

RESEARCH ARTICLE

The metabolic cost of carrying a sexually selected trait in the male fiddler crab *Uca pugilator*

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ABSTRACT

Models proposed to explain sexually selected structures assume that these traits are costly. However, studies investigating the impact of such structures on locomotory costs have produced inconsistent results. Male fiddler crabs possess a large sexually selected claw and are ideal for assessing the impact of a sexually selected trait on the cost of locomotion. Here, we measured the energy expenditure of clawed, declawed and artificially loaded crabs during sustained exercise by measuring oxygen consumption and blood lactate levels. We also measured blood lactate levels of clawed and declawed crabs following a sprint and forced walk to assess energy expenditure during non-sustainable, strenuous locomotion. Clawed and declawed crabs consumed the same amount of oxygen and had the same blood lactate concentration during sustained locomotion, suggesting that the large claw does not increase energetic costs during sustainable locomotion. Following non-sustainable, strenuous locomotion, however, there was a trend for clawed crabs to have higher concentrations of lactate in their blood than declawed crabs, suggesting that bearing a large claw may increase energetic costs during strenuous locomotion and lengthen recovery time. Artificially loaded crabs produced more lactic acid than clawed and declawed crabs during sustainable locomotion, suggesting that compensatory traits help mitigate the energetic cost of carrying the large claw. Overall, our results show that the impact of exaggerated structures on energy expenditure may depend on exercise intensity and highlight the need to examine various locomotory intensities when attempting to assess the costs of bearing a sexually selected trait.

KEY WORDS: Energetic cost, Lactic acid, Locomotion, Oxygen consumption, Sexual selection, Fiddler crab

INTRODUCTION

Sexually selected traits increase an individual's attractiveness to members of the opposite sex or advertise an individual's strength to members of the same sex, thereby increasing mating success and fitness (Darwin, 1871; Andersson, 1994). To serve their purpose, these structures are often exaggerated and elaborate, as exemplified by the large, brilliantly colored train of male peafowl (peacocks) and the impressive antlers of male deer. However, even Darwin recognized that there are likely to be costs associated with such showy traits (Darwin, 1871). Models developed to explain sexual selection posit that it is precisely because these traits come with costs that they could reliably signal the bearer's quality as a potential mate or rival, as only a high quality individual could produce and

maintain a costly trait (Zahavi, 1975; Kodric-Brown and Brown, 1984; Grafen, 1990; Andersson, 1994; Kotiaho, 2001).

There is ample evidence that sexually selected structures incur various types of biological costs (Kotiaho, 2001; Husak et al., 2015). For example, although the bright color patches on the fins of male guppies (*Poecilia reticulata*) make them more attractive towards females (Houde, 1997), they also make them more vulnerable to predation (Endler, 1980, 1983; Godin and McDonough, 2003). Exaggerated traits can also compromise the development of other body parts. For example, male horned beetles (*Orthophagus* spp.) possess extremely large sexually selected horns that help attract females, but there is a negative correlation between the size of horns and the relative sizes of other body parts, including testes, eyes and antennae (Emlen, 2001; Simmons and Emlen, 2006). Compromised immune function is another potential cost of possessing exaggerated sexually selected structures, and has been documented in barn swallows (*Hirundo rustica*: Saino and Møller, 1996) and red jungle fowl (*Gallus gallus*: Zuk and Johnsen, 1998). In addition to these receiver-independent costs, there may also be receiver-dependent costs in the form of increased retaliation from conspecifics during agonistic encounters (Husak et al., 2015; Wilson and Angilletta, 2015).

Locomotory costs caused by the presence of sexually selected structures have received a considerable amount of attention, which is not surprising given that these exaggerated structures seem well poised to impair locomotion, which could reduce fitness if an individual's ability to escape predators, forage and find mates is compromised (Oufiero and Garland, 2007). Locomotory costs can manifest themselves in two ways – impaired performance and increased energy expenditure (Irschick et al., 2007) – and both costs are important to consider as both could impact fitness. Multiple studies investigating locomotor costs have focused on various performance capabilities, such as escape performance and maximum sprint speed. Results thus far have been equivocal, with some studies showing that sexually selected traits negatively impact performance (malachite sunbird, *Nectarinia johnstoni*: Evans and Thomas, 1992; barn swallow: Møller and de Lope, 1994; Montezuma swordtail, *Xiphophorus montezumae*: Kruesi and Alcaraz, 2007), while several others demonstrate no decline in performance (green swordtail, *Xiphophorus helleri*: Royle et al., 2006; Baumgartner et al., 2011; rhinoceros beetle, *Trypoxylus dichotomus*: McCullough et al., 2012; threadfin rainbow fish, *Iriatherina werneri*: Trappett et al., 2013; peafowl, *Pavo cristatus*: Askew, 2014). As an example, the elongated caudal fin of male Montezuma swordtails decreases maximum sustainable swimming speed (Kruesi and Alcaraz, 2007), while the sword of green swordtails does not impair, and may even enhance, escape behavior (Baumgartner et al., 2011; Royle et al., 2006).

