Published in final edited form as:

Comp Biochem Physiol A Mol Integr Physiol. 2018 July ; 221: 24–31. doi:10.1016/j.cbpa.2018.03.006.

A comparison of hatchery-rearing in exercise to wild animal physiology and reflex behavior in *Aplysia californica*

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Abstract

Aplysia californica was hatchery-reared in two turbulence protocols intended to imitate the intermittent turbulence of the native habitat and to promote development of the foot muscle from the exercise of adhering to the substrate. Hatchery-reared animals in turbulence regimes were compared to siblings reared in quiet water, and to wild animals, using noninvasive assessments of the development of the foot muscle. The objective was to learn if the turbulence-reared phenotype mimicked laboratory-targeted aspects of the wild phenotype, that is, reflex behavior, swim tunnel performance, and resting oxygen consumption (MO_2). No group exhibited different MO_2 . MO_2 values for all of the compared groups of animals followed the power law, with an exponent of 0.69, consistent with this relationship throughout the animal kingdom. Turbulence-induced exercise did not affect the righting reflex or the tail withdrawal reflex, standard behavioral tests that involve the foot muscle, compared to quiet water-reared siblings. Wild individuals had significantly shorter time-to-right than all hatchery reared animals, however, wild animals did not perform better in flume tests. That turbulence-reared hatchery- or wild animals lacked superior in flume performance suggests that this species may shelter from intertidal wave energy to remain near its optimal feeding areas.

Keywords

Time to right; marine invertebrate; isometric; behavior; aquaculture; nervous system

Introduction

The marine invertebrate, *Aplysia californica* (*Aplysia*) is renowned as a model of the nervous system due to its advantages of a simple, well-mapped brain and capabilities for learning. The National Resource for *Aplysia* at the University of Miami in Miami, Florida, USA, has >25 years' experience growing this opisthobranch species for the scientific

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community (Capo et al 2002, 2003, Stommes et al. 2005). The normal habitat for this species is the rocky intertidal of the North American Pacific coast, where their red algal food is abundant (Audesirk, 1979, Carefoot, 1987). Wave energy in this environment likely promotes isometric contractions, particularly in foot muscle of *Aplysia*, during adherence to the rock substrate. This is a form of exercise that is absent in rearing conditions at the Resource. There, a gentle flow of fresh seawater with no additional sources of water movement produces less water energy, and thus would promote less isometric exercise than would the rocky intertidal. As a result of these differences, muscle mass during somatic growth, as well as sensory stimulation, may differ between hatchery and wild conditions. The goal of experiments summarized here was to estimate the difference the environment might make in the outcome of common neurophysiological protocols used on this animal model.

Isometric contractions in human skeletal muscles elicit the activity of motoneurons during muscle training, especially at the beginning of a strength improvement program (reviewed in Griffin and Cafarelli, 2005, Folland and Williams, 2007). The effect is believed to be due, in part, to enhanced recruitment of muscle fibers to the task via enhanced muscle fiber responses to neural stimuli. Rearing *Aplysia* in conditions that stimulate isometric contraction of the foot muscles similarly may affect the central nervous system. If so, the neural stimulation provided by isometric exercise could impact learning paradigms in which these animals are regularly used.

Examples in which muscle actions can recruit neurons or modulate neuronal firing patterns as part of the initiation or modulation of motor programs, respectively, exist in *Aplysia*. The neurophysiological control and modulation of the *Aplysia* buccal smooth muscle model is well studied, with many neural influences described (Weiss et al. 1978; Hurwitz et al. 2003; Brezina et al. 2003). Foot muscle neuromotor patterns focused on locomotion (Fredman and Jahan-Parwar, 1980; Jahan-Parwar and Fredman, 1980; McPherson and Blankenship, 1992; Bruno et al. 2017) have also received attention. These studies were centered on neuromuscular control that changes the length of muscles, rather than isometric contractions. Finally, there is evidence that some aspect of a turbulent water location within its normal habitat might influence how *Aplysia* respond to tactile stimuli. Carew and Kupfermann (1974) recorded that animals found in areas described as turbulent were less responsive to initial and repeated siphon withdrawal stimuli, intended to evoke habituation, than individuals found in quiet water tide pools, as though they were already habituated to tactile stimuli. These examples provide evidence that muscle activity and sensory experience provided by the local environment can influence neural activity and behavior in this model gastropod.

