

## Liver size reveals social status in the African cichlid *Neolamprologus pulcher*

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Wild groups ( $n = 167$ ) of the cooperatively breeding Lake Tanganyika cichlid, *Neolamprologus pulcher*, were used to investigate how social status and sex influence liver investment. In contrast to expectations, males and females (controlling for body size) had similar liver investment and subordinates (both sexes) had relatively larger livers compared with dominants. Three hypotheses were considered for why social status results in liver size disparity: liver mass might reflect status-dependent differences in (1) energy expenditure, (2) energy storage and (3) energy acquisition. First, dominants performed more energetically costly behaviours (e.g. social policing and care) compared with subordinates, supporting the notion that energy expenditure drives liver investment. Moreover, dominants in large groups (with many subordinates to monitor) and those holding multiple territories (with large areas to patrol), tended to have smaller livers. Second, subordinates did not appear to use the liver as a strategic energy storage organ. In laboratory and field experiments, subordinates ascending in rank had similar or larger livers during periods of rapid growth compared with non-ascending controls. Third, although subordinates fed more frequently than dominants, a negative relationship was found between feeding rates and liver size. Hence, these results contrast with previous liver studies and suggest that liver investment patterns were linked to status-driven differences in energy expenditure but not to energy intake or storage in *N. pulcher*.

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### INTRODUCTION

The liver has a wide range of physiological functions including detoxification and the synthesis, regulation and secretion of proteins, glucose, hormones, bile and lipids (Marshall & Hughes, 1980). One of the liver's most important functions is as an

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energy reservoir or fuel generator for costly processes such as reproduction and growth. Despite its well-understood role and importance in regulating these fundamental physiological processes, little is known about liver function in highly social species, where reproduction and growth are mediated by social factors. In this study, a highly social fish species was used to explore the tandem effects of sex and social status on liver investment patterns and how energy intake and expenditure affect liver size.

Sex differences in liver mass are common, with females having larger livers than males across a wide variety of taxa, e.g. snakes *Vipera aspis* (Bonnet *et al.*, 1998), house sparrows *Passer domesticus* (Chappell *et al.*, 1999), lake whitefish *Coregonus clupeaformis* (Mitchill) (Casselman & Schulte-Hostedde, 2004) and voles *Microtus agrestis* (Klemola *et al.*, 1997). These sex-specific investments in liver mass are thought to reflect the differential demands of reproduction; relatively larger livers in females result from the additional energy and protein needed to produce large, energetically rich gametes (Bonnet *et al.*, 1998; Casselman & Schulte-Hostedde, 2004). Indeed, the fats and proteins needed for egg production are processed and transferred by the liver (Henderson *et al.*, 1996; Dahle *et al.*, 2003; Guijarro *et al.*, 2003). Organ size plasticity is evident in many vertebrates. Muscle, bone, stomach, gastrointestinal tract, kidney, liver as well as gonad size can fluctuate in response to variation in feeding, reproduction and migration, and these changes occur over long or short periods of time (Piersma & Lindström, 1997; Garland & Kelly, 2006). In both males and females, the onset of gonad maturation is coupled with an overall decrease in liver mass as well as decrease in liver lipids, glycogen, protein and catabolic activity (De Vlaming, 1972; Larson, 1991; Huntingford *et al.*, 2001; Resende *et al.*, 2005). Hence, energy invested into the gonads is expected in both sexes to trade-off with energy invested in the liver.

Another factor that may result in larger liver size is increased energy intake (greater access to food or better body condition) (Allen & Wootton, 1982; Rideout *et al.*, 2004). In most group-living organisms, dominant individuals have greater access to food (Appleby, 1980; Carrascal *et al.*, 1998; Gilmour *et al.*, 2005) and hence show pronounced growth and reproduction compared with subordinates (Abbott & Dill, 1989; Ellis, 1995; Sloman *et al.*, 2000a, 2001; Heg *et al.*, 2004). Subordinates are subject to frequent attacks and chronic social stress affecting their growth rates and reproductive capacity (Sloman *et al.*, 2000a; Buston, 2003; Hamilton *et al.*, 2005; DiBattista *et al.*, 2006; Fitzpatrick *et al.*, 2006; Wong *et al.*, 2007). In fact, variation in somatic growth, common in individuals within social hierarchies, is dependent on social status and can even occur when access to food is held constant (Abbott & Dill, 1989; Heg *et al.*, 2004; Wong *et al.*, 2008). All of these patterns suggest that liver size will be indicative of social status, but this assumption has not been explicitly tested previously. Status-specific liver size differences should be evident depending on (1) the degree of social stress experienced (Sloman *et al.*, 2001); (2) the amount of energy consumed to maintain rank, somatic growth and reproduction; or (3) the amount of resources acquired by individuals of varying social rank (DiBattista *et al.*, 2006).

