The Preference for Social Affiliation Renders Fish Willing to Accept Lower O_2 Levels

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ABSTRACT

Animals are bombarded with information about their environment and must select and interpret the relevant cues to make behavioral adjustments critical to survival. How animals integrate and balance the many signals they receive about their environment is rarely assessed. We investigated how signals from the social and physical environment interact to influence environmental preferences in the endemic Tanganyikan cichlid *Neolamprologus pulcher*. Specifically, we explored how fish respond to the physiological challenge of declining O_2 levels in light of embedded social preferences using a modified shuttle box apparatus to test O_2 preferences. In the presence of a conspecific, the average (preferred) partial pressure of oxygen (P_{O_2}) and minimum P_{O_2} experienced were significantly lower (14.90 ± 2.13 and 12.35 ± 3.15 kPa, respectively) than in trials without a conspecific (17.18 ± 2.55 and 15.62 ± 3.09 kPa, respectively). Fish with conspecifics also spent more time in the low P_{O_2} zone of the shuttle box and moved between the high and low P_{O_2} zones less frequently. Hence, O_2 preferences were modified, and fish willingly remained in an area of continuously declining O_2 availability to associate with a conspecific. The O_2 preferences of an individual during social trials correlated with its excess post-exercise O_2 consumption following an exhaustive chase but not with its aerobic scope, routine O_2 consumption rate, or body mass. These results suggest that some aspects of respiratory and metabolic physiology (such as the propensity to use anaerobic metabolism) but not others (such as O_2 transport capacity) underpin some variation in social behavior under environmental stress.

Keywords: behavior, preference, shuttle box, oxygen consumption, respirometry, fish.

Introduction

Animals constantly take in information about their social and physical environments and make use of these signals to survive, cope, and thrive (Fernald 2015). However, the manner in which animals integrate information across different signal types or balance contrasting signal inputs from their social and abiotic environments to modulate behavior is rarely assessed.

While group living has considerable benefits, such as reduced individual predation risk, it can also exacerbate social stressors, such as competition with rivals for resources (Pitcher and Parrish 1993). To minimize the costs of chronic social competition, many species that live in social groups have evolved coping strategies, such as the formation of dominance hierarchies that dampen intragroup conflict (Chase 1980). Many social species may also employ affiliation and comfort behaviors to buffer against stress. These behaviors have been linked to the release of endogenous opiates and stimulation of brain reward centers to improve health and well-being (Fabre-Nys et al. 1982; DeVries et al. 2003). Such coping strategies allow animals to reap the benefits of group living while minimizing its potential fitness costs.

The physical environment (temperature, light conditions, O_2 tension, salinity, etc.) that individuals encounter also strongly influences survival, growth, physiology, and reproduction. Most animals will avoid or move away from challenging physical environments when given the opportunity. For example, many aquatic species actively avoid areas of low dissolved O_2 (hypoxia), which occurs on both seasonal and diurnal timescales (Kramer 1987; Chapman and McKenzie 2009; Diaz and Breitburg 2009). Even when escape from such areas of low O_2 is impossible, changes in behavior can minimize the physiological consequences of exposure to hypoxia. For instance, the facultative air-breather *Clarias gariepinus* surfaces more frequently in hypoxic water, a risky behavior that increases O_2 uptake but exposes the individual to aerial predators. Interestingly, air gulping is reduced in daylight conditions, a situation with increased vulnerability to aerial predators, further demonstrating the importance of context in behavioral responses to environmental stress (McKenzie et al. 2015).

Few studies to date have explored how environmental stressors such as low O_2 influence group dynamics and social interactions. It is foreseeable that the maintenance of social behavior, social hierarchies, and investment in relationship building may need to be put on hold when individual survival is threatened. In support

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of this idea, aggressive interactions decreased in haplochromine cichlids performing hypoxia-induced aquatic surface respiration, likely due to the increased investment of time and/or finite energy stores into O_2 uptake (Melnychuk and Chapman 2002). Similarly, the frequency of an aggressive signal, opercular flaring, decreases in *Betta splendens* during exposure to hypoxia (Abrahams et al. 2005). Social grouping could also be less stable in hypoxia, reducing fish school size and density and influencing the position of individuals within a school (Domenici et al. 2013). Alternatively, strong preferences for social affiliation or a dominant position may prompt individuals to remain in suboptimal environmental conditions or perform risky actions. For example, under hypoxia groups of the South American catfish *Hoplosternum littorale* will synchronize their air gulping and surface to breathe air at higher O_2 tensions than isolated animals (Sloman et al. 2009). Group coordination of aerial O_2 uptake likely diffuses individual predation risk during air gulps but also compels some individuals to surface more frequently than their physiological needs would otherwise require. Delaying exposure to aerial predators until absolutely necessary may also influence smaller oscars (*Astronotus ocellatus*) to begin aquatic surface respiration at a more severe level of hypoxia than larger fish, despite the higher energy demands of smaller body size, when held individually (Sloman et al. 2006). Clearly, the relationship among social cues, environmental cues, and behavior is complex and important. Moreover, the value placed on maintaining a specific social environment relative to individual physical demands will vary between individuals.