In cases where there is no measurable decrease in performance capability, sexually selected traits may still impose locomotory costs by increasing the metabolic energy required to achieve the same

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level of performance. Relative to the number of studies investigating performance, comparatively few have examined the impact of exaggerated structures on energy expenditure, and results thus far have been inconsistent. Although a number of studies demonstrate that a sexually selected trait increases energy expenditure during locomotion (barn swallow: Barbosa and Møller, 1999; Montezuma swordtail: Basolo and Alcaraz, 2003; hummingbird, *Calypte anna*: Clark and Dudley, 2009; stag beetle, *Cyclommatus metallifer*: Goyens et al., 2015a,b), several other studies demonstrate no change (rhinoceros beetles: McCullough and Tobalske, 2013; threadfin rainbow fish, *Iriatherina wernerii*: Trappett et al., 2013; peafowl: Askew, 2014), and one even shows that the presence of a sexually selected trait causes a decrease in energy expenditure (peafowl: Thavarajah et al., 2016). The reason for the lack of consensus is unclear, but it could include the method used to determine cost (e.g. indirect estimates through mathematical modeling versus more direct assessment through metabolic measurements), the group used for comparison (e.g. comparing males with and without the structure of interest versus comparing males with females), and the locomotor function investigated (e.g. endurance capacity versus sprint performance).

Male fiddler crabs of the genus *Uca* are ideal for addressing questions related to the cost of bearing a sexually selected structure. Unlike females, which possess two small feeding claws, male fiddler crabs possess one small claw and one extremely large claw that they use both as a weapon to fight other males and as a signal to attract females (Crane, 1975; Hyatt and Salmon, 1978; Pope, 2000). Comprising up to 40% of an individual's body mass (Crane, 1975), the major claw confers fitness advantages: larger males with relatively larger claws win more fights in agonist encounters with other males (Hyatt and Salmon, 1978; Jennions and Backwell, 1996; Pratt and McClain, 2002; Pratt et al., 2003), and in some cases, females prefer males with larger claws (Backwell and Passmore, 1996; Milner et al., 2010). These fitness benefits, however, come with costs. For example, the claw-waving display that male crabs use to attract females is energetically costly (Matsumasa and Murai, 2005; Jennions and Backwell, 1998), and the agonistic encounters between males can lead to injury or death (Jennions and Backwell, 1996). Moreover, because the major claw cannot be used for feeding, male crabs exhibit decreased feeding efficiency relative to females (Weissburg, 1992, 1993).

Previous studies have demonstrated that the major claw can also lead to locomotory costs in the form of reduced performance. Specifically, while male crabs with and without their major claw have comparable sprint speeds on a level substrate (Jordão and Oliveira, 2001; Allen and Levinton, 2007; Gerald and Thiesen, 2014), the presence of the major claw reduces the sprint performance of crabs running on slopes (Gerald and Thiesen, 2014), and decreases endurance capacity (Allen and Levinton, 2007; Gerald and Thiesen, 2014). Although no study to date has examined the impact of the major claw on the metabolic cost of locomotion, crabs with their major claw have been shown to have an 8% higher resting oxygen consumption than crabs lacking the claw (Allen and Levinton, 2007).

The aim of the present study was to examine the impact of the major claw on the metabolic cost of locomotion in male fiddler crabs, *Uca pugilator* (Bosc 1802). In light of the finding that exercise intensity may influence how the major claw impacts cost (e.g. Gerald and Thiesen, 2014), we examined sustainable and non-sustainable locomotion, both of which are used routinely by fiddler crabs (Crane, 1975; Murai et al., 1982; Frix et al., 1991; Weinstein, 1998). We assessed metabolic costs by measuring oxygen

consumption as an index of aerobic metabolism and lactic acid production as an index of anaerobic metabolism. Given previous results showing that the major claw increases resting oxygen consumption (Allen and Levinton, 2007), we hypothesized that the major claw would increase energy expenditure during both sustainable and strenuous locomotion. In addition to our main objective of comparing energy expenditure of clawed and declawed crabs, we also assessed locomotory costs of declawed crabs carrying an artificial load on their carapace in the sustainable exercise experiments. This third group was included as a way to investigate the possible role of compensatory traits in reducing the energetic cost of carrying a large sexually selected structure (Husak and Swallow, 2011).

MATERIALS AND METHODS

Animal care

Uca pugilator (2.88±0.60 g, mean±s.d., $n=104$) were obtained from Sachs Systems Aquaculture (St Augustine, FL, USA) and shipped to the University of Puget Sound. Housing conditions and experimental procedures conformed to the University of Puget Sound Animal Care and Use Committee guidelines. Three to five crabs each were housed in plastic tubs (38 cm long×18 cm wide×13 cm deep) containing a layer of sand and seawater. Tubers were tilted such that crabs could move freely between the water and a sand 'beach'. As a result of the tilting, the beach was sloped and there was a maximum water depth of 3–4 cm at the lowest end. 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. Tubers were cleaned the day after feeding. Each animal was used in a single treatment combination (e.g. sustained or strenuous exercise combined with claw, no claw or load), with the exception of three crabs which were each used in two treatment combinations.