The cichlid *Neolamprologus pulcher* (Trewavas & Poll), from Lake Tanganyika, was used to investigate the effect of social status and sex on liver investment. This group-living fish is a useful model study species, as individuals in social groups live in permanent social hierarchies (a dominant breeding pair and one to 20 subordinates

of both sexes) (Taborsky & Limberger, 1981; Balshine *et al.*, 2001; Heg *et al.*, 2005). These subordinates typically do not reproduce in the wild (Fitzpatrick *et al.*, 2006; Stiver *et al.*, 2009) and strategically undergo rapid growth only when more dominant group members are substantially larger (Heg *et al.*, 2004). Experimental removals of dominant males from social groups result in subordinate ascension in social status and a rapid and dramatic increase in reproductive investment (Balshine-Earn *et al.*, 1998; Stiver *et al.*, 2006; Fitzpatrick *et al.*, 2008). Given the liver's well-known function in regulating growth and reproduction, such high-speed alterations in testicular investment and growth suggest a potential role for the liver in mediating social status changes in *N. pulcher*. Furthermore, breeding and care of young occur year round in this species following a lunar cycle (Nakai *et al.*, 1990; Balshine-Earn *et al.*, 1998), suggesting that liver energy stores may be mobilized at specific periods within a lunar cycle. Finally, dominant males can hold one or multiple territories each with their own dominant female breeder and a set of helpers (Limberger, 1983; Desjardins *et al.*, 2008a). Patrolling several spatially distinct territories may necessitate increased use of energetic stores and may also relate to increases in reproductive investment (Desjardins *et al.*, 2008b).

Using *N. pulcher*, the following specific predictions concerning liver size were examined. (1a) Females would have larger livers (controlling for body size) compared with males to support their greater gametic investment. (1b) In both sexes, as investment in gonads increased, investment in liver mass would decrease and this inverse relationship would be influenced by the reproductive–lunar cycle. (2a) Given dominants' greater energetic demands (*e.g.* reproduction, policing subordinates and defence of young and territory), dominants would have relatively larger livers when compared with subordinate individuals. (2b) Controlling for body size, fish that were more active (*e.g.* performed more care or defended more) would have smaller livers. (3) While controlling for social status, fish with more resources (*e.g.* that fed more, had larger territories, larger groups or more mates) would have larger livers.

## MATERIALS AND METHODS

To test these predictions, social groups of *N. pulcher* were studied in the field and in the laboratory.

### FIELD STUDY: CORRELATES OF LIVER INVESTMENT

In Kasakalawe Bay (8°46' S; 31°46' E), Lake Tanganyika, Zambia, 167 social groups were observed and sampled between 29 January and 29 April 2004 and between 2 February and 28 April 2005. All behavioural and group composition data were collected using scuba and recorded on polyvinyl chloride (PVC) plates. Mating and pairing patterns, group composition and size were determined by multiple visits to each group (Desjardins *et al.*, 2008b). Fish were caught using nets and transparent PVC tubes and sexed on the basis of examination of the genital papilla. Body size was measured: standard length ( $L_S$ ) to the nearest mm. Fish were marked with three colours of non-toxic acrylic paint (injected subdermally or under a scale) in any combination of 16 possible locations on the body. Social status was assigned on the basis of behavioural observations,  $L_S$  and operculum colouration (Buchner *et al.*, 2004). At the end of the study, fish were caught using a conical tent net placed over the territory and a small volume (3–7 ml) of the fish anaesthetic quinaldine was injected into the brood chamber. Fish were brought to the surface and on shore body mass ( $M$ ) was measured to the

nearest 0.001 g and  $L_S$  to the nearest mm. Fish were anaesthetized in benzocaine and killed *via* cervical severance. Their gonads ( $M_G$ ) and liver ( $M_L$ ) were removed and weighed to the nearest 0.001 g. For a sub-set of fish collected ( $n = 330$ , all from monogamous groups), the phase of the lunar cycle (full moon, first half moon, new moon and second half moon) on the basis of the lunar fraction (the fraction of the moon that was illuminated) was recorded. Note that all fish used in this study were collected as part of a number of other studies. For details of these studies and further information of the study area and field methods, see Balshine-Earn *et al.* (1998), Balshine *et al.* (2001), Fitzpatrick *et al.* (2006, 2008), Stiver *et al.* (2006, 2007, 2008, 2009) and Desjardins *et al.* (2008a, b, c).