In this study, we explored how the preference to affiliate in the highly social cichlid *Neolamprologus pulcher* influences the behavioral response to decreasing O_2 levels, and we examined whether these responses were related to hypoxia tolerance and/or to individual variation in physiology. *Neolamprologus pulcher* are found in the upper layers (<45 m depth) of Lake Tanganyika, a deep and thermally stratified lake in the African Rift Valley (Verburg et al. 2003; Williamson et al. 2009). Though the upper layers are generally well oxygenated, seasonal changes in wind patterns can cause occasional upwelling of anoxic hypolimnic waters. These surges can trap fish as they move toward the shore and into shallower regions of the lake. Excluding this study, hypoxia tolerance has been experimentally quantified in only seven Tanganyikan cichlids. Compared with species from Lake Victoria, which more regularly encounter hypoxia in the course of their life history, virtually all of the Tanganyikan cichlids studied to date are considerably more sensitive to experimentally induced hypoxia (Verheyen et al. 1994; Chapman et al. 1995).

Neolamprologus pulcher is rapidly becoming a model for behavioral studies of fish cooperation (Wong and Balshine 2011). These animals live in social groups consisting of a dominant breeding pair and subordinate helpers that assist the breeders with territory defense, maintenance of the shelter system, and direct brood care (Taborsky and Dominique 1981; Taborsky 1984, 1985; Wong and Balshine 2011). Individual *N. pulcher* are highly motivated to remain with a social group, as group membership provides access to a shelter system and protection from predators (Balshine et al. 2001; Heg et al. 2005; Jordan et al. 2009; Wong and Balshine 2011; Groenewoud et al. 2016). Laboratory and field experiments confirm that individuals spend more time near conspecifics than on their own, with large individuals than with small individuals, with large groups than with small groups, and with familiar fish than with unfamiliar fish (Jordan et al. 2009; Reddon et al. 2011). This clear preference for social interaction provides a useful model system for testing how physiological and social cues are used to make decisions about acceptable habitat quality.

This study explored how the presence of a conspecific affects the behavioral response to decreasing O_2 and whether the individual responses were related to hypoxia tolerance and/or individual variation in physiology in the cooperative breeding fish *N. pulcher*. We predicted that fish would be willing to accept lower O_2 levels (e.g., a reduced O_2 preference) in order to remain near a conspecific. We also examined the resting O_2 consumption rate ($\dot{M}O_{2,\mathrm{rest}}$) as an index of general energetic demands, hypoxia tolerance (critical O_2 tension [P_{crit}] and the O_2 tension at loss of equilibrium [partial pressure of oxygen (PO_2) at LOE]), and whole-animal measures of O_2 transport capacity and metabolism (maximal O_2 consumption rate [$\dot{M}O_{2,\mathrm{max}}$], aerobic scope, and total excess postexercise O_2 consumption [EPOC]) to provide a physiological context to the behavioral responses to hypoxia.

Methods

Animals

Laboratory-reared *Neolamprologus pulcher* that were the descendants of wild fish caught from Lake Tanganyika in Africa were maintained at McMaster University (Hamilton, Ontario). The fish were housed in 527-L communal stock aquariums filled with aerated, dechlorinated water maintained at 26° ± 2°C. The stock tanks contain large, high-density groups of fish of mixed sexes and ages to prevent hierarchy formation. Social bonds between specific individuals under such holding conditions are not obvious. Fish were fed commercial food 6 d a week.

Preference Trials

Preference trials were conducted using a shuttle box apparatus (Loligo Systems, Viborg, Denmark) that dynamically regulates the PO_2 in response to fish position and thus allows fine-tuned measurement of O_2 preference. The apparatus consists of two circular tanks connected through a narrow channel (10 cm × 8 cm; fig. 1*A*). For each trial, one circular tank was randomly designated the "decreasing zone." Water oxygen levels were measured using galvanic O_2 probes (Loligo Systems), and pumps were used to recirculate water throughout the apparatus and to ensure sufficient water flow for accurate dissolved oxygen readings. The PO_2 of the decreasing zone was maintained to always be 6.15 ± 0.41 kPa (30% ± 2% air saturation) lower than the O_2 level in the other circular tank (designated the "increasing zone"). This difference in O_2 levels between tanks was accomplished by independently and accurately maintaining the PO_2 in each buffer tank (see fig. 1*A*), as per manufacturer specifications and recommendations.

