Exercise changes behaviour

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Summary

1. Exercise, which may be defined as bodily movement produced by the contraction of skeletal muscle and which increases energy expenditure above basal levels, is essential for fitness-related activities such as foraging, migration and dispersal. The frequency with which individuals engage in exercise depends on a range of intrinsic and environmental factors. Exercise itself can modify behaviour by inducing endocrine changes and by a training effect that increases physiological capacities.

2. Here, we test the hypothesis that exercise changes behaviour and that there is positive feedback so that performing exercise increases the likelihood of performing exercise-related behaviours.

3. We show that there was a training effect that improved sustained swimming performance of mosquitofish (*Gambusia holbrooki*) exposed to chronic exercise in flowing water for 4 weeks compared to sedentary controls. Exercised fish also became bolder and were quicker to leave a refuge, showed an increasing tendency to explore unfamiliar environments, and were more aggressive than sedentary fish. These physiological and behavioural changes reverted back to control levels when exercised fish assumed a sedentary lifestyle again.

4. We used the calcium channel blocker nifedipine to decrease swimming performance of exercised fish to control levels, which allowed us to determine whether increased locomotor capacity per se influences behaviour. We show that reducing the swimming performance of exercised fish also reduced exploration and aggression, but it did not affect the latency to leave a refuge (boldness).

5. Our data show that behavioural phenotypes are not fixed. Exercise changes behaviour and may thereby alter interactions between individuals and dispersal. Environmental changes that demand increased levels of exercise induce behavioural responses that are likely to increase the success of individuals under these changed conditions.

Key-words: aggression, boldness, dispersal, locomotion, personality, physical activity, training effect

Introduction

In humans, exercise has pronounced health benefits by countering chronic diseases such as obesity and diabetes (Hawley & Holloszy 2009) and by improving cognitive function (Winter *et al.* 2007). However, exercise and locomotion are also essential components of the ecology of other animals in their natural environment. Aggressive behaviour to achieve dominance in social species, as well as reproductive behaviour, rely on endurance and sprint locomotor performance (Husak *et al.* 2006; Mowles, Cotton & Briffa 2010; Rudin & Briffa 2012). Migration and dispersal are directly dependent on endurance exercise

(Brill 1996; Gill *et al.* 2009). 'Exercise' may be defined as physical activity that involves contraction of skeletal muscle and increases energy expenditure above basal levels (Booth, Roberts & Laye 2012). The intensity and frequency of exercise is such that it can lead to a training effect which manifests as improved physical performance (Booth, Roberts & Laye 2012). In a natural context, exercise usually involves movement that is supported by sustained locomotor performance (Nathan *et al.* 2008). Migration in salmon, for example, represents sustained locomotor performance that uses up to 90% of maximal aerobic scope (Eliason *et al.* 2011).

The extent to which an individual engages in exercise-related activities depends on a variety of intrinsic factors, including satiation and motivation, and extrinsic factors,

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such as the ecological and environmental contexts (Nathan *et al.* 2008). But beyond this, there remains considerable variation between individuals in the expression of behaviour. One key source of this variation is the existence of behavioural phenotypes, or personalities, that are different from each other but consistent within individuals across contexts. Personalities are defined on the basis of individual behaviours such as exploration, aggression and boldness, which fall within the different behavioural dimensions of extraversion, openness, conscientiousness, neuroticism and agreeableness (Sih *et al.* 2004; Briffa & Weiss 2010; Dingemanse *et al.* 2010; Careau & Garland 2012). However, exercise may feed back to modulate behaviour. For example, physiological capacities may constrain locomotor performance and thereby also curtail behaviour of individuals (Mowles, Cotton & Briffa 2010). Regular exercise, on the other hand, can lead to a training effect, which enhances cardiorespiratory, metabolic and muscular function, and thereby increases locomotor performance (Davison 1997; Anttila *et al.* 2011; Atherton & Smith 2012). Even low levels of regular physical activity stimulate a multitude of physiological changes, from gene expression to whole animal oxygen consumption (Seebacher & Glanville 2010). In addition to affecting physiological capacities, exercise is accompanied by endocrine changes such as increased levels of endorphins, serotonin and testosterone, each of which can profoundly influence behaviour (Schwartz & Kindermann 1992; Meeusen & Demeirleir 1995; Emerson *et al.* 2000). Exercise can therefore induce behavioural plasticity (Hoffmann, Thoren & Ely 1987; Kramer, Erickson & Colcombe 2006; Briffa & Sneddon 2007) and potentially cause differences in relative behavioural expression between individuals if their demands for exercise vary.