## BEHAVIOURAL ANALYSES

Each fish was observed one to three times for 7–10 min per focal observation period (on average 28 min per focal fish). Behaviours performed by each focal fish were averaged across all observational periods and a composite score of overall activity levels was calculated on the basis of all non-feeding-related behaviours, including all locomotion, within-group social behaviour, defence and care. The scores for overall activity levels and feeding rates were compared across sex (male or female) and status classes (dominant or subordinate).

Within-group social behaviour can be further broken down into (1) non-aggressive behaviours (follows, parallel swims and soft nudges); (2) aggressive behaviours (bites, rams, puffed throats and chases displayed towards other group members); and (3) submission (submissive postures and displays). Fish also participate in care or allocate some time to behaviours within the (4) workload composite. Workload was defined as the sum of an individual's contribution to shelter maintenance, territory defence and frequency of visits to the brood chamber, and included all behaviours thought to improve the survival probabilities of young. Aggressive displays to heterospecifics and conspecifics outside of the group were classed as defence and included in this workload composite, not in the aggressive score. This breakdown of behavioural categories into composites has been used extensively with this species (Taborsky & Limberger, 1981; Balshine-Earn *et al.*, 1998; Balshine *et al.*, 2001; Bergmüller *et al.*, 2005; Stiver *et al.*, 2005, 2006; Aubin-Horth *et al.*, 2006; Bender *et al.*, 2006; Desjardins *et al.*, 2008a, b, c; Fitzpatrick *et al.*, 2008). For a detailed ethogram of this species' behavioural repertoire see Table I.

## FIELD AND LABORATORY EXPERIMENTS: CONSEQUENCES OF SOCIAL ASCENSION

To explore the consequences of changes of status on liver size, 25 established groups in the field (February to April 2005) and 13 groups in the laboratory at McMaster University (September 2004 to March 2005) were selected. The fish were marked as described above. These groups all contained a large subordinate male within the size range of dominant breeding males in the wild (within 90% of laboratory to 95% of field wild male breeders'  $L_S$ ; Stiver *et al.*, 2006). The dominant male was removed from 18 field groups (Fitzpatrick *et al.*, 2008) and from seven laboratory groups, whereas in seven randomly selected field groups and six laboratory groups the dominant male was not removed. Behavioural watches were performed on the large target male subordinate (on days 0, 1 and 6 in the field, and days 1, 2, 3, 8, 13 and 17 in the laboratory). Each target subordinate male was then captured, killed with benzocaine and  $M$  and  $M_L$  measured to the nearest 0.001g.

## STATISTICAL ANALYSES

All statistical analyses were performed using the programme JMP (Version 5.0.1, 2001; SAS; www.sas.com). Data and residuals were tested for normality and transformed when necessary. Two-tailed tests were used throughout. General linear models (GLM) were used when analysing liver investment across fixed effects of sex and status and also to examine

TABLE 1. Ethogram for *Neolamprologus pulcher*. Within-group social behaviour was broken down into non-aggressive and social behaviours (follows, parallel swims and soft nudges), aggressive behaviours (bites, rams, puffed throats and chases displayed towards other group members), submissive behaviours (submissive postures and displays) and workload behaviours (shelter maintenance, territory defence and visits to the brood chamber; *i.e.* all behaviours thought to improve the survival probabilities of young). Aggressive displays to heterospecifics and conspecifics outside of the group were classed as defence and included in the workload composite, not in the aggressive score. Composites of aggressive, submissive, non-aggressive, social and workload behaviours were used for statistical analyses. *Note*: micronepping and egg fanning can only be directly observed under laboratory conditions