Sustainable exercise: oxygen consumption and lactic acid production

We tested three treatment groups in the sustainable exercise experiments: (1) crabs possessing their major claw ('clawed' crabs; $n=10$); (2) crabs with their major claw removed ('declawed' crabs; $n=10$); and (3) declawed crabs loaded with a weight representing 30% of their body mass ('loaded' crabs; $n=10$). This value was chosen because the major claw of *U. pugilator* in the present study averaged 29±1% (mean±s.e.m.; $n=45$) of a crab's total body mass (including the claw). Crabs were induced to autotomize their major claw for the de-clawed and loaded treatments by gently pulling on the claw or grasping the base of the claw with scissors. Loads were composed of metal washers and molding clay wrapped in plastic wrap. The clay and washer packets were attached using cyanoacrylate to a crab's carapace behind the eyestalks, roughly over the center of mass. Crabs were given at least 48 h to recover from claw removal before being tested or before loading. Loaded crabs were given an additional 24 h to adjust to the load after it was attached.

To assess energy expenditure during sustainable exercise, we measured oxygen consumption and blood lactate levels because both aerobic and anaerobic metabolic pathways are used to fuel sub-maximal, sustainable activity in *U. pugilator* (Full and Herreid, 1984). At the start of each trial, a crab was placed on a treadmill housed in an airtight Lucite respirometer (internal dimensions: 4 cm wide×6 cm high×8.5 cm long) maintained at 24.5°C, and allowed to adjust for 20 min. Crabs were then exercised for 14–15 min at 0.10 km h⁻¹, which represents a moderate, sustainable walking pace for the crabs (Full and Herreid, 1984). If an animal needed some

encouragement to exercise, the tread belt was pulsed very quickly into reverse for a very brief moment (~1 s). If this did not successfully encourage a crab to continue exercising, the trial was not included in the study.

Oxygen consumption was measured using open-flow respirometry according to established procedures (Herreid et al., 1981; Full and Herreid, 1984; Tullis and Andrus, 2011). Ambient air was drawn through the respirometer with an air pump (model R-2, AEI Technologies, Pittsburgh, PA, USA) at a flow rate of 52 ml min⁻¹ (TSI F-4140 mass flow meter, AEI Technologies). Before entering the respirometer, air passed through filters containing Drierite (Acros Organics, Morris Plains, NJ, USA) and soda lime (Fisher Chemicals, Fair Lawn, NJ, USA) to remove water vapor and carbon dioxide, respectively. The oxygen content of the air was measured with a dual-channel electrochemical oxygen analyzer (model S-3A/II, AEI Technologies), which was calibrated with ambient air prior to data collection. One channel of the analyzer served as a reference while the other measured the oxygen content of the air exiting the respirometer. Data from the analyzer were collected at 2 Hz with a PowerLab 15T equipped with LabChart version 7.2 (ADInstruments, Colorado Springs, CO, USA). Oxygen consumption during rest was determined from the readings over the last 4–5 min of the 20 min adjustment period when the animals were quiescent. Oxygen consumption during exercise was determined from the final approximately 4 min of an exercise bout after the oxygen content leaving the chamber had plateaued. Trials in which crabs could not keep pace with the tread belt were excluded from the study.

Immediately after exercising, crabs were removed from the treadmill respirometer and 20–40 µl of blood (hemolymph) was drawn using a 27 gauge needle attached to a 0.5 ml syringe from the membrane between two of the crab's walking legs. The time between the cessation of exercise and the blood draw was generally less than 40 s, and samples were placed on ice until processing (see below). To determine resting blood lactic acid concentration prior to exercise, blood was also drawn from a separate group of crabs not used in the exercise trials ($n=14$ clawed, $n=8$ declawed and $n=11$ loaded). Clawed and declawed crabs were weighed to the nearest 0.01 g following each exercise bout, and loaded crabs were weighed prior to loading.