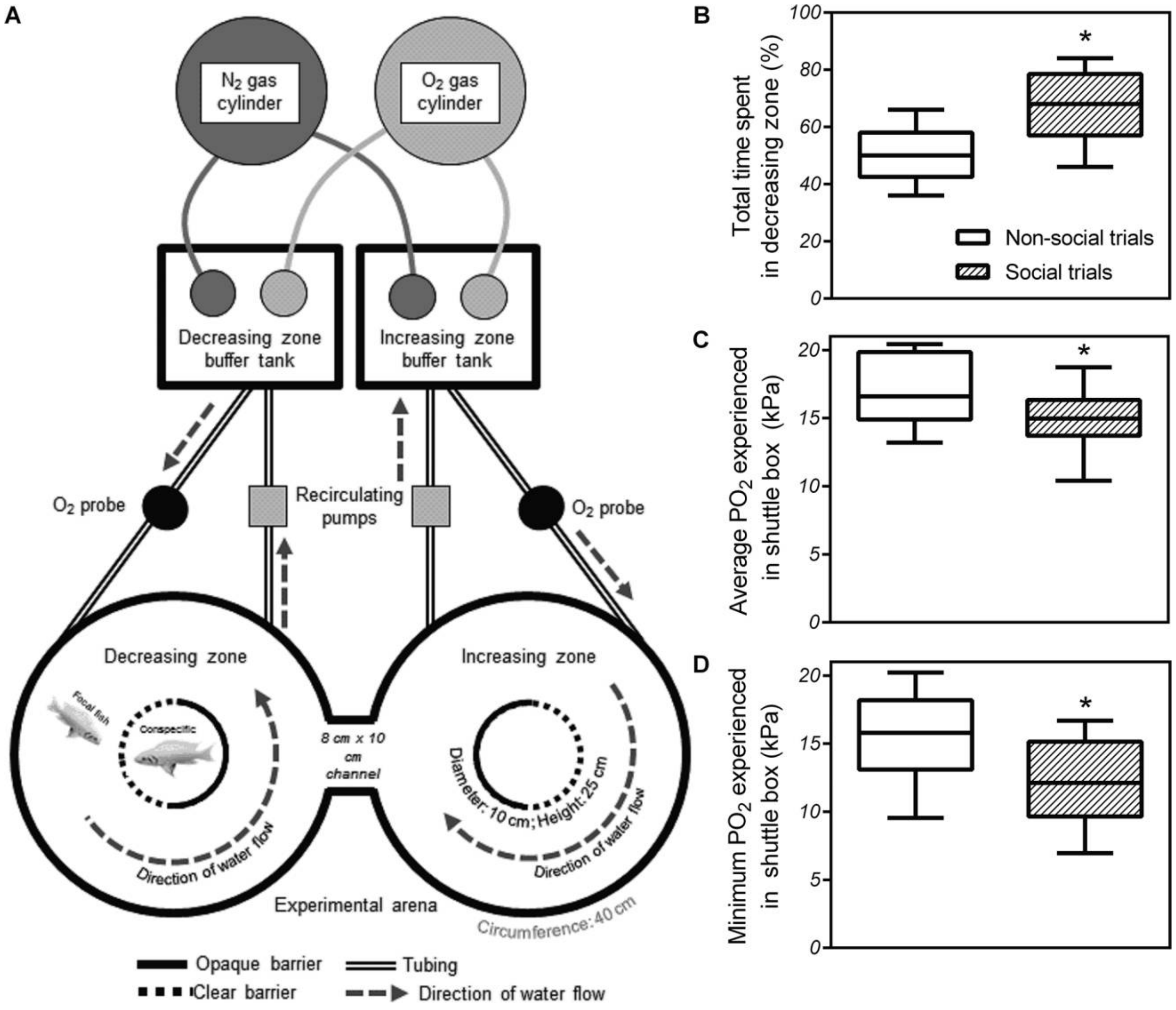

Figure 1. *A*, Overhead view of the modified shuttle box apparatus used in this study. During social trials in the presence of a conspecific, fish spent more time in the decreasing zone (*B*) and experienced lower average partial pressure of oxygen (P_{O_2}; *C*) and minimum P_{O_2} (*D*). Boxes are divided by quartiles, and whiskers indicate the range of the data. Detailed statistics are included in table 1. Asterisks indicate a significant difference between trial types. Data are expressed as mean ± SEM. 20.5 kPa = 100% air saturation.