| Behaviour (Code)          |                       | Description  |
|---------------------------|-----------------------|--|
| Non-aggressive and social | Quivering             | Two fish quiver together or one does to the other; the whole body trembles. Often happens most intensely during or just before spawning                      |
|                           | Soft touch            | Fish nips or softly makes contact with another individual  |
|                           | Following             | Focal fish follows another in the group  |
|                           | Group meeting         | Multiple fish of a social group meet each other and swim together  |
|                           | Parallel swim         | Female and male swim upwards together in parallel  |
|                           | Chase                 | Focal fish quickly darts towards another fish  |
|                           | Bite                  | Focal fish bites another fish  |
|                           | Ram                   | Focal fish makes contact with another fish using the head or mouth region, but no obvious bite is taken (jaws remain closed)                                 |
|                           | Puffed throat         | Fish flares out its operculum and lower jaw cavity. Often associated with a posture where the head is pointed downwards; a threat display                    |
|                           | Mouth-fighting        | Focal fish and another fish lock jaws and push against one another   |
| Aggressive                | Aggressive posture    | The focal fish lowers its head and raises its tail in front of the fish to which has directed the posture  |
|                           | Pseudo-mouth-fighting | Back and forth movement between two fish with open mouths (as if about to mouth-fight), but no physical contact is established                               |
|                           | Hook and 'J' display  | Focal fish swims towards another fish, bites or rams it, then turns away and quivers. Always displayed by subordinate fish to a fish higher in the hierarchy |
|                           | Head shake            | Fish thrashes its head from left to right repeatedly   |

TABLE I. Continued

| Behaviour (Code)   | Description   |
|--------------------|---|
| Submissive         | The head of the focal fish is directed upwards (sometimes entirely vertical) and the tail is downwards  |
| Submissive posture | Focal fish is positioned with a submissive posture accompanied by a quivering tail (and sometimes the entire body quivers)  |
| Submissive display | Focal fish quickly swims away from another fish   |
| Flee or chased     | Focal fish is bitten by another fish  |
| Bitten             | Focal fish takes in a mouthful of sand and spits it out (looks like feeding except that sand comes out of the fish's gills or mouth). Used to prepare shelters to escape from predators or strangers and used to prepare and clean brood chambers before fry are born |
| Digging            | Focal fish, often an individual which digs, vibrates or quivers its body on the substratum within the shelter and displaces amounts of the substratum   |
| Workload           | Focal fish takes in a mouthful of something ( <i>e.g.</i> a rock) and swims to a different area before spitting it out  |
| Body digging       | Focal fish defends the brood chamber by hovering over it, keeping other fish from entering  |
| Carrying           | Focal fish enters the brood chamber (this is a measure of parental care and defending the brood). Usually only the most dominant fish may enter the brood chamber   |
| Hover              | Focal fish appears to be eating the eggs, but is actually tasting them to remove eggs with fungus, and turning the eggs on the substratum to ensure proper development  |
| Brood chamber      | Fanning of the eggs using the pectoral fins   |
| Micronipping       | Focal fish orients towards food and opens its mouth to take in particles  |
| Egg fanning        | Slow locomotion using the pectoral fins   |
| Feed               | No locomotion or movement of the pectoral fins  |
| Swim               | A spontaneous, quick swim not directed at anything, not an obvious flee from a fish   |
| Still              | Big stretch of the mouth  |
| Darting            | Focal fish very quickly scrapes its side or underside against a surface   |
| Yawn               |   |
| Scraping           |   |



how liver investment differed across the lunar reproductive cycle, between males holding multiple *v.* single territories, and how relative liver size was influenced by group dynamics (subordinate size and group size) and behaviour.  $\log_{10} M_L$  and  $\log_{10} M_G$  were used as the dependent *y*-variable for all GLM analyses. To control for *M*, soma mass ( $M_S$ ) was included as a covariate throughout GLM analyses. Body mass was measured as  $M_S$ , which is *M* minus  $M_L$  or  $M_G$  (Tomkins & Simmons, 2002). To investigate the effects of body condition on  $M_L$ , Fulton's condition factor (*K*) was used:  $K = 10^6 M L_S^{-3}$  (Neff & Cargnelli, 2004). Low values of *K* indicate 'poor' body condition and high values indicate good body condition. Tukey HSD tests were used for *post hoc* comparisons following the parametric GLM. All non-significant interactions were removed while calculating GLM and were excluded from tables. All measurements reported are means  $\pm$  s.e.

## ETHICAL NOTE

Individuals and their liver and gonads were collected over two field seasons. Great care was taken not to collect fish from small subpopulations (<10 groups). Neighbouring groups were left intact and new fish would often occupy the empty territories within a few days of group removal. Information on other tissues from these individuals has been used in other published studies (Fitzpatrick *et al.*, 2006, 2008; Stiver *et al.*, 2006, 2007, 2008, 2009; Desjardins *et al.*, 2008*a, b, c*). All research conformed to the protocols approved by the Animal Research Ethics Board of McMaster University (AUP # 06-10-59) and comply with Canadian Council for Animal Care guidelines.

