Titles are concise and focus on the biological question or finding. Also note that including the common species name and the scientific species name (Latin binomial) is very useful in the title. The scientific name shoud be typed in *Italics* text.

Large Claws of Male Fiddler Crabs (*Uca pugilator*) Do Not Exhibit an Anaerobic Metabolism Cost.

Alyce DeMarais, Sue Hannaford, Greg Johnson, Leslie Saucedo, Alexa Tullis, and Carrie Woods

Include all people that contributed to the work, but identify the primary author by underlining or using an *. You do not need to include your instructor or IAs as authors.

Biology 333

Summer, 2018

The abstract should be written last and concisely summarize the key points of the intro, M&M, results, and discussion. In this abstract, the first two sentences reflect the intro, followed by one sentence concerning the method, two sentences about the results, and concluding with two sentences capturing the discussion.

*Note the use of single spaced lines to make the abstract stand out from the rest of the text.

Abstract

Male fiddler crabs use their large, sexually-dimorphic claws to attract potential mates and to battle other prospective suitors. Because this sexually-selected trait adds considerable body mass, we hypothesized that it would come with an energetic cost. Specifically, we examined whether the presence of large claws decreases sprint performance and increases lactate levels of males relative to those with their large claws removed. Our results yielded a 10-fold increase in lactate levels upon induced exertion, indicating we successfully stimulated anaerobic metabolism. However, we found that intact claws did not significantly affect either sprint duration or lactate levels after exercise. It may be that males have evolved compensatory traits that diminish the anaerobic cost of their large claw. Alternatively, the cost of possessing a large claw may persist even when the claw is removed.

*Also note that quantitative results and significant directions of change (effects) are very useful for the reader. However, p-values and the names of statistical tests are not required.

It is important to consider the broader implications of the results.

Introduction

Start by providing context and background for the central question or objective of the study.

A particular form of natural selection, sexual selection, occurs when males or females are attracted to and thereby select certain characteristics in a potential mate. More widely known examples of such sexually selected traits in males include the showy feathers of peacocks, shaggy manes of lions, and the pronounced antlers of elk. Sexually selected traits may arise due to within-sex competition for limited resources including access to mates, and due to mate choice for individuals with a particular trait (Darwin, 1871). However, it seems probable that such traits may disadvantage the male under some circumstances as these traits could exert a cost on physiological function (Oufiero and Garland, 2007).

The cost of sexually-selected traits has been characterized in relation to movement (Oufiero and Garland, 2007). While it may seem obvious that these traits could impair movement, no clear trend is apparent as studies have found conflicting results. For the Montezuma swordtail, *Xiphophorus montezumae,* sexually-selected traits show a negative impact as

Information about what is currently known should be written in present tense.

Continue by providing details of what is currently known with regard to your question or objective.

These details should be properly cited from previous peer-reviewed literature.

Work to cite the right papers in the right places. In this section, we are citing the papers that originally showed the finding. We are NOT citing papers that simply mentioned that finding in their own introductions nor textbooks. To support your statements, you should use the references that will be of greatest value to the reader who wants to learn more.

males with longer swords faced higher costs in terms of swimming speed than males with shorter swords (Kruesi and Alcaraz, 2007). Peacocks, *Pavo cristatus*, showed no effect as birds with and without the showy train showed no significant difference in take-off performance (Askew, 2014). Finally, the green swordtail, *Xiphophorus helleri*, showed a positive impact as males with longest swords had the greatest escape ability (Royle et al., 2006). Further studies have sought to quantify the energetic cost of sexually selected traits by measuring aerobic respiration with some studies showing an increase (Montezuma swordtail, *Xiphophorus montezumae*, males with longer swords consume oxygen than males with shorter swords: Basolo and Alcaraz, 2003), and others showing no effect (peacocks, *Pavo cristatus*, with and without the showy train showed no significant difference in change of potential or kinetic energy upon take-off: Askew, 2014). While most previous results examine the effect of sexually selected traits on aerobic respiration, little is known about the anaerobic cost of sexually selected traits.

Identify the gap in knowledge with regard to your question or objective.