Strenuous exercise: lactic acid production

We compared clawed ($n=25$) and declawed ($n=21$) crabs in the strenuous exercise experiments. To assess the metabolic cost, we focused on blood lactic acid levels because anaerobic metabolism provides much of the fuel during strenuous, non-sustainable exercise as, at this point, animals are exercising above their maximal rate of oxygen consumption. This approach has been used in past studies investigating exhaustive exercise in crustaceans (e.g. Henry et al., 1994; Full, 1997). To begin each trial, a crab was placed in a plastic tub (45.5 cm long × 32.5 cm wide × 11.5 cm deep) at 25°C that contained a layer of damp, packed fine sand. Once in the tub, we induced crabs to sprint by chasing them continuously with a hand, and timed the duration of the sprint with a stopwatch. Sprints were characterized by fast, relatively smooth movement away from an experimenter's hand. At the end of a sprint, there was a distinct change in gait as the crabs slowed to a walk; unlike sprints, walks were characterized by a slow, somewhat lurching, pattern of movement. We continued to chase the crabs as they transitioned to a walk and for an additional 10 min. Continual chasing ensured that crabs were not able to slow or stop in the corners of the tub for more than a few seconds during the 10 min trial. Blood samples

(20–30 µl) were drawn at one or two of six different time points: after the sprint, after the 10 min walk, and at 5, 15, 30 or 60 min during recovery following exercise. In most cases, one blood sample was collected per animal at a single time point ($n=14$ clawed, $n=12$ declawed); in some cases, two samples were drawn at different times during recovery ($n=8$ clawed, $n=9$ declawed). Because of the small size of the crabs, we could not draw more than two samples from each individual without risking harm to the animal.

Blood processing and lactic acid determination

Processing of blood was based on a procedure modified from Adamczewska and Morris (1994). Samples were precipitated by adding 15% aqueous trichloroacetic acid to the blood in a 3:1 v/v ratio. Following centrifugation to remove protein, the supernatant was collected and the pH neutralized with 2.5 mol l⁻¹ K₂CO₃ and analyzed for L-lactic acid concentration with a standard kit (K-LATE, Megazyme, Wicklow, Ireland).

Data and statistical analyses

Mass-specific oxygen consumption (ml O₂ g⁻¹ h⁻¹, corrected to standard temperature and pressure, dry – STPD) was calculated using the following equation:

$$\dot{V}_{O_2} = \left(\frac{\dot{V}_E \times \Delta F_{O_2}}{(1 - F_{I_{O_2}}) \times m} \right), \quad (1)$$

where \dot{V}_E is the flow rate of air exiting the respirometer, ΔF_{O_2} is the difference in oxygen content of air entering and exiting the respirometer, $F_{I_{O_2}}$ is the oxygen content of the air entering the respirometer and m is body mass (Withers, 1977). Oxygen consumption per crab (ml O₂ h⁻¹) was calculated as above without dividing by body mass.

Statistical analyses were performed using R (version 3.3.1; <http://www.R-project.org/>). To assess the normality and variance of the sustainable exercise datasets, we visually inspected density plots of residuals. The equal variance assumption was met in all cases and the normality assumption was met in all but two. We log transformed these two datasets to improve normality. Separate one-way ANOVA tests were used to determine whether oxygen consumption differed among clawed, declawed and loaded crabs during rest and during sustained exercise. Separate one-way ANOVA tests were also used to determine whether blood lactate concentrations differed among clawed, declawed and loaded crabs following rest and sustained exercise. When a significant difference was indicated, the ANOVA was followed by a *post hoc* Tukey test. We used linear regression analyses to examine the relationship between body mass and oxygen consumption for clawed, declawed and loaded crabs. For these analyses, log transformations of mass and mass-specific oxygen consumption were used. We also analyzed these data with an ANCOVA, with mass as the continuous variable and claw treatment as the fixed factor, to determine whether the relationship between mass and mass-specific oxygen consumption during sustained locomotion differed among the three treatment groups.

For the strenuous exercise part of the study, a two-tailed *t*-test was used to determine whether sprint duration differed between clawed and declawed crabs. Although overall sample sizes were sufficiently large, small sample sizes at some time points precluded meaningful statistical evaluation of blood lactate levels of clawed and declawed crabs following strenuous exercise. Therefore, we examined the overall trends in blood lactate levels of the crabs for this part

of the study. Differences were considered significant if $P < 0.05$, and means \pm s.e.m. are presented.

RESULTS

We detected no obvious systematic behavioral differences among clawed, declawed and loaded crabs, either in their holding bins or during the exercise trials.

Sustainable exercise: clawed, declawed and loaded crabs

Resting oxygen consumption did not differ significantly among clawed, declawed and loaded crabs (one-way ANOVA: $F_{2,27} = 1.63$, $P = 0.21$). During exercise, oxygen consumption increased 4–5 times from resting levels in all treatment groups (Fig. 1A). Similar to resting oxygen consumption, oxygen consumption during sustained walking was not significantly different among clawed, declawed and loaded crabs (one-way ANOVA: $F_{2,27} = 2.10$, $P = 0.14$; Fig. 1A), although clawed crabs used about 17% more oxygen while walking than did crabs in the other treatment groups.