## RESULTS

### MALE AND FEMALE INVESTMENT IN LIVER TISSUE

In general, larger fish had larger livers (linear regression, adjusted  $r^2 = 0.66$ ,  $M_L = 0.85 K - 4.91$ ;  $n = 713$ ,  $P < 0.001$ ), but fish in good body condition did not have larger livers than those in poor body condition (adjusted  $r^2 = 0.001$ ,  $n = 713$ ,  $P > 0.05$ ). In absolute terms, males (usually larger) had larger livers (male  $M_L = 20 \pm 1$  mg or *c.* 17% larger than female  $M_L = 17 \pm 1$  mg). Once differences in  $M_S$  were controlled, males and females in the field did not differ in  $M_L$  [GLM, sex:  $F_{1,710}$ ,  $P > 0.05$ ; soma:  $F_{1,710}$ ,  $P < 0.001$ ; Fig. 1(a)]. Given that lack of a sex difference in liver size, sex was not included in subsequent analyses of liver size. In line with expectation, females had significantly larger gonads [sex:  $F_{1,679}$ ,  $P < 0.001$ ; soma:  $F_{1,679}$ ,  $P < 0.001$ ; Fig. 1(b)].

### RELATIONSHIP BETWEEN LIVER, GONADS AND REPRODUCTIVE CYCLE

In the field, there was no evidence of a trade-off between liver and gonad investment as the two were positively, but weakly, correlated (linear regression,  $\log_{10} M_L = 0.06 M_G - 0.01$ ;  $n = 682$ ,  $r_2 = 0.01$ ,  $P < 0.05$ ). Among monogamous breeders, liver investment patterns varied across the lunar and reproductive cycle, with investment highest just before the full moon when spawning peaked (GLM, lunar fraction:  $F_{1,134}$ ,  $P < 0.05$ ; soma:  $F_{1,134}$ ,  $P < 0.001$ ; lunar fraction  $\times$  soma:  $F_{1,134}$ ,  $P < 0.05$ ; Fig. 2). This pattern was not found among subordinates (lunar fraction:  $F_{3,196}$ ,  $P > 0.05$ ; soma:  $F_{1,196}$ ,  $P < 0.001$ ).

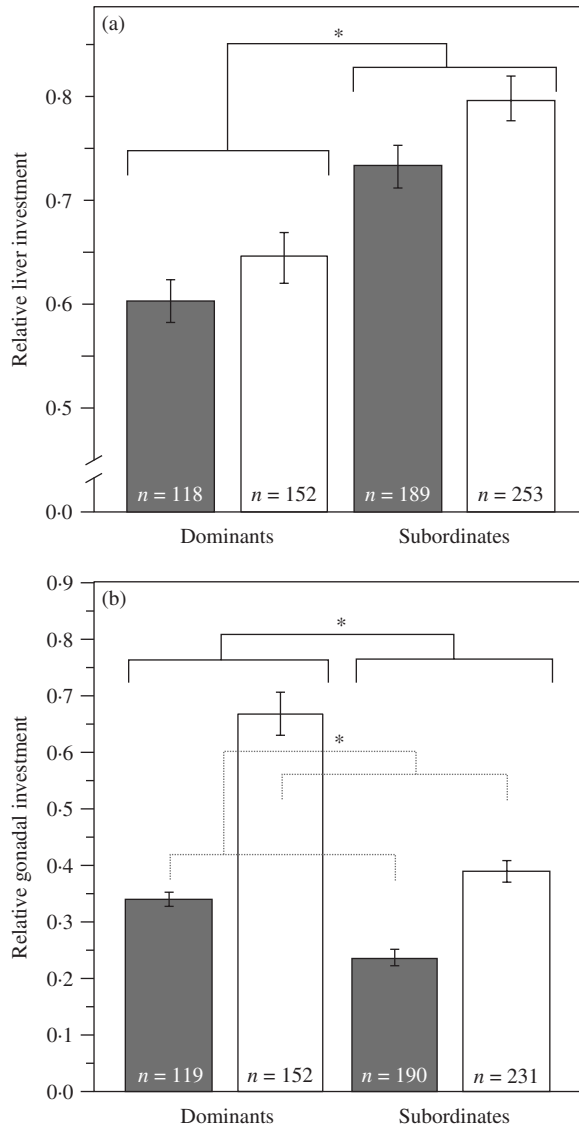


FIG. 1. Comparison of mean  $\pm$  s.e. relative (a) liver and (b) gonad investment between social status class (dominant breeders and subordinate helpers), for male (■) and female (□) *Neolamprologus pulcher*. Data were derived from analyses based on general linear models with appropriate control for allometric scaling.  $n$ , number of fish within each category; \*,  $P < 0.05$ .