Intriguingly, exercise-induced physiological responses could cause behavioural changes that reinforce the tendency of individuals to perform behaviours that require high levels of exercise. For example, increased levels of testosterone resulting from exercise may subsequently increase the motivation of individuals to perform exercise, improve athletic performance and increase aggression (Clobert *et al.* 2009; Wood & Stanton 2012). Similarly, increased locomotor performance resulting from training effects may make animals more likely, or at least less constrained, to disperse or migrate (Alerstam, Hedenström & Åkesson 2003). In other words, individuals that have started to perform exercise-related behaviours, for example as a result of changes in environmental water flow in aquatic organisms, become more likely to continue to do so.

Our aim was to determine whether regular exercise changes behaviour in the mosquitofish *Gambusia holbrooki*. We tested the hypothesis that boldness, tendency for dispersal and aggression are expressed to a greater extent in individuals that perform a regular level of exercise by living in flowing water relative to sedentary individuals living in still water.

Materials and methods

ETHICS STATEMENT

All procedures were approved by the University of Sydney Animal Ethics Committee (approval # L04-6-2008-3-4825), and fish were collected under the NSW Department of Primary Industries Scientific Collection Permits # P08/0028-1·0 and # P08/0028-2·0.

STUDY ANIMALS AND ACCLIMATION

Adult mosquitofish (*Gambusia holbrooki*, <25 mm) were caught from the wild at Manly Dam in the Sydney metropolitan area, Australia (33º46′35″S, 151º14′51″E). Fish were housed at 25 °C, with a 14 h light, 10 h dark photoperiod throughout the holding period and all experiments. Water was filtered and aerated continuously, and a 30% water change was performed each week. Fish were monitored and fed to satiation on standard aquarium fish flakes each morning (Optimum Freshwater Flakes, New Life Spectrum, Homestead, FL, USA). Only males were used in trials, because the state of pregnancy affects swimming performance of females, which store sperm and are almost always pregnant upon reaching maturity (Pyke 2008). However, during holding periods before and between experiments, males and females were housed together to provide social conditions similar to those at the capture sites. After 1 week in captivity, experimental fish were weighed, measured and fish used to test for repeatability of behaviour were tagged by injection of inert polymer dye under the skin, while lightly anaesthetised with clove oil (1 mL L^{-1}). Fish were given 5–7 days recovery before acclimation treatments commenced, and we observed no side effects of tagging on fish. All trials were observed remotely with a camera connected to a computer.

We weighed fish with a balance (PL602-S, Mettler Toledo, Melbourne, Vic, Australia) and measured their standard length using calipers. We calculated condition factors as $k = 100$ M L^{-3}, where M = mass in g and L = standard length in cm for fish in all experimental groups before and after treatments. There was no significant difference in condition factor between exercised fish and control fish (*t*-test $t = 0.369$, $P = 0.548$, mean = 1·671, SE = 0·026) and no significant effect of time in captivity on condition factors of the fish ($t = 0.026$, $P = 0.873$). Individual fish were used only once in experiments, except for measurements of repeatability of behaviour when individuals were used twice. We used a total of 594 fish, of which 280 fish were used in the experiments testing for the effect of exercise and detraining on swimming performance and behaviour, 280 fish were used in the nifedipine experiments and 34 were used to measure repeatability of behaviour; more details of sample sizes are given below. At the end of all experiments, fish were euthanized with an overdose of buffered MS222 (0·25 g/L, pH 7·0; Sigma, Sydney, NSW, Australia).

EXERCISE, CONTROL AND DETRAINING TREATMENTS

Fish were kept in opaque plastic tanks (645 × 423 × 276 mm; 35 males and 35 females in each tank at the start of each experiment) that contained an elliptical flume created with plastic barriers to exclude fish from the centre and corners of the tank. In the exercise treatments (*n* = 5 tanks per experiment), a submersible pump (5W Resun SP-900, China) placed at one corner of the tank created a water flow (6·6 cm s^{-1}; *c.* 3–4 body lengths s^{-1}, measured with a flow meter [FP101, Global Water, Gold River, CA, USA]) against which fish had to swim continuously. The flow rate was within an ecologically relevant range, and *G holbrooki* inhabit waters moving at up to 20 cm s^{-1} (Congdon 1994). Control tanks (*n* = 5 per experiment) were identical to the exercise tanks but did not contain the pump. After 28 days, swimming and behavioural

responses were determined using 13–15 fish per tank from both the control and exercise treatments. The remainder of the fish were kept in the experimental tanks but the pumps in the exercise tanks were turned off and fish from both control and exercise treatments were kept for a further 21 days in still water to test whether any effects of exercise could be reversed (=detraining treatment). We extended the still-water control treatment for the duration of the detraining period to ensure that the increased period in captivity did not affect responses. Treatments contained a slightly larger number of fish than we used in experiments in case of mortality.