The automated program tracked the position of the focal fish by means of an overhead camera. When the fish entered the decreasing zone, the P_{O_2} of the entire apparatus would decrease continuously (while maintaining the P_{O_2} difference between the zones). However, when the fish shuttled into the increasing zone, the P_{O_2} of both zones would increase continuously. Therefore, P_{O_2} was always changing in response to fish location, forcing the fish to reinforce its preferred P_{O_2} by regularly shuttling between zones and eventually spending equal amounts of time in the two zones during the trial. In contrast, if the fish lacked sensitivity to changes in P_{O_2}, this would be indicated by minimal shuttling (leading to very high or very low P_{O_2} values) or random shuttling between zones.

We modified the shuttle box to examine how the presence of a conspecific affects O_2 preference. A clear acrylic cylinder was installed in the center of each circular tank (fig. 1*A*) so that a conspecific could be housed in the center of the tank. Water in this cylinder was isolated so it could be aerated without affecting the P_{O_2} in the surrounding tank. This allowed us to investigate the O_2 preference of focal fish specifically in response to the presence of the conspecific and avoid the confounding effects of any perceived effects of hypoxia on the conspecific on the behavior of the focal fish. The channel-facing half of these tubes was opaque, forcing the focal fish to fully enter the zone and move deep into the circular tank in order to view the contents of the tube from the transparent side. Both tubes remained empty during nonsocial trials. During the social trials, a conspecific (5%–10% larger by standard length than the fish tracked by the shuttle box; sex was not determined) was placed in the tube in the decreasing zone while

Table 1: Effects of trial type, body mass, and the trial type × body mass interaction on O_2 preference behavior tested in the shuttle box

	Trial type				Body mass		Type × mass	
	Nonsocial	Social	$F_{1,22}$	P	$F_{1,22}$	P	$F_{1,22}$	P
No. trials	13	13	...	...	...	...	...	...
Body mass (g)	10.02 ± 0.76	7.58 ± .62	6.21	.02*	...	...	...	...
Total time in deceasing zone (%)	50.9 ± 2.6	67.1 ± 3.5	14.98	<.001*	2.08	.16	2.85	.11
Average Po_2 experienced (kPa)	17.18 ± 2.55	14.90 ± 2.13	5.60	.03*	<.0001	1.00	.10	.75
Minimum Po_2 experienced (kPa)	15.62 ± 3.09	12.35 ± 3.15	6.72	.02*	<.001	.98	.46	.50
No. zone transitions	504 ± 47	340 ± 33	7.67	.01*	.25	.62	.26	.61
Average velocity (cm/s)	2.49 ± .38	1.68 ± .26	3.64	.07	2.11	.16	3.62	.07
Total distance traveled (m)	26.8 ± 4.1	17.6 ± 2.7	3.98	.06	2.13	.16	3.57	.07

Note. Data are presented as means ± standard error. 20.5 kPa = 100% air saturation. Po_2 = partial pressure of oxygen.
*Significant effect ($P < 0.05$) via linear models.

the tube in the increasing zone remained empty. We alternated between social and nonsocial trials ($N = 13$ of each type of trial, using fish of mixed sexes and body masses; see table 1). Fish were habituated to the shuttle box apparatus for 10 min in normoxic water before the trial started. Fish readily crossed the channel between circular tanks without training, and 10 min was sufficient time for them to explore the entirely of the apparatus.

Using linear models in R (ver. 3.2.1; R Development Core Team 2015) within RStudio (2015), we assessed whether the presence of a conspecific influenced the time spent in the decreasing zone, the O_2 levels experienced, and the general activity of focal fish. Since fish size varied (see table 1), body mass and interaction effects were included in our analysis. We used a significance level of $P < .05$ for all statistics.

Correlating Behavior and Physiology

To follow up on the behavioral data, we related a number of physiological traits to the preference behavior observed in the shuttle box of a set of fish that completed only social trials (fig. 2*A*). To measure $\mathrm{Mo}_{2,\mathrm{max}}$, fish were chased with a net for 2 min followed by air exposure for 1 min (this approach induces higher O_2 consumption rates than chasing alone; Roche et al. 2013). The Mo_2 of exhausted fish was then measured continuously during normoxia using stop-flow respirometry (Borowiec et al. 2015) until recovery to routine Mo_2 ($\mathrm{Mo}_{2,\mathrm{routine}}$; fig. 2*A*). Mass-specific Mo_2 values (μmol/g/h) were calculated by dividing the rate change in chamber O_2 concentration over time (μmol/h) by individual fish body mass (g). Factorial aerobic scope (the ratio of the measurements of maximal and routine Mo_2) and absolute aerobic scope (the absolute difference between maximal and routine Mo_2) were calculated as described elsewhere (Clark et al. 2013). Pilot experiments confirmed that this protocol reliably stimulated $\mathrm{Mo}_{2,\mathrm{max}}$. The EPOC, an index of anaerobic metabolism use during exercise, was calculated as the area under the curve of Mo_2 over time following the chase (see fig. 2*A*), as has been done previously (Scarabello et al. 1992; Lee et al. 2003; Genz et al. 2013; Brennan et al. 2016). For this investigation, EPOC was defined as Mo_2 values ≥5% of the resting value measured for that individual during normoxia. Average Po_2 and minimum Po_2 experienced in the shuttle box was correlated with $\mathrm{Mo}_{2,\mathrm{max}}$, $\mathrm{Mo}_{2,\mathrm{routine}}$, the aerobic scopes, and EPOC using GraphPad Prism (La Jolla, CA).

