significance where a statistical significance occurs between the bars at which the lines starts and ends. Data are means ± S.E.

Dominants of size-matched pairs of helpers housed together for two days (experiment 1) having higher concentrations of liver glycogen, suggests the use of energy stores by subordinates or a significant short-term physiological cost associated with subordinate status. Surprisingly, subordinates of these paired fish in experiment 1 also had higher protein concentrations. Fishes show a continual cycle of synthesis and degradation of protein (Houlihan *et al.*, 1995) and tissue concentrations of protein can vary in response to changes in the feeding success of a fish (McLaughlin *et al.*, 1995). Therefore, dominants were expected to have higher protein stores than subordinate fish that grow less and acquire less food. The response of protein concentrations may lag behind changes in food availability (McLaughlin *et al.*, 1995) and so it is likely that the results seen in these longer-term experiments are more indicative of the

TABLE III. Physiological variables measured in the livers of ranks of fish housed without breeders for 57–58 days, where rank 1 = dominant (experiment 2). Plasma cortisol concentrations are also given. Data are given as means ± S.E.

	Rank (1 = most dominant)					
Physiological variable	1	2	3	4	ANOVA	P
Glucose (μmol g^{-1})	19·33 ± 5·36	13·22 ± 3·12	8·00 ± 1·79	18·35 ± 5·56	$F_{3,20} = 1{\cdot}275$	0·31
Lipid (mg g^{-1})	0·19 ± 0·04	0·24 ± 0·05	0·22 ± 0·03	0·15 ± 0·04	$F_{3,32} = 0{\cdot}924$	0·44
ATP (μmol g^{-1})	6·93 ± 0·97	7·30 ± 1·31	13·35 ± 2·54	9·36 ± 2·02	$F_{3,31} = 2{\cdot}734$	0·06
CrP (μmol g^{-1})	37·14 ± 6·08	42·63 ± 6·08	69·98 ± 20·97	34·25 ± 4·96	$F_{3,32} = 1{\cdot}699$	0·19
Lactate (μmol g^{-1})	6·43 ± 1·51	8·01 ± 1·09	9·93 ± 1·82	6·64 ± 3·03	$F_{3,32} = 0{\cdot}814$	0·50
Glycogen (μmol g^{-1})	180·20 ± 43·75	65·96 ± 18·46	87·14 ± 21·25	141·42 ± 43·94	$F_{3,29} = 2{\cdot}594$	0·07
Protein (mg g^{-1})	169·17 ± 31·46	84·67 ± 23·66	69·68 ± 20·97	90·07 ± 30·65	$F_{3,30} = 3{\cdot}758$	0·02*
Plasma cortisol (ng ml^{-1})	38·08 ± 9·63	44·12 ± 16·77	42·7 ± 12·83	47·19 ± 17·81	$F_{3,28} = 0{\cdot}020$	0·996

*, Significantly different ($P < 0{\cdot}05$).

TABLE IV. Physiological variables measured in the livers of dominant and subordinate fish housed with breeders (experiment 3). Plasma cortisol concentrations are also given. Data are given as means ± S.E.

Physiological variable	Dominants	Subordinates	*t*	*P*
Glucose (μmol g^{-1})	9·59 ± 1·12	9·21 ± 1·23	0·103	0·92
Lipid (mg g^{-1})	0·07 ± 0·01	0·08 ± 0·01	0·99	0·35
ATP (μmol g^{-1})	4·04 ± 0·18	4·25 ± 3·25	0·067	0·95
CrP (μmol g^{-1})	1·84 ± 5·44	31·36 ± 12·14	1·172	0·29
Lactate (μmol g^{-1})	13·29 ± 3·69	24·99 ± 15·64	0·438	0·67
Glycogen (μmol g^{-1})	77·2 ± 20·69	149·23 ± 72·66	1·041	0·33
Protein (mg g^{-1})	27·75 ± 2·80	20·27 ± 1·95	2·368	0·039*
Plasma cortisol (ng ml^{-1})	43·75 ± 20·69	15·59 ± 2·68	2·369	0·045*

*, Significantly different ($P < 0{\cdot}05$).

effects of social status on liver protein. Furthermore, although this result is difficult to explain, as changes in protein stores are commonly associated with a longer-term stress than the 2 day confinement of these fish, it is likely that this result is an artifact of the prior condition of the fish.