## DOMINANT AND SUBORDINATE INVESTMENT IN LIVER TISSUE

Wild-caught subordinates (males and females combined) had larger livers than dominants [status:  $F_{1,710}$ ,  $P < 0.01$ , Fig. 1(a)]. As expected, dominant breeders had larger gonads (controlling for body mass differences) when compared with subordinates [status:  $F_{1,679}$ ,  $P < 0.001$ , Fig. 1(b)].



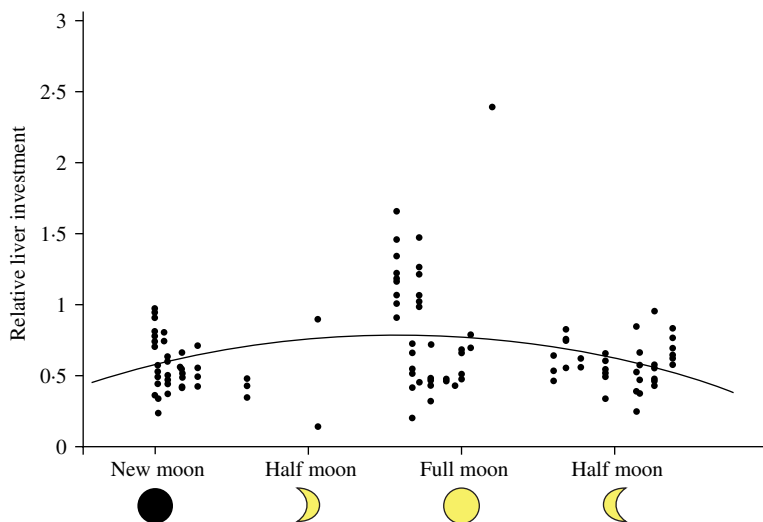


FIG. 2. The relationship between *Neolamprologus pulcher* relative liver investment and lunar phase. Young emerge from the brood chamber at the darkest part of the lunar cycle (around new moon). Young take c. 7–10 days to develop into free-swimming fish from fertilized eggs, thus most of the courtship and spawning will occur between full moon and mid-cycle. Analyses were based on general linear model.

#### WITHIN-CLASS LIVER INVESTMENT AND FEEDING

In the field, feeding represented >90% of the behaviours recorded. In natural groups, dominants fed less than subordinates (status:  $F_{1,436}$ ,  $P < 0.05$ , soma:  $F_{1,436}$ ,  $P > 0.05$ ), but as feeding rates increased liver size decreased (multiple regression, feeding:  $F_{1,439}$ ,  $P < 0.05$ ; soma:  $F_{1,439}$ ,  $P < 0.001$ ).

#### ENERGETIC DEMANDS, ACTIVITY LEVELS AND LIVER INVESTMENT

Wild dominant males that held multiple territories tended to have smaller livers (mean  $\pm$  s.e. liver mass =  $27 \pm 1$  mg) compared with dominant socially monogamous males holding only a single territory ( $32 \pm 1$  mg, GLM, mating behaviour:  $F_{1,116}$ ,  $P > 0.05$ ; soma:  $F_{1,116}$ ,  $P < 0.001$ ). Polygynous males are required to swim longer distances across several territories each day (Limberger, 1983; Balshine *et al.*, 2001; Desjardins *et al.*, 2008a). Dominant individuals (males and females) in larger groups with many individuals to monitor tended to have smaller livers (group size:  $F_{1,243}$ ,  $P > 0.05$ ; soma:  $F_{1,243}$ ,  $P < 0.001$ ).

After feeding, the most common class of behaviour observed in the field was workload (territory defence, brood care and shelter maintenance), followed by within-group social interactions. These behaviours did not significantly correlate with liver size (Table II). Across all individuals, however, liver investment decreased as overall activity levels increased (activity:  $F_{1,438}$ ,  $P < 0.05$ ; soma:  $F_{1,438}$ ,  $P < 0.001$ ). Within a status class, the most active helpers had the smallest livers (activity:  $F_{1,210}$ ,  $P < 0.05$ ; soma:  $F_{1,210}$ ,  $P < 0.001$ ), but this was not observed among dominant breeders. Overall, dominant breeders (males and females combined)