Provide information about your study organism and the details necessary to understand why it was chosen with regard to your question or objective.

To examine anaerobic costs of sexually-selected traits, we chose to work with male fiddler crabs (*Uca pugilator*) that exhibit a large sexually-dimorphic claw that may constitute up to 40% of body mass (Crane, 1975) and which appears to enhance fitness. These large claws are used by male fiddler crabs both to fight other males and also in a waving presentation to attract females (Crane, 1975). We hypothesized that this large claw may induce an energetic cost on male fiddler crabs under anaerobic exertion when fleeing a predator. We examined this possibility by measuring the energetic cost of carrying a major claw to adult male fiddler crabs both at rest and after an anaerobic sprint. Given this hypothesis, we predicted that male fiddler crabs with an intact major claw relative to declawed male crabs would show (a) decreased sprint performance as indicated by shorter sprint durations and (b) a higher metabolic cost as indicated by higher lactate levels when exposed to a simulated predator.

Information about your organism should be properly cited with peer-reviewed literature.

Inclusion of specific predictions based on your hypothesis is a good way to finish the introduction. It provides your reader with a clear understanding of the experiment(s) that will be described in the Materials & Methods section that follows.

Since you are writing this report after you have completed your experiments and analysis, any reference to the experiment should be past tense.

Use subheadings to organize the information. In most biological papers, the methods sections will include at least 3 subheadings: 1) the subjects (e.g., animals or plants, ecological study sites, cell lines), 2) experimental procedures, and 3) data analysis subsections.

Materials and Methods

Collection and maintenance of animals

We recommend using an active voice (We did x…) rather than passive voice (X was placed…) in biological writing, because it reflects the scientist's agency and human process of doing science. However, professors and disciplines have different preferences; we recommend asking for your professor's preference.

We obtained adult intermoult male fiddler crabs, *Uca pugilator*, from Sachs System Aquaculture (St. Augustine, FL). Animals were shipped to the University of Puget Sound one month before the experiment where they were held in groups of 3-4 in plastic tubs with artificial seawater (~2.1 cm) and PVC tube habitats. Crabs were maintained at 22°C on a 12 h:12 h light:dark cycle and fed sinking shrimp pellets (Aquarian, Chalfont, PA, USA) twice per week.

Write out numerals below 10 unless it is a measurement or in combination with other numbers or is at the start of a sentence. For example: 6 mm, 5 crabs, 35 sites, 10 x 5 m. The use of numerals in scientific writing can be different than in other areas: https://www.enago.com/academy/numbers-in-writing-guidelines-life-sciences-medicine/

In addition to pure methods, explanatory phrases are sometimes included in the Materials and Methods section to clarify why a particular step was taken.

Fiddler crabs autotomize (i.e. shed) their claws as a means of self-defense to evade predators. We exploited this behavior to remove claws from half of the sample population 3 to 7 days prior to testing by gently pulling the claw across the animal's body until the claw was released. In some cases where autonomization proved difficult, the claw was quickly excised with scissors at the base of the limb.

Subheadings continue. In this lab report, the experimental protocol is broken into 2 subsections, but you could have a single subheading titled Experimental procedure with the two paragraphs under it.

Exercising the crabs

Fiddler crabs, with and without major claws, were placed into the exercise arena (a tub filled with damped sand to mimic their natural environment) and chased around with our hands until the crab stopped sprinting and slowed to a walk as described in the lab manual (Biology Faculty, 2017). The sprint duration was recorded. Crabs were then encouraged to move for an additional 2 minutes following their sprint. Following exercise, 20-40 ml of blood was drawn from the crabs by inserting a chilled 27-gauge needle attached to a 0.5 ml syringe through the membrane between two of the crab's walking legs.

Never have naked decimal places. It should be "0.5 ml", not ".5 ml"

Your materials and methods will typically be drawn from a lab manual. It's good practice to cite it in the methods section, usually when you first introduce your protocol, but you do not need to recite it for each subsequent step.