SWIMMING PERFORMANCE

We determined critical sustained swimming speed (U_{crit}; Kolok 1999) of 20 fish per treatment group (control, exercise, detraining control, detraining exercise) according to the published protocols (Sinclair, Ward & Seebacher 2011). U_{crit} is a standard measure of prolonged swimming performance in fish during which the animals are forced to swim against an incrementally increasing water flow until fatigue (Kolok 1999). U_{crit} was measured in a swimming flume consisting of a 150×50 mm clear plastic (Perspex) cylinder tightly fitted over the intake end of a submersible inline pump (12V DC, iL500, Rule, Hertfordshire, UK). A plastic grid separated the Perspex swimming flume from the pump, and a bundle of hollow straws at the inlet helped maintain laminar flow; the flume was contained in a plastic tank ($645 \times 423 \times 276$ mm). We used a variable power source (MP3090, Powertech, Sydney, NSW, Australia) to adjust the flow speed, which was calibrated using a flow meter (FP101, Global Water, Gold River, CA, USA). Fish were allowed to settle in the flume for 20 min at a flow rate of 6 cm s^{-1} before flow rates were increased. U_{crit} was determined as $U_{crit} = U_f + T_f/T_i \times U_i$, where U_f is the highest speed maintained for an entire interval ($T_i = 5$ min), T_f is the time until exhaustion in the final speed interval. A fish was defined to be exhausted when it could no longer keep its position in the water column. U_i is the speed increment (2 cm s^{-1}, or *c*. 1 body length s^{-1}).

LATENCY TO LEAVE A REFUGE AND EXPLORATION

Fish ($n = 20$ per treatment group) were placed individually into one corner of a 730×730 mm tank behind a transparent, perforated screen for 5 min before trials commenced. The tank was filled with aged water to a depth of 100 mm and one litre of water from the mosquitofish holding tank was added to introduce familiar chemical cues into the water. The screen was then lifted allowing fish access to a refuge, consisting of sand and plastic weed, and to the rest of the tank beyond the refuge; the tank outside the refuge was an open field that contained no structures. Fish always entered the refuge first, that is the whole body of the fish was within the area demarcated by the sandy substrate and weed. We determined boldness as the latency to leave the refuge by measuring the time taken from the moment the screen was lifted until the whole body of the fish was in open water (Wilson, Godin & Ward 2008).

Exploration was recorded in an open-field test by measuring the distance moved by the fish in the first 50 s after leaving the refuge. A grid on the bottom of the tank marked 73×73 mm sections, and distance was measured as the number of times the fish crossed over a line of the grid. We verified that the number of lines crossed was tightly correlated with the total distance travelled by fish under control conditions in pilot trials using 35 fish not elsewhere used in experiments. We filmed fish and analysed the total distance moved and the number of lines crossed in video analysis software (Tracker; available at www.opensourcephysics.org); $n = 35$ individuals; $R^2 = 0.86$, $F_{1,35} = 204.5$, $P < 0.0001$).

We ascertained that latency to leave refuge and exploration were repeatable over time by conducting a separate experiment comparing responses of the same fish kept under control conditions twice, 27 days apart ($n = 34$ individuals). Repeatability scores were calculated according to the methods given in Lessells & Boag (1987).

AGGRESSION

Pairs of size-matched males (difference in standard length <2 mm) from control or exercise treatments ($n = 15$ pairs [i.e. control-control and exercise-exercise pairs] per treatment group) were filmed during dyadic interactions. Each pair was introduced into a $127 \times 92 \times 95$ mm tank containing 200 mL of water sourced from a tank that previously housed mosquitofish. Fish were filmed for the duration of the trial (with a Logitech C905, USA, camera). We recorded the time from when the fish first entered the tank until the first aggressive behaviour occurred and then scored aggressive behaviours for 15 min. We recorded the frequency with which displays, lunges, chases, bites and holds were displayed, which represent behaviours along a scale of escalating aggression, that is, displays are least aggressive and holds are most aggressive. A display was defined as a lateral undulation of the body while aligned parallel to the opposing fish. A lunge was defined as a fast, but not sustained, movement directly towards another fish without, however, making contact. A chase was defined as swimming directly towards a conspecific resulting in the approached fish swimming away; the chased animal could escape the aggressor, and this escape behaviour ended the interaction. A bite was defined as an individual closing its mouth around any part of another fish but letting go immediately. A hold was defined as an individual closing its mouth around any part of another fish and not letting go immediately; during all observation periods, fish only bit or held the fins of other fish so that these behaviours did not result in injury other than fin damage. Fin damage is a naturally occurring phenomenon that does not cause bleeding, and fins regenerate over time (Johnson & Denton 1977). Fish were observed continuously during behavioural trials, and we planned to terminate trials if injuries other than fin damage occurred; in the event, this was not the case in any of the trials. All observations were made by a single observer so that scores were consistent.