The apparent trend for clawed crabs to have higher oxygen consumption than declawed and loaded crabs (Fig. 1A) could be due to their greater body mass; because they possessed their major claw, the body mass of clawed crabs was $\sim 30\%$ greater than that of the other two groups. To determine whether the trend towards higher oxygen consumption was simply a result of this greater body mass, we also examined oxygen consumption per gram (Fig. 1B). This comparison reduced the difference shown in Fig. 1A, and showed no significant difference in mass-specific oxygen

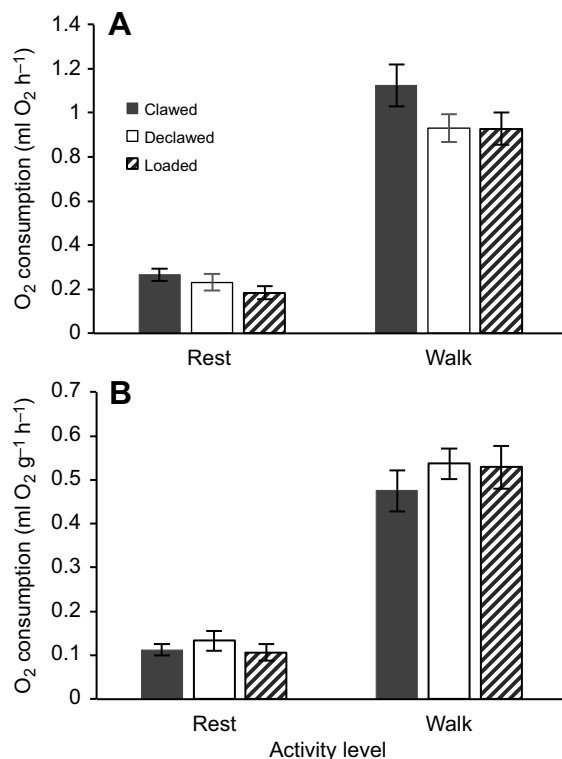


Fig. 1. Oxygen consumption of clawed, declawed and loaded male *Uca pugilator* during rest and sustainable exercise. Oxygen consumption (means \pm s.e.m.) was calculated per crab (A) and per gram body mass (B). Oxygen consumption increased during exercise, and there were no significant differences among the three treatment groups during rest or during exercise on a per crab (rest, $P = 0.21$; exercise, $P = 0.14$) or per gram (rest, $P = 0.58$; exercise, $P = 0.57$) basis. $n = 10$ for each treatment group. P -values are from one-way ANOVA.

consumption among clawed, declawed and loaded crabs (one-way ANOVA: rest, $F_{2,27} = 0.55$, $P = 0.58$; walk, $F_{2,27} = 0.57$, $P = 0.57$).

As oxygen consumption scales with body mass (Schmidt-Nielsen, 1997), we also plotted mass-specific oxygen consumption against body mass to further examine the influence of the enlarged claw on oxygen consumption (Fig. 2). Despite a small size range, our analysis showed that mass-specific oxygen consumption decreased significantly with an increase in body mass (ANCOVA: $F_{1,24} = 9.36$, $P < 0.05$), and that the influence of body mass on oxygen consumption was the same for all three treatment groups ($F_{2,24} = 0.18$, $P = 0.84$). Finally, there was no significant difference in oxygen consumption among clawed, declawed and loaded crabs at any given body mass ($F_{2,24} = 0.48$, $P = 0.62$), indicating that mass-specific oxygen consumption was unaffected by the major claw or the artificial load.

We also examined blood lactate concentration in crabs after the bout of sustained exercise. As with results for oxygen consumption, there was no significant difference in blood lactate levels among resting clawed, declawed and loaded crabs (one-way ANOVA: $F_{2,31} = 0.87$, $P = 0.43$; Fig. 3). Following exercise, however, treatment did have a significant effect on blood lactate concentration (one-way ANOVA: $F_{2,26} = 7.25$, $P < 0.01$; Fig. 3), with loaded crabs having a significantly higher blood lactate concentration than clawed ($P < 0.01$) and declawed ($P < 0.05$) crabs, whose levels were not significantly different ($P = 0.77$).

Strenuous exercise: clawed and declawed crabs

The major claw had no significant effect on sprint duration, with clawed crabs sprinting for 25.4 ± 1.51 s and declawed crabs sprinting for 25.3 ± 2.14 s before slowing to a walk (two-sample t -test, $t = 0.04$, d.f. = 37.2, $P = 0.97$).

Blood lactate concentration of clawed and declawed crabs changed similarly following a sprint and forced walk, and during recovery (Fig. 4). In both groups, blood lactate concentration more than quadrupled immediately after the sprint, increased further after 10 min of forced walking, and continued to increase 5 min into the recovery period. After 15 min of recovery, blood lactate levels decreased noticeably in both groups and continued to decrease throughout the 60 min recovery period. Overall, there was a general trend for clawed crabs to have a higher blood lactate concentration than declawed crabs following 10 min of forced walking and during