Aplysia were reared in conditions intended to imitate the turbulence of the rocky shoreline to begin to address the effects on foot muscle development of the routine exercise of adhering firmly to the substrate. We tested for indices of positive foot muscle development noninvasively, by challenging animals to perform at steadily increasing speeds in a swim tunnel and by measuring oxygen consumption. We monitored reflex behaviors that involve foot muscle, to learn if exercise affected their execution.

Materials and Methods

Husbandry and turbulence rearing regimes

Animals from a single cohort spawned from wild-caught brood stock were reared at ~100 individuals per 16 L cage starting several weeks post-metamorphosis and fed standard ration (<ad lib.) *Aghardhiella subulata* until age 4 months and ~5 g. Animals were placed at a density of 10/cage into the exercise protocols illustrated in Fig. 1, and fed nominally *ad lib.*, which provided satiation rations over 4 hr with little uneaten food. Three cages were set up for controls and for each of 2 exercise regimes such that n=30 for each treatment. All these animals are referred to as cohort animals.

Cohort animals were reared under 3 different protocols. Fresh, 15°C seawater flowed into cages at ~2 L per minute from a corner valve in the still water controls (Fig. 1A), or at ~1 L per minute flow from the corner valve, plus intermittent turbulent flow from either electric pumps or dumpbuckets (Fig. 1B and C). All cage bottoms were perforated with 6 discontinuous, 0.5 cm slits along the 43 cm long axis, and cages were suspended in fiberglass reservoirs to allow single-pass seawater flow. Each turbulent flow protocol, pumps or dumpbuckets, was intended to deliver approximately the same volume of fresh seawater flow as for control cages during each hr. For the pump exercise program, seawater was delivered to each pump cage during a 30-sec period at 2.8 l/min, every 5 min, from a 2400 gal/hr capacity aquarium pump set into a ~400-l reservoir located below the cages. Six, ~3-cm aperture duckbill valves placed around the inside perimeter of each rectangular rearing cage created chaotic turbulence from the pump flow, and minimized cage areas without flow (Fig. 1B, showing small animals with flared parapodia as the flow dislodged and moved them). For the dumpbucket exercise program, seawater was delivered to each dumpbucket cage via a ~3 cm diameter PVC pipe attached to a 20-l bucket suspended above the cage, that filled during the non-dump period, and discharged during ~35 sec every ~7.5 min (Fig. 1C).

Assessment schedule

The assessment scheme is shown in Fig. 2. At one month in the experiment, when the cohort animals were ~age 5 months and ~19 g (Fig. 2, row 2), mass and seawater volume displacement measurements were made on them all to estimate density. Reflex behaviors that involve the foot muscle exercised in dumpbucket and pumps exercise were assessed on 12 cohort animals haphazardly chosen from each exercise regime and still-water controls, alongside 14 wild-caught animals of unknown age that arrived in the hatchery that week, mass ~120 g. Single trials for righting (time-to-right, TTR) and tail withdrawal reflexes (TWR) were executed as in Kempzell and Fieber (2014). Flume tests for muscle performance were executed on these 36 cohort animals and on 12 of the wild animals, either the same day or the following day.

Five weeks later, at ~2 months in the experiment, when the cohort animals were age ~6 months and ~140 g (Fig. 2, row 3), morphometric and behavioral assessments were made on all cohort animals in the experiment. Flume test assessments were made on 12 cohort animals chosen haphazardly from controls and each exercise regime, alongside 12 recently

wild-caught animals of unknown age but similar mass as the hatchery animals. Oxygen consumption (MO_2) was measured in 11 of these cohort animals following their flume tests, and in 5 of the wild animals. The cohort animals reached sexual maturity 2 d prior to these final tests. Exercise rearing ceased, and all cohort animals were returned to still-water rearing but maintained as distinct groups.

Three weeks later, at age ~7 months and ~230 g (Fig. 2, row 4), morphometric and TWR measurements were made on 8 cohort animals that had been in each treatment, but that had been maintained in still water rearing conditions since the 2 month assessments.

Four months later, when the hatchery cohort was age ~11 months and ~300 g (Fig. 2, row 5), morphometric, behavioral, muscle performance and MO_2 measurements were made on 12, still water-reared (control) animals. Reflex behavior assessments indicated these animals had reached aging stage AII (Kempson and Fieber 2014).