NIFEDIPINE TREATMENT

We used the calcium channel blocker nifedipine to reduce the locomotor performance of exercised fish to control levels. Nifedipine is a muscle-specific calcium channel blocker which reduces force production in both cardiac and skeletal muscle (Foster *et al.* 1983), and administration of nifedipine results in a reduction in swimming performance in fish (Seebacher, Pollard & James 2012; Seebacher & Walter 2012). Hence, nifedipine treatment can be used to reduce the increased swimming performance in exercised fish back to control levels. We were thereby able to eliminate the differences in locomotor performance between control and exercised fish, which allowed us to test the hypothesis that behavioural changes following exercise resulted from increased locomotor capacity. In other words, we predicted that if increased locomotor performance influenced behaviour, behavioural differences between control and exercise treated fish should disappear following nifedipine treatment.

We repeated the exercise and control treatments described above with a new set of fish. After 28 days in the exercise or control treatments, swimming performance, latency to leave refuge and exploration, and aggression were measured in fish either exposed to nifedipine (50 μmol L^{-1} in DMSO) or to the DMSO vehicle only (Seebacher, Pollard & James 2012). Immediately upon removal from their treatment tanks, fish were exposed to nifedipine or DMSO in water from their treatment tanks for

45 min before behavioural or swimming trials were conducted. Nifedipine acts within minutes and has a half-life of 10–12 h in mammals (Foster *et al.* 1983), and all measurements were completed within 1–2 h of treatment. The sample sizes were the same as in the experiments described above for similar response measures, that is $n = 20$ for swimming trials, $n = 20$ for measures of latency to leave refuge and exploration, and $n = 15$ pairs for measures of aggressive interactions within each of the four treatment groups (control plus nifedipine or DMSO only, and exercise plus nifedipine or DMSO only).

STATISTICAL ANALYSES

We used independent *t*-tests to compare swimming performance, latency and exploration between exercised fish and control fish, before and after detraining. To test whether time in captivity affected responses during the exercise and detraining periods, we compared swimming performance of control groups immediately after exercise training to their performance time after the detraining period (i.e. after 28 days and 49 days in treatments). We used two-way analyses of variance to determine the effects of exercise and nifedipine on swimming performance, latency to leave refuge and exploration. Behavioural data were square root-transformed to achieve homogeneity of variance. We analysed simple main effects by *t*-tests following ANOVA (Quinn & Keough 2004). The repeatability of latency to leave refuge and exploration behaviours within individuals were analysed by Spearman rank correlations. The same individuals were measured 27 days apart, and we correlated the ranks of individuals within the group of 34 fish between the first and second trials. The average responses did not differ between the two trials (paired *t*-tests: latency $t = 0.33$, $P = 0.75$; exploration $t = -0.075$, $P = 0.94$).

The frequency of the different types of behaviours representing escalating aggression (display, lunge, chase, bite, hold) was compared between control and exercise fish by generalized linear models with a Poisson loglinear distribution with exercise/control as fixed factor and the different types of behaviour as dependent variables. Similarly, we compared the total number of aggressive interactions displayed between nifedipine-treated and untreated controls by generalized linear model. We compared the time taken until the first aggressive behaviour was displayed between control and exercised fish with independent *t*-tests. All statistical analyses were performed in SPSS 20 software (IBM Software, New York, NY, USA).

We used the truncated-product method (Zaykin *et al.* 2002) to assess the effect of multiple comparisons on the validity of *P*-values. When multiple tests are performed, the probability of achieving at least one significant result is greater than the significance level for each test. Therefore, there is an increased probability of rejecting a null hypothesis when it would be inappropriate to do so as the number of statistical tests increases (Zaykin *et al.* 2002; Moran 2003). The truncated-product method considers the distribution of all *P*-values to provide a table-wide *P*-value for the overall hypothesis that there is no bias in *P*-values which could lead to the inappropriate rejection of null hypotheses (type 1 errors)(Zaykin *et al.* 2002). In our experiments, multiple hypothesis testing did not bias the statistical results presented here ($P < 0.0001$). All data are presented as means ± SE.