As subordinates are typically harassed more by individuals above them in the ‘pecking order’, it was expected that subordinates would use energy stores and not grow as quickly as dominant fish. The short term nature of experiment 1 was not sufficient to study changes in growth. Growth differences were verified in experiment 2, in which dominant fish had significantly higher growth rates when compared with all other ranks of fish. The most subordinate fish grew the least. Such positive correlations between growth rate and dominance have been demonstrated in previous studies on salmonids (Abbott & Dill, 1989; Sloman *et al.*, 2000). Dominant fish in experiment 2 also performed far less submissive behaviour compared to all other ranks of fish. Here, along with higher growth rates, dominant fish had higher liver protein concentrations but no other physiological differences were detected. Perhaps dominants, in the absence of breeders, could channel all of their energy into growth as there were no other fish above them in the hierarchy (no breeder or other helper) to appease. Subordinates in this experiment grew very little, maybe because they were not buffered from aggression and competition by the presence of other helpers and breeders as in the last experiment. From the results of this study it cannot be determined whether dominance status resulted in increased growth or whether increased growth resulted in dominance. The cortisol levels in this experiment were higher than those seen in experiments 1 and 3 but fish still grew well, suggesting that the cortisol levels may not have been high enough to interfere with growth rate.

Interestingly in experiment 3, the dominance rank of a pair of fish maintained in the presence of group members (breeders and other helpers) did relate to cortisol levels. Experiment 3 was arguably the most ‘natural’ as sampled individuals were housed long-term, in family groups with breeders as they would in the wild. Interestingly, Fox *et al.* (1997) found that basal cortisol levels differ with rank but only under natural and stable social conditions once the dominance relationships are well sorted. These authors also did not detect any cortisol differences directly after the tank was set up when the social hierarchy was highly labile.

In marked contrast to Fox *et al.* (1997) and most of the previous studies utilizing other species of fishes, plasma cortisol concentrations were significantly higher in dominant fish. Previous teleost research has demonstrated that subordinate fishes tend to have higher levels of plasma cortisol associated with the stress of being the submissive individual (Peters *et al.*, 1980; Abbott & Dill, 1989; Fox *et al.*, 1997; Sloman *et al.*, 2001*a*). Hence results of this study were similar to behavioural endocrinological studies on mammalian cooperative breeders, where higher cortisol levels in dominants compared to subordinates have also been found (Creel *et al.*, 1996, 1997). Although higher cortisol levels are typically viewed as negative (a physiological cost), it is possible that such costs need to be incurred in order to improve an individual's rank and realize future reproductive success.

Neolamprologus pulcher and other cooperative breeders live in social groups with a strict dominance hierarchy (Balshine *et al.*, 2001), for most other species the dominance hierarchies are more transient. Some of the previous work on social status in which subordinate fishes are shown to have higher cortisol levels is based on placing two unfamiliar captive animals together for a short time and observing them fight (Adams & Huntingford, 1996; Øverli *et al.*, 1999*a*, *b*; Sloman *et al.*, 2001*a*). Typically, winners are called dominants, losers are called subordinates. These fairly unnatural conditions may not provide much insight as to what stress levels are likely to be experienced by subordinates that live in permanent groups. It appears as if dominant animals generally have higher stress levels among cooperative breeders. It may be that, although dominants typically enjoy access to resources or mates, they may also have to bear hidden physiological costs of stress that may influence metabolism or immune function. It may be that high quality individuals (*e.g.* larger, older and better body condition) can bear the costs that social stress places on metabolism and immune functions.