Muscle performance flume test protocols

Individual flume tests assessing the ability of the animals to adhere to a smooth surface were executed three times during the experiment, at times that corresponded to the morphometric and behavioral measurements (TTR, TWR) described above. All flume tests were conducted at 16°C. At one month in the experiment (Fig. 2, row 2), 12 cohort animals from the still-water control and each turbulence treatment, and 12 wild animals, were placed one at a time into 5.3-l Brett-type swim chamber respirometers (Loligo Systems ApS). The working section of each of the 3 identical respirometers measured $28 \times 7 \times 7$ cm. The flume speed protocol began with orientation of the animal in the center of the flume chamber at a flow speed of 5 cm/s until it adhered to the chamber bottom, then the flume chamber lid was attached. The test consisted of 1 min at, consecutively, 10, 19, 25, 30, 35, 40, 45 cm/s, and finally 4.5 min at 50 cm/s. The protocol ran until the animal either lost contact with the bottom and was pinned by the flow to the rear wall of the chamber, or the protocol was completed (11.5 min). If the animal completed the protocol without losing its hold on the chamber bottom, it was considered a no-failure trial. Fluming experimenters were unaware of the treatment group or wild identity of animals studied.

Five weeks later, when the cohort animals were age ~6 months (Fig. 2, row 3), fluming exercise was repeated for a haphazardly chosen 12 cohort animals per treatment and 12 wild animals. Experimenters were blind to the group. Orientation of the animal in the center of the flume chamber at a flow speed of 20 cm/s adhered it to the bottom, then the flume lid was attached. The 14 min protocol was 2 min at, consecutively, 20, 35, 50, 65, 80 cm/s, and 4 min at 95 cm/s, or until the animal lost contact with the bottom and was pinned by the flow to the rear wall of the chamber. If the animal reached 14 min, water velocity was increased to 100 cm/s and held at this speed until failure. This fluming protocol was repeated on aged II, still water controls ~5 months later (Fig. 2, row 5).

Respirometry protocol

MO_2 (mg O_2 /kg/hr, or mg O_2 /hr) measurements followed the age 6 months flume muscle performance trials (Fig. 2, row 3). A subset of the flumed animals from each treatment (n=11 for control, dumpbucket, or pump animals; n=5 for wild animals) were subjected to

MO₂ trials for 4 hrs or overnight. The MO₂ trials were conducted using the AutoResp™ 2.1.0 software (Loligo Systems ApS) in combination with the swim chamber respirometers used in the flume test protocols noted previously. MO₂ was measured using a Pt100 fiber-optic probe connected to a Fibox 3 minisensor oxygen meter (PreSens Precision Sensing GmbH) equipped with temperature compensation. Intermittent respirometry was executed, with 30 min measurement loops divided into 240 s flush (F), 300 s wait (W), and 1260 s measurement (M) periods. MO₂ values for each 30 min loop were averaged to obtain mean MO₂ for each animal. The respirometers were cleaned thoroughly every few days using a dilute bleach solution to limit sources of background respiration in the system, and blank trials were conducted in each chamber over the course of the trials. Mean background respiration values were subtracted from individual animal MO₂ data prior to analyses. Water velocity during MO₂ trials was maintained at 6.6 cm s⁻¹ and temperature was maintained at the same setpoint used in the flume tests (16°C).

MO₂ trials were executed on aged II, still water controls ~5 months later (Fig. 2, row 5).

Statistics

Statistical tests were executed in the R statistical environment (Team 2013).

Results

Overview

A systematic comparison of reflex behaviors, flume performance, and MO₂ was made among the different rearing regimes of hatchery cohort animals, as well as their comparison to these parameters in wild animals, to determine if turbulence-rearing mimicked aspects of the wild phenotype. Times for the reflex behaviors of cohort animal TTR and TWR at both the 1- and 2-month assessments were consistent with these values in mature, hatchery-reared *A. californica* (Kempsell and Fieber, 2014, 2015a and b). TTR and TWR were not different among the different exercise regimes of control, dumpbucket and pump exercise cohort animals (Fig. 3B; ANOVA, log TTR at 1 month: $F(2,33)=1.54$, $p=0.229$, $\eta^2=0.086$; TWR at 1 month: $F(2,33)=0.224$, $p=0.8$, $\eta^2=0.013$; TTR at 2 months: $F(2,21)=1.013$, $p=0.38$, $\eta^2=0.088$; TWR 2 months: $F(2,21)=0.32$, $p=0.73$, $\eta^2=0.030$). The grand mean of all cohort animals $\pm$ SD was TTR of 11.7 ± 1.5 s at 1 month (mean $\pm$ SD; $n=36$), and 13.2 ± 3.2 s at 2 months ($n=62$). TWR was 14.0 ± 1.3 s at 1 month ($n=36$) and 17.0 ± 5.7 s at 2 months ($n=62$).