Results

EXERCISED FISH HAVE GREATER SWIMMING PERFORMANCE

Exercised fish had significantly greater U_{crit} than control individuals ($t_{37} = -2.30$, $P < 0.01$; Fig. 1). The exercise-
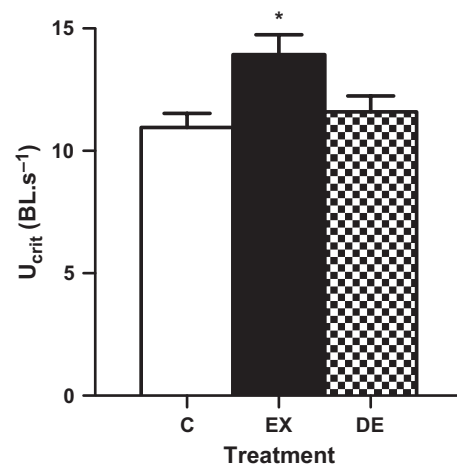

Fig. 1. Chronic exercise (EX) induced by swimming against a current increased critical sustained swimming speed (U_{crit}; means ± SE) compared to control fish (C). The exercise-induced increase in U_{crit} was reversed after exercised fish were held in still water for 21 days (detraining, DE). Significant differences between treatments are indicated by an asterisk, and $n = 20$ fish per treatment group.

induced increase in U_{crit} was reversed after fish were kept in still water for 21 days detraining (control cf detraining: $t_{38} = 0.80$, $P = 0.43$; Fig. 1). U_{crit} of control fish did not change during the detraining period (mean = 12.45 ± 0.87 [SE]; control after 28 days vs. 49 days, $t_{19} = -1.54$, $P = 0.14$).

EXERCISE INCREASES BOLDNESS AND TENDENCY FOR EXPLORATION

Fish from the exercise treatment displayed significant differences in two repeatable personality traits compared to control fish that were kept in still water. Exercised fish were quicker to leave the refuge than control fish, indicating increased boldness ($t_{48} = 2.05$, $P < 0.05$, Fig. 2a). Exercised fish also travelled a greater distance in a novel area after leaving their refuge than control individuals, indicating increased exploration and tendency for dispersal ($t_{48} = -2.44$, $P < 0.02$, Fig. 2b). Under control conditions, the expression of both 'latency to leave a refuge' and 'exploration' had high repeatability scores of 0.83 and 0.79, respectively, and the behaviours were significantly correlated between trials (latency, Spearman $r = 0.48$, $P < 0.01$; Fig. 2c; exploration, Spearman $r = 0.42$, $P < 0.02$; Fig. 2d), which indicates that the traits were stable over time within the same treatment.

These changes in behaviour following exercise were reversible, and when fish were kept for 21 days in still water following the exercise treatment (detraining treatment), detrained fish were slower to leave refuge than control fish ($t_{48} = -2.62$, $P < 0.02$, Fig. 2a) and there was no significant difference in exploration ($t_{48} = -0.22$, $P = 0.83$, Fig. 2b) between detrained and control individuals. In the control groups, the expression of these
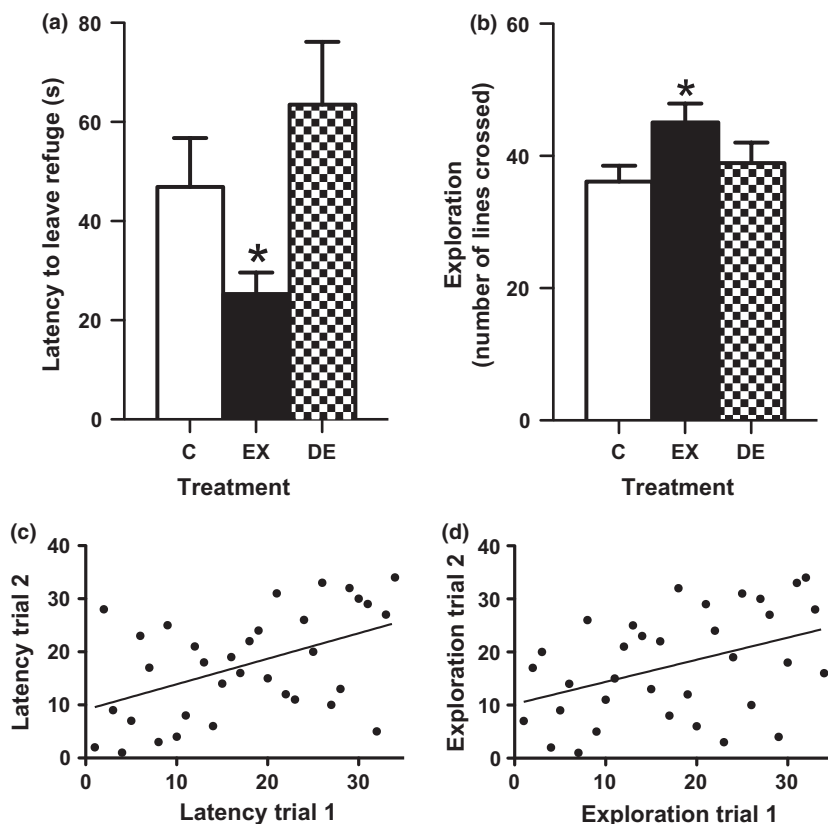
Fig. 2. Boldness and the tendency for dispersal (means ± SE) increased following chronic exercise (EX). The latency to leave a refuge was significantly shorter in exercised fish compared to controls (C; panel a), and exercised fish moved further in an open arena (exploration) than sedentary control fish (panel b). Returning exercised fish to still water for 21 days (detraining, DE) reversed the exercise-induced changes in boldness (panel a) and exploration (panel b). Significant differences are indicated by an asterisk and $n = 20$ fish per treatment group. The ranking of individuals ($n = 34$ fish) with respect to latency to leave a refuge (panel c) and exploration (panel d) was repeatable over time, and ranks of individuals were significantly correlated (Spearman $r = 0.42$ for latency and $r = 0.41$ for exploration) between two trials that were conducted 27 days apart.
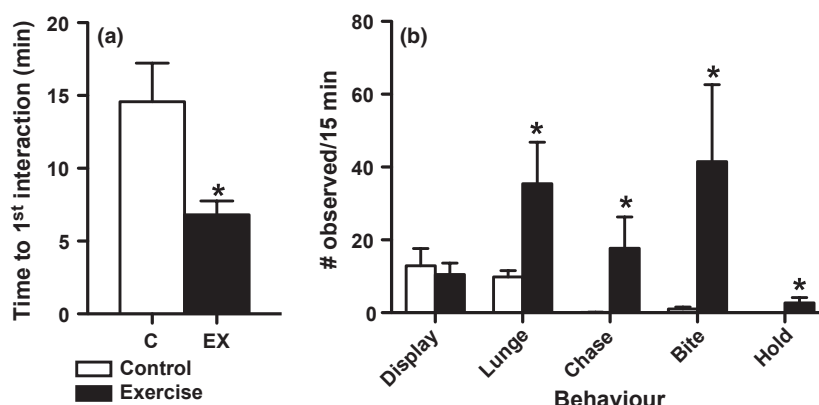