It was expected that dominants in experiments 2 and 3 would grow more than subordinates. Such growth differences could exist because: 1) dominants monopolize food or 2) subordinates are 'too stressed' to eat. In this study, excess of food was fed to each tank and this food was distributed randomly throughout the tank, hence it is unlikely that there was an insufficient amount of food for all the fish. In wild *N. pulcher*, group members typically feed in the water column in large aggregations without any overt signs of interference competition (Balshine *et al.*, 2001). Thus dominants were unlikely to directly monopolize food, however, dominants did monopolize shelter. *Neolamprologus pulcher* compete for space and dominants prevent subordinates from freely moving around the tank (Werner *et al.*, 2003). Hence subordinates may not have eaten as much due to 'psychological stress' or the perceived threat of drawing the dominant's attention and being attacked (Abbott & Dill, 1989). Subordinates moved around less and may not have eaten or searched for food as much as dominant fish. In fact, in this study only in experiment 2 did dominants grow significantly more than subordinates.

Lowered growth rates observed in subordinates in experiment 2 undoubtedly would translate to true fitness costs for these individuals. *Neolamprologus pulcher* helpers are often unrelated to the breeders they are assisting (K.A. Stiver, P. Dierkes, M. Taborsky, L. Gibbs & S. Balshine, pers. comm.) so it would be advantageous to grow quickly, reach breeding size and gain an independent breeding spot. The longer an individual must survive before reaching breeding size, the greater its chances of being predated before realizing direct fitness. There are additional costs of slow growth for males and females. In other species of cichlids, females with low growth will not achieve a large body size and will lay fewer eggs compared to larger females (Balshine-Earn, 1995). Since large males are preferred by females (Balshine-Earn & McAndrew, 1995; Balshine-Earn, 1996), a small male may not be able to attract a mate.

Most of the previous studies conducted have been restricted to short-term experiments (typically 2 weeks) and have not considered effects over longer time periods. The fact that the experimental duration was altered, as well as the social setting, however, is a potential weakness of this study. In the future, it would be useful to vary duration and social setting independently. Another potential problem with this study results from the fact that, in experiment 3, individuals were examined in a social group. Because of this, the dominance relationships and the potential physiological effects may not have been independent of other dominance relationships within the group. The fish in experiments 1 and 3 (matched pairs) were taken from natural groups and were likely to be siblings. In experiment 2, however, the fish were taken from a stock tank so they would have been potentially familiar with each other but not likely to be related. In the wild, groups are composed of relatives and non-related individuals (Stiver *et al.*, 2004). It would be expected that competition for food and position would be most severe among non-relatives. As several factors (*e.g.* duration, maturity, relatedness and social setting) varied among the experiments it is difficult to make strong conclusions from a direct comparison of measures across experiments. For example, it remains unclear which factors accounted for the variation in protein levels across experiments.

Finally, basal cortisol levels were determined from blood collected only at the end of the experiments as only terminal sampling could ensure sufficient blood for analysis from such small fishes (1–5 g). Stress-free non-invasive methods of hormonal analysis have not been optimized for this species but these would allow examination of changes in cortisol over time. Overall, few physiological differences were found with respect to social rank. It can be concluded that, in the cooperatively breeding cichlid, *N. pulcher*, there is evidence of benefits of dominance (increased growth and higher energy stores) and few physiological costs to being dominant in a stable social group.

The authors would like to thank K. Persaud, K. Stiver and S. Marsh for help with data collection and sample processing, J. Richards for help with physiological methods and analysis, C. Wood for the use of his laboratory and S. Marsh for fish care. Funding for this project was kindly provided by an NSERC research grant to SB. The procedures described have been reviewed and approved by the McMaster University's Animal Research Ethics Board and adhere to animal handling guidelines specified by the Canadian Council for Animal Care.

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