Reflex comparisons

TTR of wild animals was significantly shorter than all cohort treatment groups: 8.9 ± 0.02 in wild animals measured alongside the 1-month cohort treatment assessment (TTR data for 1 month were log normalized, Student's t -test, $t(38.76)=5.30$, $p=0.01$, $d=1.29$, 95% CI [0.56, 2.01]) and 9.8 ± 2.2 s in different wild animals at the 2-month assessment (Student's t -test, $t(32.53)=10.31$, $p=0.01$, $d=2.43$, 95% CI [4.17, 6.23]; $n=12$ for each set of wild animals). This is consistent with past studies comparing wild with hatchery-reared animals (Kempsell and Fieber 2014). TWR was not different between any cohort treatment or between cohort treatments and wild *Aplysia* at either 1 or 2 months in exercise (Fig. 2, rows 2-3). Furthermore, there were no differences in TWR after 2 months in exercise compared to

three weeks after return to still-water conditions (Fig. 2, rows 3 vs. 4) of controls (Student's *t*-test, $t(12.086)=0.08$, $p>0.1$, $d=0.02$, 95% CI $[-6.6, 7.1]$), dumpbucket ($t(14)=1.35$, $p>0.1$, $d=0.34$, 95% CI $[-1.99, 8.75]$) or pump ($t(10.9)=1.9$, $p>0.05$, $d=0.475$, 95% CI $[-0.84, 11.85]$) treatments.

Mass

The wild animals were of significantly higher mass than the cohort animals at the 1 month assessment (119.9 ± 14.4 vs. 18.6 ± 5.8 g, wild vs. hatchery grand mean; $p=0.001$ Wilcoxon); this was due merely to no selection for the size of the wild animals that were collected. No differences in the mass of cohort animals emerged between treatments after 1 or 2 months in exercise protocols (Fig. 3A). The mean mass of a separate set of wild animals was not different from cohort animals at their 2 months assessment (Fig. 3A; Student's *t*-test, $t(26.8)=-0.58$, $p>0.5$, $d=-0.097$, 95% CI $[-34.7, 19.5]$), moreover, estimated animal density at 1 and 2 months was not different between any cohort exercise treatment and controls, or between cohort treatment and wild animals (Fig. 3A).

Flume Performance

All wild animals and nearly all cohort animals from the turbulence and still water rearing regimes achieved at least 1 min at the maximum current speed of 50 cm/s in flume tests conducted after 1 month. The lone exception was a single control animal that failed during the second highest flow speed of 45 cm/s. Furthermore, at least half of the cohort animals in each treatment reached the end of the protocol, which included 4.5 min at 50 cm/s (Table 1), and were scored no failure. Thus no differences in flume test performance of animals were found for any treatment at 1 month in the experiment.

After two months in the exercise regimes, flume performance was assessed again. As mentioned above, masses of wild and cohort animals at the 2 months assessment were not different. It is also important to note that there were no relationships in any 2 months cohort treatment for mass of animals with either duration in the flume or fail speed (Fig. 4B and C). This observation held when considering all cohort animals combined. This implies that adherent foot muscle size, which was assumed to be proportional to mass, did not affect flume performance of either control, dumpbucket or pump animals at 2 months. Therefore performance in each treatment can be considered independent of the mass of animals in that treatment.

The proportions of animals achieving fail speeds greater than 50cm/s were compared via pairwise Chi-Square test between 2 month cohort treatment groups, wild animals, and aged controls, with Bonferroni correction for multiple testing ($\alpha=0.05/10$). No comparison proved significant. Comparisons between dumpbucket and control and between aged II control and 2 month control, however, revealed skewed confidence intervals (99.5% CI $[-1, 0.04]$ and $[-1, -0.08]$) and large effect sizes (Cramer's $V=0.44$ and 0.49), suggesting the sample size was too small to detect differences in these specific cases. No significant differences in total duration in the flume was observed between any cohort treatments, or between treatments and wild animals (Kruskal-Wallis rank sum test, $X^2(3, n=36)=3.1$, $p>0.1$). Tests of duration at fail speed failed both Shapiro-Wilk normality test and the Fligner-Killeen test of

homogeneity of variances and thus was not assessed. Although TWR involves the foot muscle, TWR of individual animals was not significantly correlated with their flume performance (data not shown).