Fig. 3. Aggression increases following chronic exercise. Exercised fish (solid bars) initiated aggressive interactions significantly faster than control (open bars) fish (a; means ± SE) and escalated aggression to a greater extent (b; means ± SE). The aggressiveness of behaviours increased from displays to lunges, chases, bites, and finally holds, and exercised fish displayed lunges, chases, bites and holds significantly more often within a 15-min trial period than sedentary control fish (b). Significant differences are indicated by asterisks, and $n = 15$ pairs per treatment group.

behaviours did not change over the experimental time period (latency, mean after 49 days = $40.33 ± 7.40$; control 28 days vs. 49 days, $t_{24} = -1.70$, $P = 0.10$; exploration, mean after 49 days = $38.04 ± 2.65$; control 28 days vs. 49 days, $t_{24} = -0.68$, $P = 0.50$).

EXERCISE INCREASES AGGRESSION

In staged dyadic trials, pairs of exercised fish engaged in aggressive interactions sooner ($t_{28} = 2.77$, $P < 0.01$,

Fig. 3a) than pairs of control fish. Exercised fish also displayed greater numbers of aggressive acts within 15 min trials and escalated aggression to a greater extent than control fish (Fig. 3b). There was no significant difference in the number of low risk displays ($\chi^2 = 3.48$, $P = 0.062$), but lunges ($\chi^2 = 189.9$, $P < 0.0001$), chases ($\chi^2 = 31.06$, $P < 0.0001$), bites ($\chi^2 = 203.22$, $P < 0.0001$) and holds ($\chi^2 = 38.48$, $P < 0.0001$) were displayed significantly more often by exercised fish compared to control fish (Fig. 3b).

DECREASED ATHLETIC PERFORMANCE REDUCES EXPLORATION AND AGGRESSION BUT NOT BOLDNESS

As above, the exercise treatment resulted in faster U_{crit} ($F_{1,76} = 4.72$, $P < 0.03$) before nifedipine treatment. Nifedipine reduced swimming performance in both control and exercised fish, and nifedipine-treated fish had significantly lower U_{crit} ($F_{1, 76} = 8.40$, $P < 0.005$, interaction $F_{1,76} = 0.021$, $P = 0.89$; Fig. 4a). Administration of nifedipine allowed us to manipulate exercised fish in such a way that their U_{crit} was reduced to control levels (control vs. exercise $t_{38} = 0.51$, $P = 0.61$; Fig. 4a).