Hypoxia Tolerance

We evaluated hypoxia tolerance by quantifying $\mathrm{Mo}_{2,\mathrm{rest}}$, P_{crit}, and Po_2 at LOE in a separate group of fish ($N = 11$, mixed sexes, 12.31 ± 1.32 g) taken from the same stock aquariums. P_{crit} represents the lowest Po_2 at which resting O_2 demands are met and likely reflects the limits of O_2 transport capacity during hypoxia. Po_2 at LOE provides a proxy for the limits of survival during severe acute hypoxia. Similar to previous studies (Borowiec et al. 2015; Crans et al. 2015), fish were fasted and acclimated overnight to an acrylic respirometry chamber that was situated in a buffer tank and continuously flushed with normoxic water. The following morning, $\mathrm{Mo}_{2,\mathrm{rest}}$ was measured using stop-flow respirometry by the selective activation and deactivation of pumps to sequentially isolate the respiratory chamber (allowing measurement of fish O_2 consumption) and flush it with water from the buffer tank (allowing control of the Po_2 of the water in the chamber). The Po_2 of the buffer tank was reduced in 2-kPa steps by the controlled bubbling of nitrogen gas. After measurement of fish O_2 consumption at 2 kPa, fish were allowed to consume the remaining O_2 in the chamber until LOE occurred. P_{crit} was determined using REGRESS software (Yeager and Ultsch 1989).

Results

In nonsocial trials, *Neolamprologus pulcher* always preferred remaining in areas with high O_2 levels (fig. 1), but this preference was altered in trials in which a conspecific was present in the decreasing zone. In nonsocial trials, fish spent 51% of the trial time in the decreasing zone (fig. 1*B*), compared with 67% during social trials. Correspondingly, fish made fewer shuttles between zones in social trials (table 1) than in nonsocial trials. Since regular shuttling is required to maintain a stable Po_2 in the shuttle box, the increased time in the decreasing zone and decreased number of shuttles resulted in lower average Po_2 and minimum Po_2