Flume duration and fail speed *were* dependent on mass in aged II animals. Mass was inversely correlated with these parameters (Fig. 4B and C, insets). The aged II cohort animals tested were reared in still water, and at ~300 g were twice the mass of the 2-months assessed cohort animals. They achieved significantly greater durations at 50 (Fig. 4A) and 65 cm/s flume speeds (data not shown) compared to their younger sibling, still water controls. Five of 8 (62%) Aged II animals reached 80 cm/s, whereas only 5 of 36 cohort animals tested after 2 months in the treatments (14%) reached this speed ($X^2(1, N=41) = 6.3, p = 0.05, \phi = 0.39, CI [0.056, 0.92]$, data not shown).

MO₂

A positive linear relationship with a slope near 0.7 emerged when $\log_{10}(\text{MO}_2)$ was expressed as a function of $\log_{10}(\text{mass})$, regardless of treatment (Fig. 5). There were no differences in MO₂ with treatment. Furthermore, no patterns were evident between an individual animal's MO₂ and performance in flume tests, for any group.

Discussion

Turbulence in pump or dumpbucket rearing protocols provided eight weeks of isometric foot muscle training that was expected to have induced development of Aplysia foot attachment muscles. Changes in mass of the foot muscle were not evaluated in this study, however, flume performance and MO₂ were used as proxies for measuring the effects of turbulence-induced exercise on muscle development. No variable among exercise treatment, advanced age, or an origin as wild was associated with distinctive flume performance. Neither were they suggestive of attachment muscle that was developed by exercise. Coincidentally, neither exercise treatment nor age produced a distinctive resting MO₂ that might have been expected to reflect differences in foot muscle mass.

Expectations of MO₂ differences in wild and hatchery Aplysia in turbulence or still water stemmed from studies in humans and fish suggesting that respiration becomes more efficient in exercise. Holloszy and Coyle (1984) summarized studies documenting increased mitochondrial respiratory capacity in exercised human skeletal muscles, including the first study to demonstrate an increase in mitochondrial content (Holloszy, 1967). Similarly, white muscle of exercise-trained fish exhibit higher oxidative capacity and also increased contraction efficiency (Anttila et al 2006; 2008). Boska (1994) summarized research describing how the increased mean rates of ATP production in skeletal muscle during isometric force development can derive from all 3 sources of creatine kinase activity, anaerobic glycolysis and oxidative phosphorylation. Moreover, endurance training supported faster rates of muscle oxidative phosphorylation (Lanza et al. 2005). Although such studies do not always demonstrate that MO₂ varies from resting rate during exercise or after a fitness regime, they document that exercise increases both the efficiency and rate of oxygen use by skeletal muscle. Endurance exercise-trained human subjects exhibited increased skeletal muscle fiber diameter, and shifts in fiber type abundance that suggested a shift in

expression of myosin heavy chain isoform (Coggan et al. 1992), which may account for aspects of the changes in mitochondrial respiration rate and efficiency. These changes in muscle respiration predict, at least, differences in resting MO_2 between animals that exercise vs. those that do not.

Returning to mollusks, Kraffe et al. (2008) reported that valve adductor muscle of scallops metabolically taxed by the demands of spawning displayed increased standard metabolic rates and thus these animals had higher O_2 maintenance requirements than immature scallops. Although our study focused on resting metabolic rate and did not measure MO_2 during fluming trials, it is apparent that changes in muscle, such as in exercise, or activities that diminish energetic reserves, can influence resting MO_2 . Greater time in exercise may have revealed differences in resting MO_2 between turbulence treatment *Aplysia* and still water siblings. Fluming MO_2 measurements vs. resting MO_2 for each treatment also may have revealed differences between exercised and unexercised hatchery cohort animals, and perhaps compared to wild specimens. Finally, there may be little similarity between the energetics of vertebrate skeletal muscle and the network of longitudinal and transverse muscle fibers that form the foot muscle of *Aplysia*.