Nifedipine reduced exploration in exercised and control fish (main effect $F_{1, 76} = 7.50$, $P < 0.01$; interaction $F_{1,76} = 0.12$, $P = 0.73$; Fig. 4b). As above, exercised fish not treated with nifedipine explored further than sedentary controls ($F_{1, 76} = 4.33$, $P < 0.05$; Fig. 4b), and nifedipine treatment reduced exploration of exercised fish to those of untreated controls ($t_{38} = 0.68$, $P = 0.50$; Fig. 4b). Similarly, exercised fish not treated with nifedipine displayed more aggressive behaviours than control fish ($\chi^2 = 150.57$, $P < 0.0001$, Fig. 4c), but fish that were treated with nifedi-pine displayed significantly fewer aggressive behaviours than untreated fish ($\chi^2 = 270.67$, $P < 0.0001$, Fig. 4c). Nifedipine treatment did not affect latency to leave refuge ($F_{1, 76} = 2.39$, $P = 0.13$, interaction $F_{1,76} = 0.93$, $P = 0.34$; Fig. 4d).

Discussion

Movement of organisms is fundamental to life and understanding the constraints and motivation of organisms to move is essential to understanding processes ranging from ecosystem function to human health. The theory of movement partitions the organism-specific dimension of movement into internal state, movement capacity and navigation capacity (Nathan *et al.* 2008). We show that there is a positive feedback from movement per se on locomotor capacity, thereby decreasing constraint. Additionally, there were behavioural changes that occurred independently from locomotor capacity, which may reflect changes in motivation. Our results thereby reveal a dynamic interaction between physiology and behaviour that has implications for behavioural interactions and movement ecology.
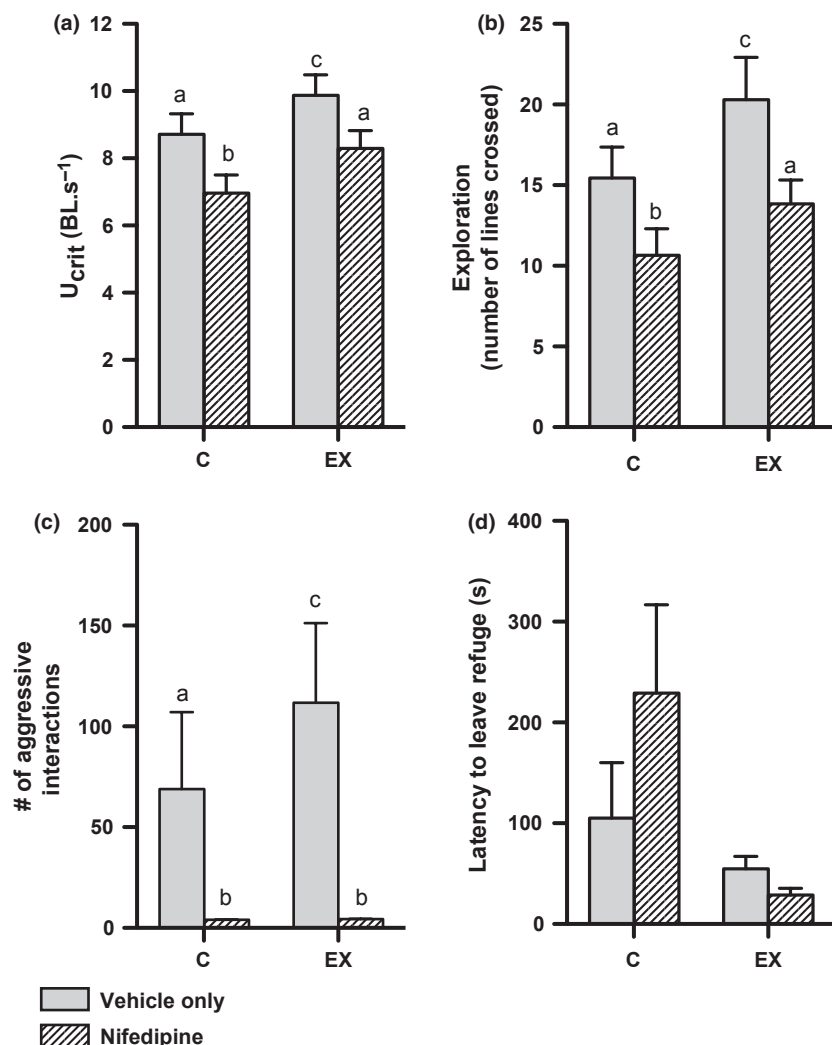

Fig. 4. Nifedipine (grey hatched bars) reduced sustained swimming performance (U_{crit}, means ± SE; a) in sedentary control (C) and exercised fish (EX) compared to fish that were administered with the vehicle (DMSO) only; U_{crit} of exercised fish that were given nifedipine was reduced to untreated control levels (a). Following the same pattern as U_{crit}, nifedipine reduced exploration, and exploration in exercised fish was the same as in control fish following nifedipine treatment (b). Nifedipine also reduced aggression (panel c), but it had no effect on the latency to leave refuge (d). Different letters indicate significant differences between treatment groups, and $n = 20$ fish per treatment group.