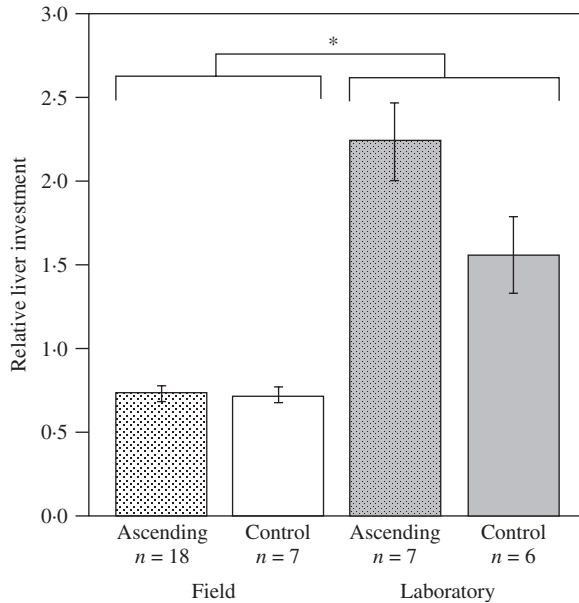


FIG. 3. Comparison of *Neolamprologus pulcher* mean  $\pm$  S.E. relative liver investment between ascending and non-ascending control subordinate helper males from field and laboratory experiments. Analyses were based on general linear model controlling for allometry. *n*, number of fish within each category; \*,  $P < 0.05$ .

had higher activity levels than subordinate helpers (status:  $F_{1,434}$ ,  $P < 0.01$ ; soma:  $F_{1,434}$ ,  $P > 0.05$ ).

In both the laboratory and the field, ascending and non-ascending males had similar liver investment patterns (ascending *v.* non-ascending  $F_{1,53}$ ,  $P > 0.05$ ; laboratory and field:  $F_{1,53}$ ,  $P < 0.001$ ; soma:  $F_{1,53}$ ,  $P < 0.01$ ; Fig. 3). This result, coupled with the results above, suggests that livers probably fuel the costs of maintaining rank but not the costs of rank ascension.

## DISCUSSION

In contrast to initial predictions, highly social *N. pulcher* males and females had similar liver investment, and liver and gonad investment were weakly correlated. Subordinates, not dominants, had larger liver investment. The available evidence indicates that energy expenditure needed to maintain (but not achieve) high social rank is associated with smaller livers. Despite having access to more resources, dominants had relatively smaller livers. Moreover, dominant breeding males with two or more territories (polygynous males) tended to have smaller relative liver investment compared with males holding only one territory (socially monogamous males with smaller areas to patrol). In the field, dominants had highest liver investment just before the full moon and spawning, and lowest at the darkest part of the cycle when fry emerge (Nakai *et al.*, 1990; Balshine-Earn *et al.*, 1998). It is well understood that moonlight improves the hunting success of nocturnal predators,

common in Lake Tanganyika (Rossiter, 1991; Daly *et al.*, 1992). Interestingly, liver size did not differ between males that ascended in social rank and those males that did not ascend. The much larger overall investment in livers observed in laboratory fish compared with field fish was probably a result of greater food availability and higher food quality, a finding also observed in haddock *Melanogrammus aeglefinus* (L.) (Rideout *et al.*, 2004).

Poor liver condition (less liver glycogen and smaller hepato-somatic index) has been shown to be a physiological consequence of lower social status (Ejike & Schreck, 1980; Fernandes & Volpato, 1993; Sloman *et al.*, 2001). Unlike the species used in most previous studies of liver size, *N. pulcher* live in permanent, cooperatively breeding, highly social, groups. Subordinates in the wild are often sexually mature but rarely reproduce; instead they care for the offspring of the breeding pair (Balshine *et al.*, 2001; Stiver *et al.*, 2009). In *N. pulcher*, growth and breeding are socially sanctioned. Dominants actively defend the territory (Desjardins *et al.*, 2008a) and they regularly police subordinates (*via* aggression), enforcing subordinate reproductive suppression and reinforcing and stabilizing the social hierarchy (Fitzpatrick *et al.*, 2008; Heg, 2008; Heg & Hamilton, 2008). More active fish had smaller livers, but when behaviours were broken down into functional categories, within-group aggression (or workload) did not relate to liver size (Table II).

In general, high activity levels and agonistic behaviours both can raise metabolic rates (Grantner & Taborsky, 1998; Taborsky & Grantner, 1998), and higher metabolic rates have been reported in dominant Atlantic salmon *Salmo salar* L. (Metcalf *et al.*, 1995), brown trout *Salmo trutta* L. (Sloman *et al.*, 2000b) and white throated dippers *Cinclus cinclus* (Bryant & Newton, 1994). Increased metabolic rates associated with maintaining dominance as well as breeding itself can quickly deplete energy stores; hence, dominant individuals within a territory or in the population may have had smaller livers because of these greater long-term greater energetic demands. The social ascension experiments suggest that the short-term costs of acquiring rank are not fuelled by liver stores.