We drew blood from each crab only once, at one of three different time points: immediately after exercise (n = 9 with claw, n = 8 without claw), 10 minutes after exercise (n = 7 with claw, n =7 without claw) and 30 minutes after exercise (n = 7 with claw, n = 8 without claw). Blood samples were then placed into separate microcentrifuge tubes and chilled on ice for processing of blood lactate concentration. Crabs were weighed to the nearest 0.1 g following the blood draw. Blood was also taken from another set of crabs before exercise to provide a baseline for lactic acid (n = 12 with claw, n = 5 without claw).

Your experiments will typically include at least one control. Although the word control isn't used here, the phrase "to provide a baseline" alerts the reader to why this group is included.

Include the concentrations and relative amounts of reagents. This information is essential for a reader to replicate your experiment.

Determining lactate levels

We used a commercially available assay kit (Megazyme) to determine the concentration of lactic acid in each sample. To remove blood proteins, 25 ml of blood was first combined with 75 ml of 15% trichloroacetic acid and vortexed. The solution was allowed to sit on ice for 15 minutes. Following this incubation, the sample was centrifuged for 5 minutes in a refrigerated microcentrifuge (4 °C) to precipitate proteins in the sample. The remaining supernatant was then placed into a new tube and neutralized by the addition of 1 part 2.5 M K_2CO_3 to 7 parts supernatant. The resulting sample was then analyzed in accordance with the directions provided by the kit, cuvettes were prepared by adding given volumes of sample, water, buffer, NAD^+, and glutamic-pyruvate transaminase. We took an absorbance reading on a spectrophotometer (Genesys 20) set at 340 nm three minutes after mixing the contents of each cuvette. We then added 10µL of lactate dehydrogenase to each cuvette and a second reading was taken fifteen minutes later.

Include the model and manufacturer of a piece of specialized equipment.

Data Analysis

We calculated the concentration of lactic acid in the cuvette using the absorbance values before and after the addition of the lactate dehydrogenase. These values were then converted to blood lactate concentration by accounting for dilution volumes to yield blood lactate concentration in the crab's blood using the equation:

Present the methods for data analysis in the Materials and Methods section.

Here an equation used to convert the raw values collected to a concentration is presented in the Materials and Methods section. As an alternative, this equation could be presented in an appendix at the end of the lab report.

$$\frac{mg\ lactate\ in\ cuvette}{mL\ cuvette\ supernatant} * \frac{\mu L\ supernatant\ +\ \mu L\ K_2CO_3}{\mu L\ blood} * \frac{1\ g}{1000\ mg} * \frac{1\ mol}{90.1\ g} * \frac{10^6\ \mu Mol}{1\ mol} = \frac{\mu Mol\ lactate}{mL\ blood}$$

We used t-tests to determine whether crabs with and without a claw differed in their weight and whether blood lactate levels differed between resting and sprinting crabs. Linear regression was used to determine if crab weight influenced sprint duration. A 2-way ANOVA was used to determine if the way sprinting influenced blood lactate levels depended on whether crabs had a claw or not. A 2-way ANOVA was run to determine if time after sprinting influenced blood lactate levels for crabs with and without a claw. All of the statistical analyses were performed using R software (R Core Team, 2013). Graphs were prepared using Excel. A significance level of 0.05 was used for all analyses.

Start with a brief overview sentence of the investigation. Report the result in prose and support the result with a figure or table.

Reserve the term "significant/ly" for results that have statistical significance.

Results

In this experiment, we investigated the metabolic cost of carrying a sexually dimorphic major claw in male fiddler crabs by measuring blood lactate levels in crabs at rest or after sprinting. Males with intact major claws were significantly heavier than males without a claw (t-test; t = -6.53, df = 61, $p < 0.001$; Fig. 1). Male crabs with claws were 33.6% heavier than males crabs without claws (Fig. 1). The presence of a claw did not affect sprint duration (t-test; t = -.26, df = 43 $p = 0.796$; Fig. 2).

Figures are numbered consecutively based on when they are cited in the text of the report.

Include the statistical test and the *p* value parathentically in the text and in the figure legend.

Report each variable tested in a separate paragraph; use parallel construction for each paragraph.