Individuals can vary in the extent to which particular behaviours are displayed and in the ability to adjust these in response to environmental change (Reale *et al.* 2007; Wilson *et al.* 2010; Webster & Ward 2011). According to the current paradigm, the ability of animals to modify their behaviour is constrained by personality (Bell & Stamps 2004; Reale *et al.* 2007; Webster & Ward 2011). Personality refers to differences between individuals in multiple behavioural traits that are expressed consistently across contexts and at different times (Briffa & Weiss 2010). These traits (boldness, exploration, activity, aggression and sociability (Sih *et al.* 2004)) are present in many species, including humans and other mammals, birds, fish and insects (Wilson *et al.* 1994; Reale *et al.* 2007; Briffa & Weiss 2010; Dingemanse *et al.* 2010). If personality is fixed, it may prevent individuals from responding adequately to new challenges and therefore result in the expression of inappropriate or maladaptive behaviours (Bell & Stamps 2004; Dingemanse *et al.* 2010). To understand this constraint, it is necessary to determine the mutability of behavioural traits that make up personality and their underlying physiological mechanisms in response to environmental change (Careau & Garland 2012).

We have shown that the expressions of some of the behaviours that together make up personality are labile within individuals and relative to other individuals. In other words, personality is not fixed within individuals, and individuals experiencing different levels of exercise display personality differences. At an ecological level, our findings indicate that fish from streams, which experience greater levels of physical activity, may be more willing to take risks compared to fish from still water. Hence, population differences in personality may reflect a reversible response to the level of exercise imposed by the different environments experienced by individuals. We have demonstrated that these behavioural differences can be mediated by changes in the physiology of the organisms in response to exercise. Exercise-induced increases in exploration and aggression were associated with improved locomotor capacity. However, our measure of boldness (latency) was not affected by locomotor performance, and its change in response to exercise was in all likelihood mediated by some other mechanism, such as endocrine changes (Schwartz & Kindermann 1992; Meeusen & Demeirleir 1995). Interestingly, the outcome of a fight in the sea anemone *Actinia equina* influenced boldness, and losers of a fight became less bold than winners. Hence, boldness was not fixed in individuals, and it changed in response to the outcome of an interaction (Rudin & Briffa 2012). The reasons for these responses are not clear, but fighting represents a demanding physical activity so that changes in boldness following fights could also be related to the exercise performed during the fight.

Physical activity is a key part of the evolutionary history of most animal species, including humans (Booth, Chakravarthy & Spangenburg 2002; Booth *et al.* 2008). We show that a sedentary lifestyle can have important implications for behaviour. Sedentary animals can become less likely to explore and less aggressive. Conversely, in active individuals, behaviours that require exercise may be reinforced. The ecological implications of these dynamics are important for dispersal, especially in invasive species such as *G. holbrooki*. Individuals of this species travel up to 800 m per day when dispersing, which means that exercise is an intrinsic part of their lifestyle (Alemadi & Jenkins 2008). Dispersal is therefore self-reinforcing and individuals that started to disperse will become more likely to continue to do so. The increased level of aggression that accompanies exercise may make dispersing individuals more likely to dislodge resident, less aggressive animals, thereby facilitating dispersal success. Even in non-dispersing animals, more active individuals will be more aggressive and may therefore gain selective advantages by improved access to resources (Briffa & Sneddon 2007).

There are broad similarities in the behavioural dimensions that comprise personality in many animals (Wilson *et al.* 1994; Briffa & Weiss 2010; Dingemanse *et al.* 2010) and fish, in particular, are an important model for behavioural research (Wilson *et al.* 1994; Webster & Ward 2011). It is interesting therefore to speculate about the implications of our results for humans. In humans, inactivity is recognized as a factor contributing to physical and mental health issues, and consequently, the healthcare industry, sports scientists and popular media promote exercise as a way of enhancing physical and mental well-being (Hoffmann, Thoren & Ely 1987; Vuori 2004; Penedo & Dahn 2005; Booth *et al.* 2008). The likelihood of humans to engage in regular physical activity depends on behaviours analogous to boldness and exploration, and their increased expression renders individuals more likely to exercise (Rhodes & Smith 2006). If exercise affects these behavioural traits in a similar way as in *G. holbrooki*, a sedentary lifestyle would make individuals less likely to exercise unless the cycle of inactivity is broken. If sedentary humans do commence exercising regularly, then the resulting training effect could reinforce individuals to continue exercising as physical activity will become progressively less challenging and may enhance personality traits that promote energetic behaviour.

Acknowledgments

Data accessibility

Data associated with this manuscript are available on Dryad Digital Repository: http://doi.org/10·5061/dryad. 905j6 (Sinclair *et al.* 2014).

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