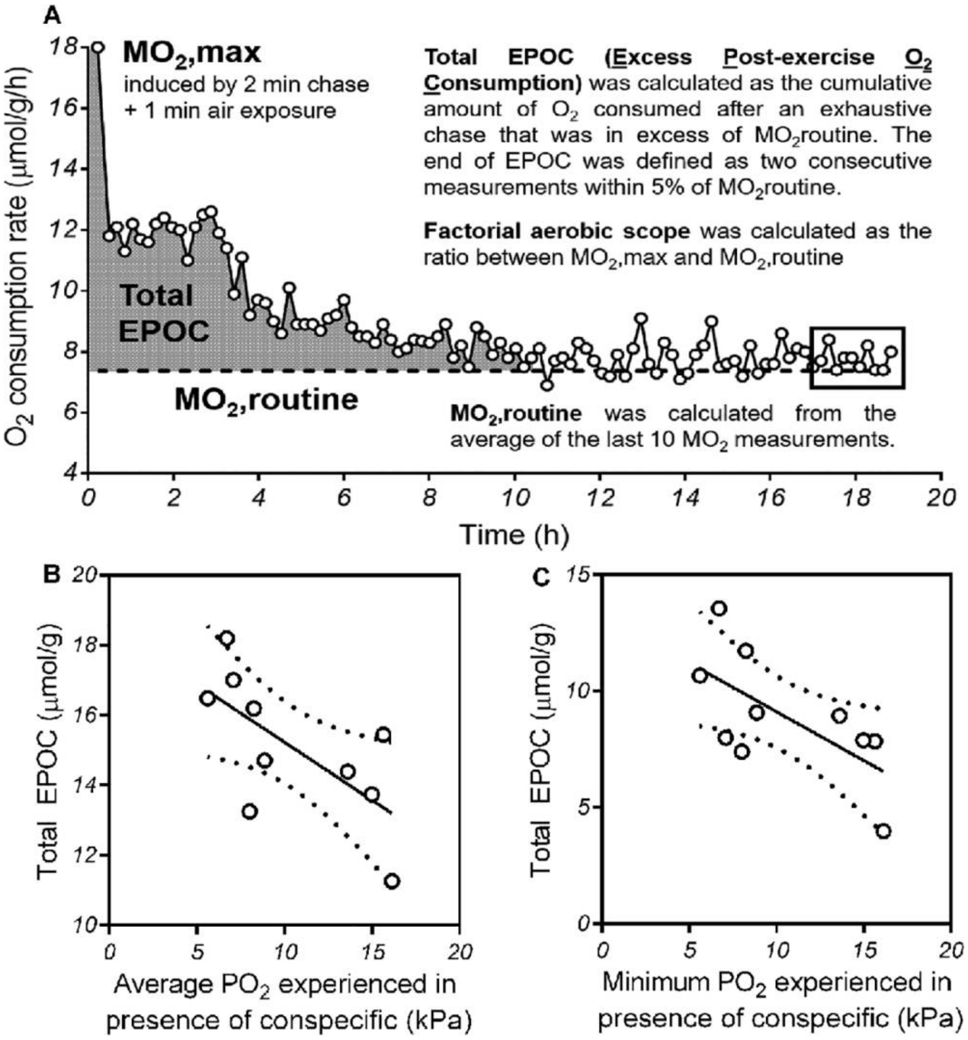

Figure 2. *A*, O_2 consumption rate during the exhaustive exercise protocol. Individual variation in average partial pressure of oxygen (P_{O_2}; *B*) and minimum P_{O_2} (*C*) during social trials correlated with total excess postexercise O_2 consumption (EPOC). Statistics for the regression line are detailed in table 2. Dotted lines indicate 95% confidence intervals. $M_{O_2,\mathrm{max}}$ = maximal O_2 consumption rate; $M_{O_2,\mathrm{routine}}$ = routine O_2 consumption rate.

experienced in the shuttle box during social trials than during nonsocial trials (fig. 1*C*, 1*D*). Along with a reduction in the number of zone transitions, there may have been a modest decrease in general fish activity during social trials. Although not statistically different, fish in the social trials tended to have a lower mean swimming velocity and to travel less total distance during the trial than fish in the nonsocial trials (table 1). O_2 preferences quantified in the shuttle box did not correlate significantly with fish body mass (fig. A1).

There was a significant negative correlation between EPOC and the O_2 levels experienced during social trials (fig. 2; table 2), suggesting that individuals that accepted deeper hypoxia to associate with a conspecific also had a greater propensity for recruiting anaerobic metabolism. O_2 preferences did not correlate with body mass, $M_{O_2,\mathrm{routine}}$, $M_{O_2,\mathrm{max}}$, or either calculation of aerobic scope (table 2).

The minimum P_{O_2} during social trials (12.34 ± 3.15 kPa) and nonsocial trials (15.62 ± 3.09 kPa) were both far above the measured P_{crit} (4.51 ± 0.48 kPa) and P_{O_2} at LOE (0.81 ± 0.08 kPa) for this species. $M_{O_2,\mathrm{rest}}$ was 4.42 ± 0.20 μmol/g/h, which was significantly lower than the $M_{O_2,\mathrm{routine}}$ values measured in a different set of fish after recovery during the exhaustive exercise experiments (5.84 ± 0.50 μmol/g/h) when assessed using a two-tailed Student's *t*-test ($P = 0.02$). Both $M_{O_2,\mathrm{rest}}$ and $M_{O_2,\mathrm{routine}}$ were within the range of M_{O_2} values reported for other African cichlids of comparable size (Verheyen et al. 1994). Neither P_{crit} nor P_{O_2} at LOE correlated significantly with fish body mass (fig. A2).

Discussion

In this study, we explored how social and physical cues integrate in the gregarious fish *Neolamprologus pulcher*. Individuals lin-

Table 2: Correlations between O_2 levels experienced during social O_2 preference trials and individual physiological measurements

	Y = average Po_2				Y = minimum Po_2			
X =	Equation (Y =)	R^2	F (df)	P	Equation (Y =)	R^2	F	P
Body mass	$-.03X + 15.56$	<.001	.01 (1, 9)	.95	$-.46X + 12.70$	.05	.44 (1, 9)	.53
$Mo_{2,\mathrm{routine}}$	$.34X + 13.35$	.07	.69 (1, 9)	.43	$.18X + 8.29$	.01	.14 (1, 9)	.76
$Mo_{2,\max}$	$-.05X + 16.04$	<.01	.06 (1, 9)	.82	$-.10X + 10.79$	.01	.10 (1, 9)	.72
Factorial aerobic scope	$-.41X + 16.52$	.02	.17 (1, 8)	.70	$-.13X + 9.82$	<.01	.01 (1, 8)	.93
Absolute aerobic scope	$-.14X + 16.46$	.05	.43 (1, 9)	.53	$-.15X + 10.56$	.03	.28 (1, 9)	.61
EPOC	$-.33X + 18.54$	.45	6.56 (1, 8)	.03*	$-.42X + 13.31$	.43	6.03 (1, 8)	.04*