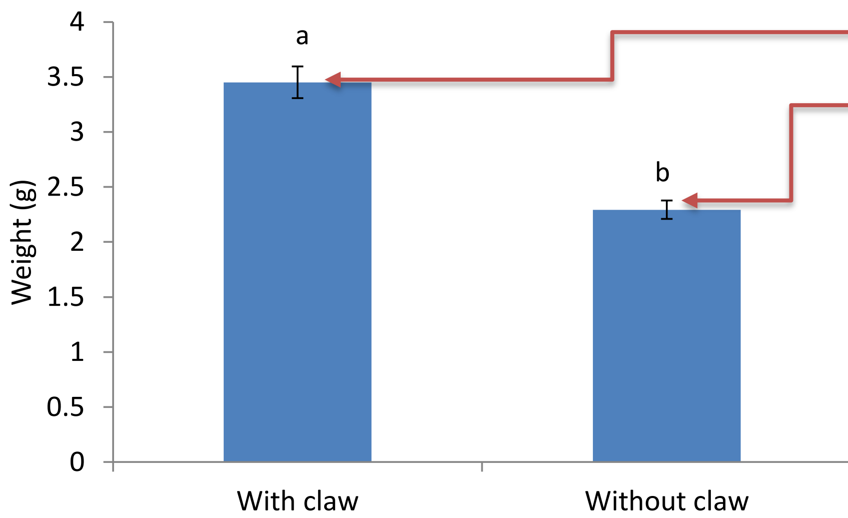
Use first person, active voice in the past tense for the results section.

To determine % difference use these two examples: 1. ((10-5)/10)*100 = 50%. You would conclude: "5 is 50% less than 10".

2. ((10-5)/5)*100 = 100%. In this second example you would say: "10 is 100% larger than 5".

Usually Figures and Tables are interspersed with the results section, appearing soon after they are described in the text. Alternatively, all the figures/tables can appear at the end of the Results text.

Bar charts should always have error bars and this should be included in the figure caption.

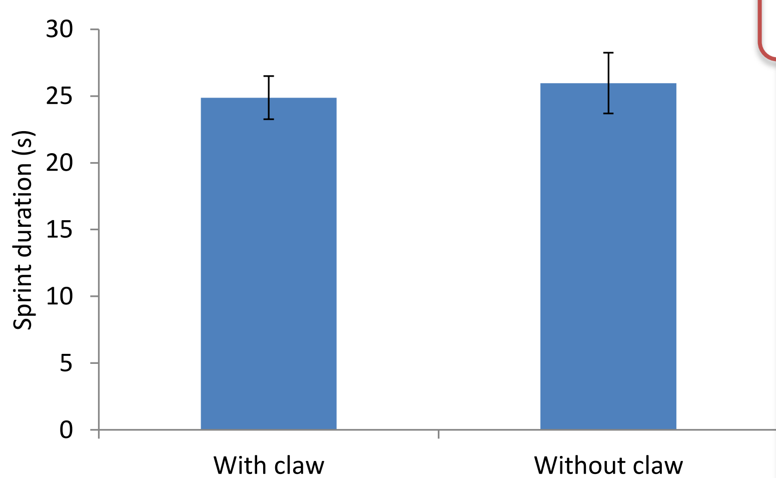


Figures are numbered consecutively based on when they are cited in the text of the report.

Figure 1. Male fiddler crabs (*Uca pugilator*) with intact claws are heavier than those without claws. The two groups of crabs used in this experiment either had intact claws ("with claw") or were induced to autotomize their major claws ("without claw"). The weight (mean ± SEM) of the crabs with intact claws was significantly greater than the weight (mean ± SEM) of crabs without claws (t-test; $p < 0.001$). Different letters (a, b) indicate statistical significance; with claw, n = 35; without claw, n = 28.

Be sure to note the statistical test used to assign letters. In this case it is a t-test. As an alternate example, see Figure 3 where an ANOVA was used.

Include the number of samples/data points (n) used.



Summarize the results shown in the figure in the title.

Figure 2. Presence of a claw does not affect sprint duration. Male fiddler crabs with and without a major claw were induced to sprint to exhaustion. Sprint duration was not affected by the presence of a claw (t-test; $p = 0.796$). With claw, n = 23; without claw, n = 23.