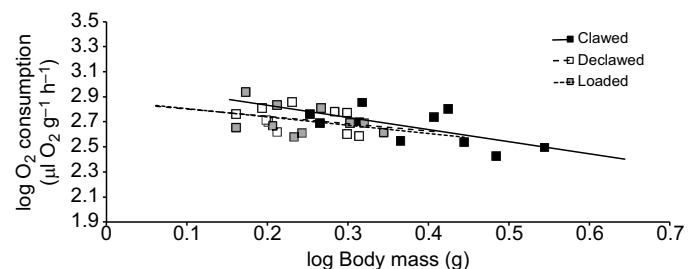


Fig. 2. Oxygen consumption as a function of body mass for clawed, declawed and loaded male *U. pugilator* during sustainable exercise. Log oxygen consumption per gram decreased significantly with log body mass for all treatment groups ($P < 0.05$). The influence of body mass on oxygen consumption did not differ among the treatment groups ($P = 0.84$) and there was no effect of treatment group on oxygen consumption at any body mass ($P = 0.62$). Regression equations: clawed, $y = -0.97x + 3.03$, $R^2 = 0.42$; declawed, $y = -0.58x + 2.86$, $R^2 = 0.12$; loaded, $y = -0.66x + 2.87$, $R^2 = 0.12$. Mass of the loaded crabs does not include the inert load. $n = 10$ for each treatment group. P -values are from an ANCOVA.

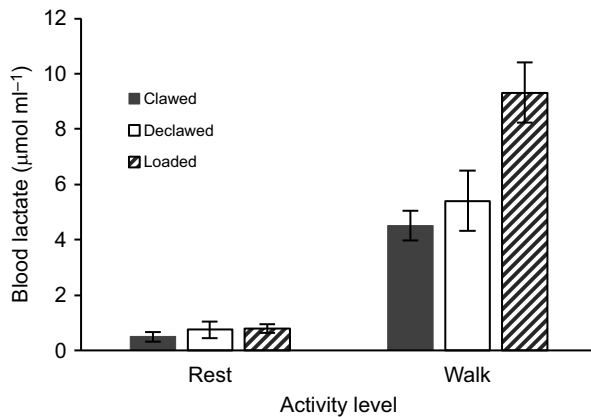


Fig. 3. Blood lactic acid concentration in clawed, declawed and loaded male *U. pugilator* during rest and following sustainable exercise. Resting blood lactate concentration (means \pm s.e.m.) was the same for all three treatment groups ($P=0.43$), but lactate levels following exercise were significantly higher in loaded crabs than in clawed ($P<0.01$) and declawed ($P<0.05$) crabs, whose levels were not different ($P=0.77$). Resting: clawed, $n=14$; declawed, $n=8$; loaded, $n=11$. Exercise: clawed, $n=10$; declawed, $n=10$; loaded, $n=9$. P -values are from one-way ANOVA.

recovery. At 60 min, blood lactate levels remained elevated relative to resting levels in both groups.

DISCUSSION

Models proposed to explain the evolution of exaggerated sexually selected traits carry the assumption that these traits are costly (Zahavi, 1975; Kodric-Brown and Brown, 1984; Grafen, 1990; Andersson, 1994; Kotiaho, 2001). Making up as much as 40% of an individual's body mass (Crane, 1975), the large major claw of *U. pugilator* seems likely to increase the energetic cost of locomotion. Contrary to our hypothesis, the results showed that the major claw did not increase energy expenditure during sustained locomotion (Figs 1, 2 and 3). However, the major claw may begin to increase energy requirements during more strenuous, non-sustainable locomotion (Fig. 4).

The results of the present study corroborate some findings from earlier work on *U. pugilator*. Similar to our results showing that clawed and declawed crabs sprinted for the same amount of time, previous studies found no influence of the major claw on sprint

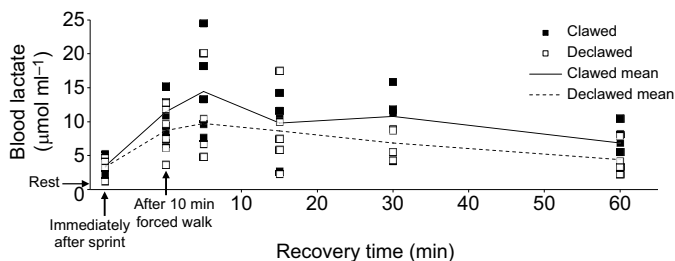


Fig. 4. Blood lactate concentration of male *U. pugilator* with and without their major claw following strenuous exercise and during recovery. Each point represents blood from a single animal. The lines connect the mean values at each time point for each treatment group and help illustrate trends. Overall, blood lactate levels in clawed and declawed crabs followed a similar pattern, and there was a trend for clawed crabs to have a higher average blood lactate concentration than declawed crabs. Clawed crabs: after sprint, $n=4$; after 10 min forced walk, $n=8$; after 5, 15, 30 and 60 min recovery, $n=6, 4, 4$ and 5, respectively. Declawed crabs: immediately after sprint, $n=4$; after 10 min forced walk, $n=8$; after 5, 15, 30 and 60 min recovery, $n=5, 5, 4$ and 4, respectively.