Note. EPOC = total excess postexercise O_2 consumption; $Mo_{2,\max}$ = maximum O_2 consumption rate; $Mo_{2,\mathrm{routine}}$ = routine O_2 consumption rate.
*Significant nonzero slope ($P < 0.05$) via an F-test.

gered in progressive hypoxia in order to remain near a conspecific, as reflected by lower average Po_2 and minimum Po_2 observed during social trials compared with their O_2 preference in the absence of a conspecific (fig. 1). Our work expands on previous research that demonstrates that *N. pulcher* prefers to associate with conspecifics (Reddon et al. 2011) to show that this strong preference to remain near other fish is maintained under abiotic stress. As *N. pulcher* recognize familiar conspecifics and are more affiliative toward their own group members (Hick et al. 2014), we expect that the hypoxia avoidance response would be disrupted even more in pairs from the same social unit. As female *N. pulcher* cooperate more than males (Stiver et al. 2005) and live in matrilineal groups (Dierkes et al. 2005), we would expect that females might accept a higher hypoxia threshold than males in order to remain near social fellows. Future research would be valuable to critically test these two predictions to further characterize the behavioral response of *N. pulcher* to decreasing O_2 levels.

In the current study, methodological considerations (such as the interference of the acrylic cylinder that prevented physical contract between animals) precluded assessment of the social interactions between the focal fish and the stimuli social fish inside the cylinder. In the future, it would be interesting to quantify the effects of hypoxia on these social interactions and to test whether the sex, dominance rank, size, or behavior of the stimuli fish influenced social interactions and the strength of a focal fish's preference for conspecifics. For example, both cichlids (*Astatotilapia* "wrought-iron" and *Astatotilapia aeneocolor*) and Siamese fighting fish (*Betta splendens*) reduce the frequency of aggressive interactions during hypoxia (Melnychuk and Chapman 2002; Abrahams et al. 2005), and this may also occur in *N. pulcher*, particularly at O_2 tensions approaching or below the critical O_2 tension, where O_2 uptake is insufficient to meet basal metabolic demands.

On the other hand, an increase in affiliative behaviors in response to hypoxia stress in the focal fish may reduce stress levels (Kikusui et al. 2006) and dampen the induction of physiological stress response systems, including activations of the hypothalamic-pituitary-interrenal axis (DeVries et al. 2003). For example, if affiliating with other individuals dampens the activation of the stress response, then social fish may have lower cortisol levels than solitary fish during hypoxia exposure, and this may feed back to influence some physiological responses to hypoxia (van Raaij et al. 1996; Herbert and Steffensen 2005; Lai et al. 2006). There are likely to be complex interactions among the level of hypoxia stress, the degree of preference to socialize, the nature of social interaction between individuals, and the physiological response to hypoxia, and this warrants further investigation.

O_2 transport capacity did not strongly influence environmental O_2 preference, as reflected by our observation that interindividual variation in $Mo_{2,\max}$ and aerobic scope did not correlate with the O_2 levels accepted in social trials. Similarly, the Po_2 experienced in the O_2 preference trials was well above the critical O_2 tension of *N. pulcher* (which was comparable to data previously reported for similarly sized African cichlids; Verheyen et al. 1994), suggesting that the shuttle box behavior was not strongly tied to hypoxia tolerance limits. Furthermore, O_2 demands did not appear to determine the degree of preferences, as neither body mass nor $Mo_{2,\mathrm{routine}}$ mapped onto any behaviors in the O_2 preference trials (table 2). Since Po_2 experienced in the shuttle box was generally well above hypoxia tolerance limits, the lack of concordance between the physiological determinants of O_2 transport capacity and behavior is perhaps unsurprising. Nevertheless, our results suggest that *N. pulcher* will endure mild hypoxia—along with the potential reduction in aerobic scope (which represents the aerobic energy production available to power routine locomotion, behavior, and many other traits important for fitness) that hypoxia has been shown to cause in other investigations (Crans et al. 2015)—in order to associate with a conspecific.