Like abstracts, figure captions are single-spaced to differentiate them from the main text.

Include a brief statement about methodology used in the figure legend.

We measured blood lactate levels in crabs, with or without major claws, at rest and immediately after sprinting. The way that sprinting influenced lactate levels did not significantly depend on whether the crabs had a claw or not (2-way ANOVA, $F = 0.0006$, df = 1,58, $p = 0.938$, Fig. 3). The presence of a claw had no significant effect on lactate levels (2-way ANOVA, $F = 0.16$, df = 2,58, $p = 0.848$). At rest, animals with and without claws had the same lactate levels (Fig. 3). Lactate levels were significantly higher (1148%) in crabs immediately after sprinting when compared with resting crabs (2-way ANOVA; $F = 46.87$, df = 1,58, $p < 0.001$; Fig. 3).

It's helpful to elaborate on statistical results with important biological results to make sure that readers notice what you want them to notice.

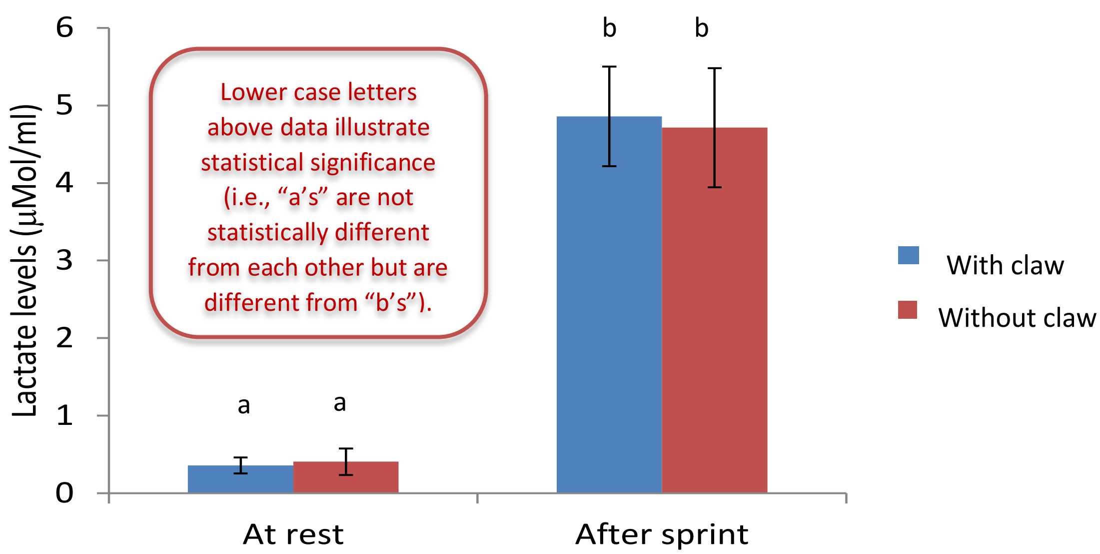


Figure 3. Sprinting increases blood lactate levels in male fiddler crabs. Blood lactate levels were measured in crabs (with and without claws) at rest and immediately after sprinting to exhaustion. Lactate concentrations (mean ± SEM) were significantly higher in crabs that had sprinted as compared with crabs at rest (2-way ANOVA; $p < 0.001$), but this was not influenced by the presence of a claw (2-way ANOVA; $p = 0.938$). Different letters (a and b) indicate statistical significance. With claw, at rest, n = 12; no claw, at rest, n = 5. With claw, after sprint, n = 23; no claw, after sprint, n = 23.

We then analyzed whether lactate levels changed over time after sprinting (10 or 30 minutes post-sprint) for crabs with and without intact claws. The way that time after exercise influenced lactate levels did not depend on the presence of a claw (2-way ANOVA, $F = 0.001$, df = 1,42, $p = 0.973$; Table 1). Blood lactate levels did not significantly vary with time after exercise (2-way ANOVA; F = 0.49, df = 1,42, p = 0.486; Table 1; Fig. 4) or presence of a claw (2-way ANOVA; F = 0.03, df = 1,42, p = 0.870; Table 1; Fig. 4).