Aplysia TWR recruits foot and tail adherence muscles used in escape locomotion (Jahan-Pawar and Fredman, 1979; Flinn et al. 2001). The same muscles are likely recruited in adherence to the substrate, such as in flume tests. Only three pedal nerves innervate the anterior, middle, and posterior portions of the foot (Jahan-Pawar and Fredman, 1978a). Thus it was presumed that fast TWR would correlate with superior adhesion, using flume performance as the proxy. It was also expected that wild animal TWR would be similar to pump or dumpbucket TWR meant to mimic the wild environment. Neither expectation was observed. TWR of wild animals was most similar to still water-reared controls. Dumpbucket animals, with unremarkable TWR, were superior in the flume.

A potential explanation lies in how TWR and adhesion are commanded neurophysiologically. Escape locomotion is initiated and maintained centrally, while adhesion is more a peripheral phenomenon (Jahan-Pawar and Fredman, 1978a, b, and c, 1979). Escape locomotion is complex, but it includes a pattern consisting of the animal detaching its tail and balling up its posterior while attached at the head (e.g., the tail shortening of TWR), and then galloping forward. This pattern is commanded by the pedal and cerebral ganglia, and modulated by the pleural ganglia, which also relay information from the pedal to the cerebral ganglia (Jahan-Pawar and Fredman, 1979). Coordination between the 2 sides of the foot is achieved via the pedal commissure connecting the left and right pedal ganglia.

Suction, on the other hand, is normally inhibited centrally by the cerebral and pleural ganglia (Jahan-Pawar and Fredman, 1979). Suction is commanded by the excitatory output of motoneurons in the pedal ganglia responding to sensory information conveyed via the three pedal nerves (Jahan-Pawar and Fredman, 1978a). The commissures connecting pedal with pleural ganglia, and pedal with cerebral ganglia relay information from the periphery, to coordinate this output to the foot muscles. Therefore TWR may not predict expected differences in foot muscularity.

It may be a misperception that wild animals have superior muscle tone advantageous in adherence in flow, or that characteristics of wild *Aplysia* have any bearing on TWR. Wild animals that survive may do so because they are adapted, or have a learned ability to minimize exposure to wave energy that might dislodge them from their feeding areas. They may survive by sheltering in areas where they are shielded from waves, for example, rather than by achieving superior muscularity. TTR results support this idea, with significantly shorter times in this reflex in wild animals. Fast righting and attachment after dislodgement from the rocky substrate might also be adaptive or a learned survival strategy in the wild.

Finally, age and the presumed attendant decline in muscle tone were mitigated by larger mass and foot area in achieving better flume performance in Aged II cohort animals. Greater mass shielding an animal's body from the brunt of the flow may have been a factor in aged animals' improved flume performance.

Published MO_2 data in opisthobranchs are sparse, but offer confirmation to our measurements of 19-32 mg $\text{O}_2/\text{kg/hr}$ in one-day fasted, *A. californica* at 15°C. Carefoot (1987) studied MO_2 in Hawaiian *A. dactylomela* fed the red alga *Acanthophoru spicifru*, after a one-day fast. Conversion of this author's data in ~70-120 g wild animals estimated MO_2 values of approximately 25-43 mg $\text{O}_2/\text{kg/hr}$ at 25°C. Bedford and Lutz (1992) estimated rates of 5.7 mg $\text{O}_2/\text{kg/hr}$ in one-day fasted *A. californica* at 22°C, and of 53 mg $\text{O}_2/\text{kg/hr}$ in *A. braziliana*, also at 22°C, while Idrisi et al. (2006) calculated rates of 21-119 mg $\text{O}_2/\text{kg/hr}$ in 2-day fasted *A. californica* at 15°C. Values reported in Donovan et al. (2010) were in line with the latter range, recording 103 mg $\text{O}_2/\text{kg/hr}$ in resting *A. braziliana* at 24-26°C. These authors noted increases to 149 mg $\text{O}_2/\text{kg/hr}$ during crawling and 172 mg $\text{O}_2/\text{kg/hr}$ during swimming.