An important caveat to this study is the assumption that liver size relates to energy stores; size may not be a sufficient energy metric. For example, enlarged livers can also indicate disease, cancer and viral infections (Majeed *et al.*, 1984; Perelberg

TABLE II. Effects of behaviour (composites are identified in the ethogram, Table I) on liver investment across sex and status of *Neolamprologus pulcher*

|          | <i>n</i> | Behaviour |          |                |          |            |          |            |          |          |          |
|----------|----------|-----------|----------|----------------|----------|------------|----------|------------|----------|----------|----------|
|          |          | Soma      |          | Non-aggressive |          | Aggression |          | Submission |          | Workload |          |
|          |          | <i>F</i>  | <i>P</i> | <i>F</i>       | <i>P</i> | <i>F</i>   | <i>P</i> | <i>F</i>   | <i>P</i> | <i>F</i> | <i>P</i> |
| Breeders |          |           |          |                |          |            |          |            |          |          |          |
| Male     | 87       | 49.23     | <0.001   | 1.44           | >0.05    | 0.19       | >0.05    | 0.07       | >0.05    | 2.34     | >0.05    |
| Female   | 137      | 34.71     | <0.001   | 0.24           | >0.05    | 0.05       | >0.05    | 0.58       | >0.05    | 0.53     | >0.05    |
| Helpers  |          |           |          |                |          |            |          |            |          |          |          |
| Male     | 98       | 132.57    | <0.001   | 0.03           | >0.05    | 0.50       | >0.05    | 0.004      | >0.05    | 1.59     | >0.05    |
| Female   | 111      | 98.36     | <0.001   | 0.03           | >0.05    | 1.25       | >0.05    | 0.78       | >0.05    | 1.17     | >0.05    |

*n*, number of individual fish within each category.

*et al.*, 2003). Moreover, in this study liver glycogen, lipid and protein levels were not measured, nor have they been directly related to size in other studies. Hence, it remains possible that energy stores vary differently with respect to sex and status than does size. Liver glycogen, protein, glucose, ATP and creatine phosphate levels have been examined previously in size-matched and sex-matched subordinate *N. pulcher* (Buchner *et al.*, 2004). Although the lowest ranking of the subordinates had the largest relative liver investment, these individuals also had the lowest liver protein levels or the lowest liver glycogen levels (Buchner *et al.*, 2004). No differences were detected in liver ATP and creatine phosphate levels in relation to social status. Although a much narrower range of the social hierarchy was examined in the previous laboratory study, future experimental work is clearly necessary to clarify the link between liver size and energy stores and energy mobilization.

No evidence of a trade-off between liver and gonad investment was observed. Thus far all observed inverse relationships between liver and gonad investment have been in seasonal breeders (De Vlaming, 1972; Larson, 1991; Huntingford *et al.*, 2001; Malavasi *et al.*, 2004). It remains possible that the liver and gonad trade-off is not a feature found in tropical species with year-round reproduction such as *N. pulcher*. Moreover, energy stores may not accumulate in the liver but in other tissues. Lipid and protein concentrations have been found to be greatest in the muscle of plaice *Pleuronectes platessa* L. and three-spined stickleback *Gasterosteus aculeatus* L.; however, depletions due to energy requirements were greatest in the liver compared with the rest of the body (Dawson & Grim, 1980; Chellappa *et al.*, 1989). Analysing the glycogen, lipid and protein levels in several storage organs such as liver, gonad and muscle at different stages of the reproductive cycle may provide a better indication of how energy reserves vary temporally. Sex differences may lie in energy mobilization rather than in size *per se*. For example, in Arctic charr *Salvelinus alpinus* (L.) the mobilization of energy reserves is significantly greater in females than in males (Jørgensen *et al.*, 1997).

In summary, the results of this study show that liver investment patterns reflect social state and the associated energy demands of group social behaviour. Future studies in the laboratory, controlling for variation in availability and quality of food, could further elucidate how restricted resources (*e.g.* food) and monopolized access to resources influence liver investment patterns as well as behaviour. Moreover, future laboratory and field studies that manipulate rank of individuals and territory-holding ability could use modern imaging and labelling technologies, including radiolabelling, magnetic resonance imaging and positron emission topography scanning to repeatedly sample liver size dynamics and experimentally test whether energy invested into growth is shunted from energy stores in the liver.

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