Typically, data should be presented in only one format – either a table or a figure, not both! Here we included it both ways to A) give you an example of what a nicely formatted table looks like and B) to demonstrate that patterns are more easily seen in the figure format. Use tables only when it is a better way to communicate your aims.

Table 1. Blood lactate levels in fiddler crabs did not vary with time after sprinting (0, 10, or 30 minutes) or vary in crabs with and without a claw.

Claw present	Blood Draw Time after Sprinting (minutes)	Blood Lactate (µMol/ml) (mean ± SE)
Yes	0	4.86 ± 0.64 (n = 9)
	10	4.89 ± 1.16 (n = 7)
	30	4.27 ± 1.40 (n = 7)
No	0	4.71 ± 0.77 (n = 8)
	10	5.52 ± 1.02 (n = 7)
	30	4.22 ± 0.84 (n = 8)

Interaction between time after sprinting and presence of a claw: 2-way ANOVA; p = 0.973
Time after sprinting: 2-way ANOVA; p = 0.870;
Presence of claw: 2-way ANOVA; p = 0.486

Table numbers and titles are placed above the table (and any statistical legends are placed below the table).

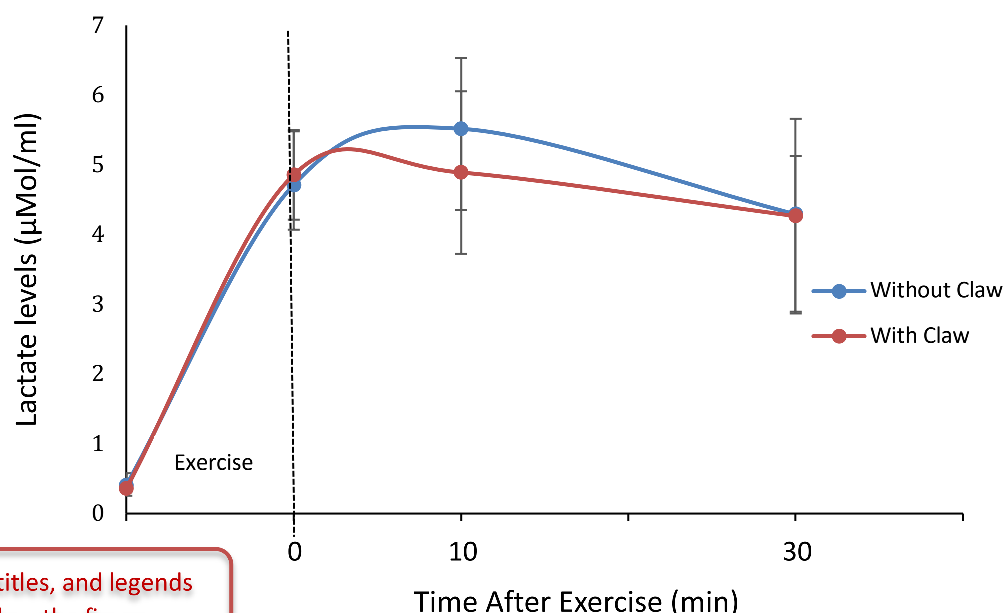


Figure numbers, titles, and legends are placed below the figure.

Figure 4. <u>Blood lactate recovery does not vary in crabs with or without intact claws within 30 minutes after sprinting</u>. Blood lactate levels (mean ± SEM) did not change over a 30 minute recovery period in crabs that had exercised to exhaustion. The presence of a claw did not affect lactate levels following exercise. With claw: at rest, n = 12; 0 min, n = 9; 10 min, n = 7; 30 min, n = 7. Without claw: at rest, n = 5; 0 min, n = 8; 10 min, n = 7; 30 min, n = 8.

Start by restating the central question or objective of the study.

Clearly state if your results support your hypothesis(es). Depending on the complexity of your results, this could come in more than one place in the paper.