speed (Jordão and Oliveira, 2001; Allen and Levinton, 2007; Gerald and Thiesen, 2014, for horizontal surfaces). However, our results showing that the major claw has no influence on oxygen consumption during rest or during exercise differ from those of Allen and Levinton (2007), who reported that the major claw increases resting aerial oxygen consumption by 8% (although they found no significant difference in resting aquatic oxygen consumption). The reason why our results for resting oxygen consumption differ from this earlier study is unclear, but could include subtle differences in the activity of the animals or methodological differences. For example, Allen and Levinton (2007) used closed-system respirometry and small respirometers (4.7 cm diameter \times 4 cm high), while we used open-flow respirometry and larger respirometers, although it is not immediately apparent how these factors would lead to different outcomes. Regardless of the exact reason for the different results, it could be argued that an 8% increase in resting oxygen consumption is relatively small given that the major claw comprised \sim 30% of body mass (Allen and Levinton, 2007).

When exercising at a sustainable speed, clawed and declawed *U. pugilator* in the present study consumed the same amount of oxygen and produced the same amount of lactic acid. Our results showing that possession of the sexually selected trait did not increase the energetic cost of locomotion are in keeping with results from some previous studies on other animals. For example, the large train of peacocks does not increase energy expenditure during terrestrial locomotion (Thavarajah et al., 2016), the elongated fin streamers of male threadfin rainbow fish do not increase oxygen consumption during swimming (Trappett et al., 2013), and the large anterior horn of male rhinoceros beetles adds only a negligible amount to the aerodynamic cost of flight (McCullough and Tobalske, 2013). It has been suggested that the reason some sexually selected structures do not lead to an increase in energy expenditure is that they make up only a small percentage of a bearer's body mass (Goyens et al., 2015b; e.g. peacock train: 6.9% of body mass: Askew, 2014; rhinoceros beetle horn, 1.5% of body mass: McCullough and Tobalske, 2013). Support for this hypothesis comes from a study on the male stag beetle, *C. metallifer*, whose enlarged mandibles and associated musculature make up 18% of body mass, and lead to a 40% increase in the cost of terrestrial locomotion relative to females, which lack the trait (Goyens et al., 2015a). However, low relative mass cannot explain why the major claw of *U. pugilator* did not increase locomotory costs in the present study as the claw made up \sim 30% of the total body mass.

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; Husak and Swallow, 2011) – is one explanation for why some elaborate sexually selected traits incur minimal locomotory costs. For example, the aerodynamic costs resulting from the long tails of male barn swallows, *H. rustica*, are partly mitigated by greater wingspans (Barbosa and Møller, 1999). Similarly, males of some species of stalk-eye flies have increased thorax size and wing length, which help compensate for increased inertia during flight (*Cyrtodiopsis whitei*: Swallow et al., 2000; *Cyrtodiopsis dalmanni*: Ribak and Swallow, 2007). Results from the sustainable exercise experiments suggest that compensatory traits may help minimize the energetic cost to *U. pugilator* when locomoting with the enlarged major claw. Although clawed, declawed and loaded crabs in the present study consumed the same amount of oxygen during sustained locomotion, average blood lactate concentration of loaded crabs was significantly higher

than that of the other two treatment groups (Fig. 3), indicating that artificially loaded crabs did require more energy to achieve the same level of performance as clawed and declawed crabs. The fact that the loads were placed on the carapace suggests that male crabs have evolved to carry the weight of the claw only in its natural location and not elsewhere on the body. Given that crab limb muscles are composed of multiple fiber types (Parsons and Mosse, 1982; Perry et al., 2009), the higher blood lactate concentration observed in artificially loaded crabs may result from additional glycolytic muscle fibers not typically used for sustained locomotion being recruited to help carry the unnaturally placed load.

Although we do not know what sort of compensatory trait could mitigate the cost of carrying the weight of the claw, possibilities include morphological, behavioral and biomechanical adaptations (Herreid and Full, 1986; Heglund et al., 1995; Kram, 1996; Møller, 1996). For example, research has shown that asymmetry in the leg structure of male *Uca* spp. is associated with species-specific claw waving patterns (Takeda and Murai, 1993), suggesting that a similar type of adaptation has also made it economical to carry the claw. If compensation for carrying the major claw includes biomechanical factors, this could help explain why claw removal did not lead to a decrease in energy expenditure during locomotion. Given that evolution acts on the whole organism, it is reasonable to assert that limb orientation, posture and stride characteristics of male fiddler crabs evolved to carry the enlarged claw efficiently. Therefore, removal of the claw could necessitate short-term modifications (as opposed to evolutionary adaptations) in one or more of these factors, which could negate any decreases in metabolism that would have been brought about by having less weight to carry. For example, crabs can conserve up to 50% of energy during walking through exchange between potential and kinetic energy, energy that would otherwise need to be generated by muscles (Blickhan and Full, 1987; Full, 1997). If removal of the major claw changed stride characteristics such that the efficiency of this energy exchange was decreased, this would increase the amount of force muscles needed to produce, thereby increasing locomotory costs. Alternatively, if claw removal led to subtle changes in joint angles, this could alter where on the length–tension curves the muscles were working. This could, in turn, decrease the amount of force individual muscles could produce (Full et al., 1990; Full and Ahn, 1995), thereby requiring activation of more muscles to produce the necessary force. Finally, if removal of the weight of the claw led to an increase in stride frequency, this could add to locomotory costs as higher rates of force production increase the cost of force production (Taylor et al., 1980; Roberts et al., 1998). Although speculative at this point, these are all testable hypotheses for future studies.