Interestingly, EPOC correlated well with O_2 preference behaviors, as individuals that accepted lower O_2 levels to associate with a conspecific used more anaerobic metabolism during exhaustive exercise. Tissue anaerobic capacity (e.g., lactate dehydrogenase activity) is often correlated with hypoxia tolerance (Mandic et al. 2013; Borowiec et al. 2015) and influences the outcome of contest behavior in many species (e.g., decapod crustaceans, redbelly tilapia, and copperhead snakes; Briffa and Sneddon 2007) but not all species (e.g., Siamese fighting fish; Regan et al. 2015). In our study, it is possible that fish with a greater propensity for using anaerobic metabolism suffered less during the repressive effects of hypoxia on aerobic metabolism and could thus remain in deeper hypoxia to associate with a conspecific. Metabolic physiology may therefore underpin some of the natural variation observed in social behavior and how social behavior is affected by environmental stressors.

The results of our study suggest that there are important interactions among social behavior, abiotic environmental conditions, and physiological function and performance. Our research emphasizes the importance of assessing integrated traits when considering responses to environmental stressors. Moreover, as aquatic habitats are increasingly affected by global change, understanding the links between responses to abiotic stressors (such as hypoxia) and behavioral disruption (such as reduced social affiliation and grouping) will help prepare us to deal with future ecological challenges.

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APPENDIX

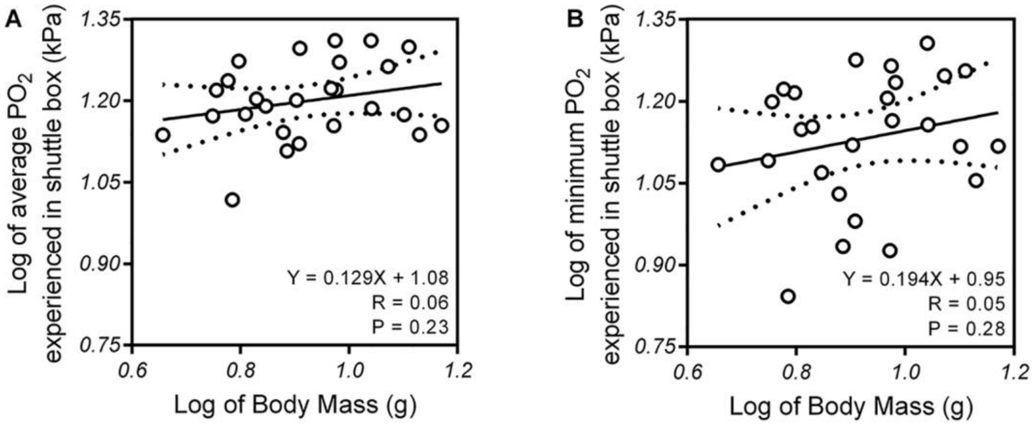

Figure A1. Effect of fish body mass on the average partial pressure of oxygen (P_{O_2}) experienced in the shuttle box (*A*) and the minimum P_{O_2} experienced in the shuttle box (*B*). Statistics for the regression line are detailed in each panel. Dotted lines indicate 95% confidence intervals.

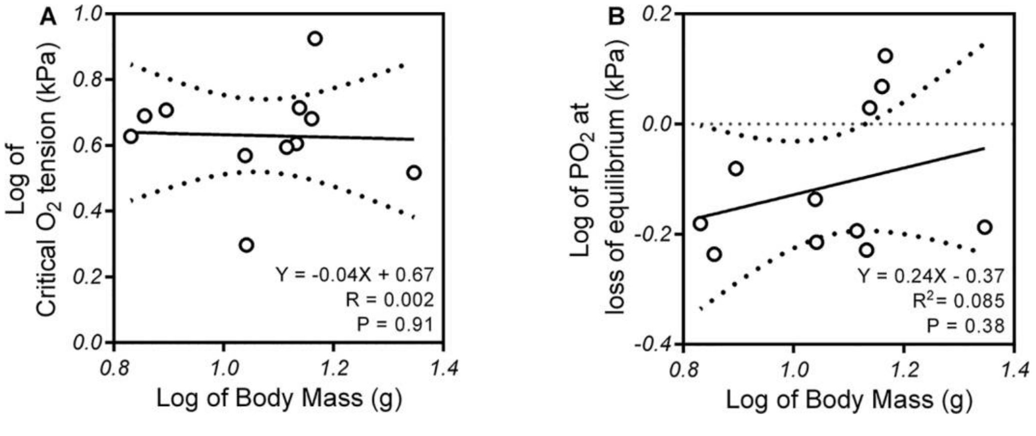

Figure A2. Effect of fish body mass on critical O_2 tension (*A*) and partial pressure of oxygen (P_{O_2}; *B*) at loss of equilibrium. Statistics for the regression line are detailed in each panel. Dotted lines indicate 95% confidence intervals.

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