Discussion

The main objective of the present study was to determine the impact of possessing a large sexually selected structure on animal performance. We did this by comparing sprint performance and blood lactate levels in male fiddler crabs with and without their major claw. Our results supported our hypothesis that blood lactate levels would increase following sprinting. Specifically, blood lactate levels in both clawed and declawed crabs increased more than 10 times over resting levels following sprinting. An increase in blood lactate concentration is expected for animals during non-sustainable locomotion (Hill et al. 2008), and a 10 times increase is similar to that found for other crabs exercised to exhaustion (Henry et al. 1994). Thus, sprinting causes crabs to use anaerobic metabolic pathways.

Compare your results with previous results from peer-reviewed literature.

By contrast, our results did not support our hypothesis that clawed crabs would show reduced sprint performance and higher blood lactate levels than declawed crabs. Clawed and declawed crabs in the present study sprinted for the same amount of time. This result is similar to that of previous studies showing that the presence of the major claw did not influence sprint speed of male fiddler crabs (Jordão and Oliveira, 2001; Allen and Levinton, 2007). Consistent with the similar sprint performance, we found no impact of the major claw on blood lactate following sprinting. This finding contrasts that of Allen and Levinton (2007) who found that clawed crabs showed an 8% higher resting oxygen consumption than did declawed crabs. The reason for these seemingly different results may be due to the fact that blood lactate levels are a measure of anaerobic metabolism while oxygen consumption is a measure of aerobic metabolism. Thus, it may be that the major claw impacts aerobic but not anaerobic processes. Future studies that investigate both aerobic and anaerobic metabolic pathways are needed to fully understand the costs of a claw.

Provide biologically-based explanations for differences between your results and those found in the literature.

Discuss what your results might mean for the particular system you're studying.

Anticipate reader's questions and address them with biologically - based testable hypotheses

A 33% difference in body mass between clawed and declawed crabs is a substantial amount. Given that clawed crabs are essentially sprinting while carrying a load equivalent to 33% of body mass, how can they do this while incurring no additional anaerobic cost? One answer is the presence of compensatory traits - traits that evolve through the process of natural selection to counter-balance costs associated with possessing an exaggerated trait (Møller, 1996). For example, studies have demonstrated that male barn swallows with long tails have greater wingspans than their female counterparts (Barbosa and Møller, 1999). It is possible that male fiddler crabs have evolved traits that enable them to carry a large load with relative ease, such as a specialized limb position or gait pattern. Interestingly, researchers have shown that there is asymmetry in the legs of male fiddler crabs based on their species-specific claw-waving patterns – it is possible that similar asymmetry enables male crabs to carry a large claw with minimal energetic expenditure.

If compensatory traits allow male fiddler crabs to carry their large claw with relative ease, why does performance not improve and costs decrease when the claw is removed? The answer to this may rest with short-term biomechanical adjustments that occur following claw removal. In other words, once the claw is removed, any energetic savings that may occur because the animal no longer is carrying the weight, may be negated by changes in posture and stride characteristics caused by claw removal that then to increase metabolic cost. Further studies comparing morphologies and gait patterns of male and female fiddler crabs from species with varying claw sizes would help identify the role of compensatory traits in helping to reduce the cost of carrying a large sexually selected trait.

Integrate ideas for future studies that stem directly from your study.

Taken together, results of the present study show that possessing a large major claw has little to no impact on sprinting cost or performance in male fiddler crabs. The presence of

End by telling readers what we know now that we didn't know before.

large sexually selected traits may indeed pose costs to organisms, but those costs do not appear to manifest themselves in all types of activities.

There are many ways to format your full citations. At minimum, you need the primary (first) author, the year published, and the name, volume and pages of the journal or textbook.

To avoid formatting errors (and save a lot of time), install the free reference editor Zotero on your computer. Ask your professor which specific citation guidelines they want you to follow. See details on proper citation format here: https://research.pugetsound.edu/citation

Also consult the following site for the proper way to cite electronic sources: https://owl.purdue.edu/owl/research_and_citation/apa_style/apa_formatting_and_style_guide/reference_list_electronic_sources.html

Citations

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You should cite the lab manual for in-class research papers in order to properly credit materials and methods. Please note that a lab manual citation is not an apporopriate citation in an introduction or discussion to properly credit background information.

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