Marsden et al. (2012) observed that resting MO_2 of >30 gastropod species was proportional to mass, with a slope of ~0.6. The data presented here corroborates this relationship for *Aplysia* of 100-500 g. The slope of ~0.7 is consistent with this relationship throughout the animal kingdom (Packard and Boardman, 1999), and supports the observation that the rate of a physiological processes is higher in larger individuals. Resting MO_2 in sea hares, however, may be higher than other species of marine mollusks of comparable size. Marsden et al. (2012) published MO_2 for dry mass values, however, using an estimate that dry mass is ~12% of wet mass (from Ganmanee et al 2010, in abalone), MO_2 in *Tritonia hombergii* of approximately the same mass as animals in our study was estimated at ~3 mg $\text{O}_2/\text{kg/hr}$ at 10°C. The implied difference in metabolism suggests that the sluggish lifestyle of *A. californica* is not associated with a low metabolic rate.

In summary, 2 months of turbulence-induced isometric exercise did not change reflex performance in hatchery-reared *Aplysia* compared to unexercised siblings, and the experience of turbulent water movement did not mimic wild origins in performance of the reflexes. Wild animals did not perform better in swim tunnel tests than exercised hatchery-reared animals or their unexercised sibling controls. While other forms of hatchery-induced exercise may have produced differences, it is possible, instead, that wild individuals may shelter from intertidal wave energy to remain near optimal feeding areas.

Acknowledgments

We gratefully acknowledge the University of Miami Aplysia Resource staff. The lead author thanks D.L. Crawford and C.B. Paris for comments on a version of the manuscript. M. Grosell is a Maytag Chair of Ichthyology.

Funding: This work was funded by the National Institutes of Health P40 OD010952.

Abbreviations

MO₂	resting oxygen consumption
TTR	Time-to-right
TWR	Tail withdrawal reflex

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A

B

C

Fig. 1.
Exercise rearing regimes. A. Still-water control cage holding 100 g *A. californica* and algal food. B. 5 g animals in pumps regime. C. 120 g animals in dumpbucket regime.

Fig. 2.

Assessment scheme for the experiment. Briefly, density, righting reflex, tail withdrawal reflex, flume test, and resting O_2 consumption measurements were made on 4 different groups of animals after two different durations of turbulence. Three groups of lab-reared animals were tested 1 month after turbulence rearing concluded. The still water controls were tested again 4 months later. See methods section for animal ages at each assessment stage.

Fig. 3.

Mean morphometric and behavioral measurements on age 6 months hatchery *A. californica* after 2 months in turbulence regimes, compared to wild animals studied at the same time, and aged siblings 5 months later. A. Mean mass and density $\pm$ SD. Sample size indicated in bars. B. Mean time to right $\pm$ SD. *denotes significantly less than controls ($p < 0.05$, one-tailed Wilcoxon test). C. Mean time to relax tail 30%, a measure of TWR, $\pm$ SD. Grey dotted horizontal lines denote mean TWR times studied in 8 animals for each treatment after return to control water conditions for 3 weeks. Mean $\pm$ SD for 3-week return were: Control, 16.8 $\pm$ 7.4; Dumpbucket, 14.5 $\pm$ 5.0; Pump, 13.4 $\pm$ 7.1.

Fig. 4.

Performance of age 6 mo hatchery *A. californica* in flume trials after 2 months in turbulence regimes, compared to aged still-water control siblings and wild animals. A. Proportion of 12 animals each treatment that attained a flume speed of 50 cm/s for 1 min. B, C. Plots of fail speed and duration in flume as a function of animal mass. No relationships of flume performance with mass were noted for any treatment except Aged II, or for the treatments groups analyzed together. Aged II animals had significant inverse relationships with mass for both fail speed and duration (insets), at $y=0.067x + 43.47$, $R^2=0.574$, $p = 0.05$ via F test; and $y=1.99x + 966.6$, $R^2=0.746$, $p = 0.001$, respectively.

Fig. 5.

Resting oxygen consumption as $\log_{10}\text{MO}_2$ after 2 months in exercise regimes for all treatments, as a function of $\log_{10}\text{mass}$. Aged II still-water controls are included. The best fit line $y=0.6946x-0.8782$ with $R^2=0.55$ is significant via ANCOVA test at $p < 0.05$.

Table 1

Summary of age 5-months *A. californica* after 1 month in exercise protocols that did not fail the 11 min flume protocol. Performance of wild animals is also provided.

Treatment	Number tested	No failure
Control	11	6
Dumpbucket	12	10
Pump	12	8
Wild	12	7

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