In the present study, clawed and declawed *U. pugilator* had equivalent blood lactate concentrations following a bout of sustainable exercise at 0.10 km h^{-1} (Fig. 3). In many animals, including crustaceans, there is a correlation between lactic acid and fatigue (Adamczewska and Morris, 1994; Briffa and Elwood, 2002; Matsumasa and Murai, 2005), although increased lactic acid itself is probably not the cause of fatigue or decreased endurance (Putnam, 1979; Allen et al., 2008). Extrapolating from what is known about the link between fatigue and blood lactate concentration, clawed and declawed crabs locomoting at 0.10 km h^{-1} should have the same endurance. Empirical tests of the influence of the major claw on endurance, however, show that this is not the case for *U. pugilator* running at faster speeds. Clawed crabs in Allen and Levinton's (2007) study had much lower endurance than declawed crabs when exercising at the moderately high speed of 0.24 km h^{-1} , a result that

was confirmed by Gerald and Thiesen (2014) for clawed and declawed crabs exercising at 0.22 km h^{-1} (Gerald and Thiesen, 2014). Combining these results with our findings suggests that as exercise becomes more intense, carrying the large major claw may become more energetically costly, ultimately leading to decreased endurance. The finding that the cost of bearing the claw can depend on the intensity of locomotion was noted by Gerald and Thiesen (2014), who found that the major claw impairs sprint performance of male *U. pugilator* running up and down slopes, but not for those running on level ground. The results from our strenuous exercise experiments support the idea that the energetic cost of bearing a large sexually selected trait could depend on the intensity or type of locomotion.

Unlike results for the sustainable locomotion experiments, results from the strenuous experiments show a clear trend for clawed crabs to have a higher energy expenditure than declawed crabs, as indicated by higher blood lactate levels after 10 min of forced walking and throughout the recovery period (Fig. 4). Although we could not assess whether the observed difference rose to the level of statistical significance, the difference between the two groups may, nonetheless, be biologically significant. High levels of lactic acid have been shown to correlate with reduced performance capabilities in crustaceans (Booth and McMahon, 1985; Briffa and Elwood, 2001; Matsumasa and Murai, 2005). Thus, the trend observed in the present study towards higher blood lactate concentrations in clawed crabs following strenuous locomotion is in keeping with observations showing that clawed crabs have lower endurance than declawed crabs when running at moderately high speeds (Allen and Levinton, 2007; Gerald and Thiesen, 2014). Higher blood lactate concentrations in clawed crabs relative to declawed crabs could also lead to longer recovery times as more lactic acid moves from the muscles to the blood for eventual clearance through gluconeogenesis or conversion back to pyruvate for further metabolism (Adamczewska and Morris, 1994; Henry et al., 1994). We can make a rough estimate of recovery time for clawed and declawed crabs from the data shown in Fig. 4. Assuming that blood lactate levels decrease linearly during recovery from 5 to 60 min, and extending this beyond 60 min, blood lactate levels should return to resting levels in approximately 93 min for declawed crabs and 107 min for clawed crabs, an increase of 15%, although these likely underestimate recovery times as blood lactate levels decrease exponentially following exercise (Full and Herreid, 1984; Booth and McMahon, 1985; Henry et al., 1994). If locomoting with a major claw does necessitate longer recovery times, this could translate into less time available for foraging and pursuing mates, both of which could have fitness implications.

The idea that sexually selected traits are costly is central to models of sexual selection. However, results from studies investigating the impact of these exaggerated traits on the energetic cost of locomotion have been equivocal. Rather than resolve the issue, results from the present study on metabolic energy expenditure of *U. pugilator* during locomotion add to the complexity. Although the major claw did not lead to increased energy expenditure during sustained locomotion, we show that costs may begin to manifest themselves during more strenuous, non-sustainable locomotion. Our results reinforce the need to examine a variety of activity types and intensities when investigating potential locomotory costs of bearing an elaborate sexually selected trait.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.T.; Methodology: A.T., C.H.T.S.; Formal analysis: A.T., C.H.T.S.; Investigation: C.H.T.S.; Writing - original draft: A.T., C.H.T.S.; Writing - review & editing: A.T., C.H.T.S.; Supervision: A.T.; Funding acquisition: A.T., C.H.T.S.

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