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The Cost of Bearing a Sword: Locomotor Costs and Compensations in Relation to a Sexually Selected Trait in *Xiphophorus*

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Evolution, Ecology and Organismal Biology

by

Christopher E. Oufiero

December 2010

Dissertation Committee:

Dr. Theodore Garland, Jr., Chairperson
Dr. David N. Reznick
Dr. Mark A. Chappell

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The Dissertation of Christopher E. Oufiero is approved:

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Committee Chairperson

University of California, Riverside

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ABSTRACT OF THE DISSERTATION

The Cost of Bearing a Sword: Locomotor Costs and Compensations in Relation to a Sexually Selected Trait in *Xiphophorus*

by

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Doctor of Philosophy, Graduate Program in Evolution, Ecology, and Organismal Biology

University of California, Riverside, December 2010

Dr. Theodore Garland, Jr., Chairperson

Some of the most compelling traits to evolutionary biologists are sexually selected traits. The 'un-natural' expression of these traits has puzzled biologists since the time of Charles Darwin. Why would a trait evolve that seems to decrease survival? Most of these traits evolve because they increase the reproductive fitness of the bearer, either through intra-sexual or inter-sexual competition. However, these sexually selected traits must meet the demands of both natural and sexual selection. It is therefore assumed that there are 'costs' associated with the trait that limits their expression. In this dissertation I examined the performance costs of a sexually selected trait, expressed as an exaggerated morphological structure.

I first introduce a more integrative approach to the study of detecting costs of sexually selected traits. This approach incorporates additional aspects of the organism's phenotype that may have evolved to offset the costs of the sexually selected trait. I show

that ignoring these ‘compensatory’ traits may mask the cost of the sexually selected trait using synthesized data.

I then set out to examine the repeatability of various swimming performance measures. Using male guppies (*Poecilia reticulata*) I demonstrate that swimming performance measures are stable over various time scales. Both aerobic and anaerobic locomotor functions were examined over time scales varying from days to more than a year later. While most performance measures were not stable over a year, at least one was repeatable. Furthermore, although most performance measures decreased over a year, one significantly increased (maximum speed, U_{max}).

I then used phylogenetic comparative methods to determine the locomotor costs of the sexually selected ‘sword’ among species of *Xiphophorus* and *Priapella*, taking into account compensatory traits. I showed that inter-specifically, the sword is not a cost to critical swimming speed among 19 species of *Xiphophorus* and *Priapella*. In fact, when compensatory traits and phylogenetic information are included, the sword had a significant positive effect on critical swimming speed among these species. This suggests that as this sexually selected trait evolved it was not a locomotor handicap.

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Introduction

Sexually selected traits have puzzled biologists for many years (Darwin 1871, Bradbury & Andersson 1987, Maynard Smith 1987, Andersson 1994, Andersson & Iwasa 1996, Meade & Arnold 2004). How can we explain the evolution of traits that seem to compromise the bearer's survival? Since Darwin first recognized the existence of such traits, including complex behaviors, songs, and/or morphological structures, many theories have been proposed to explain the origin and evolutionary maintenance of sexually selected traits. These theories range from female choice to sensory bias to runaway selection (Fisher 1930, Zahavi 1975, Smith 1976, Hamilton & Zuk 1982, Kirkpatrick 1982, Andersson 1986, Kirkpatrick 1987, Grafen 1990, Andersson 1994, Andersson & Iwasa 1996, Ryan 1998, Meade & Arnold 2004). According to most of these theories, the sexually selected trait evolves (most often in males) because it causes some sort of reproductive advantage; those that possess the trait acquire more mates and/or matings, thereby potentially siring more offspring. However, the seemingly 'unnatural' expression of phenotypes that evolve because they give a reproductive advantage must also face the rigors of natural selection. It is generally assumed that structures, such as the tails of male peacocks, impose a cost (e.g., a reduction in survival or increased energetic demands), and that this cost will mean they are disfavored by natural selection, which eventually limits their expression.

The costs of sexually selected traits have been examined in a variety of organisms and for a variety traits (Kotiaho 2001). In general, it is thought that although the sexually selected trait increases the bearer's matings, it will also compromise survival. Therefore, opposing natural selection counteracts exaggeration of the structure. Despite the costs and opposing selection, many examples of sexually

selected traits exist, including behaviors, morphological structures, and coloration (Andersson 1994). Costs associated with these traits can, in principle, be anything that reduces Darwinian fitness, i.e., lifetime reproductive success, or one of its major components (e.g., survivorship). For example, male Tungara frogs (*Physalaemus pustulosus*) call to attract females. The calls can be energetically expensive, thereby reducing resources available for other needs and indirectly reducing survival (Bucher, Ryan & Bartholomew 1982), but see (Green 1990). In addition, the calls can attract bat predators, leading to immediate mortality, i.e., a direct survival cost (Ryan, Tuttle & Rand 1982). As this example indicates, costs should be examined in traits that are biologically relevant to the particular sexually selected trait.

The potential cost of sexually selected traits has been examined using various methods, with little consistency. The first, and most common is experimental reduction or amplification of the sexually selected trait to determine if reducing the trait results in a reduction of its costliness, or vice versa for increasing the trait (Evans & Hatchwell 1992, Møller & deLope 1994, Basolo & Alcaraz 2003, Kruesi & Alcaraz 2007, Clark & Dudley 2009). Another common method is to examine costs associated with natural variation in the sexually selected trait (Ryan 1988, Nicoletto 1991, Garcia, Jiminez & Contreras 1994, Emlen 2001, Allen & Levinton 2006, Karino, Orita & Sato 2006, Royle, Metcalfe & Lindstrom 2006). Thirdly, costs of sexually selected traits have been estimated by comparing the sex with the trait to the sex without the trait (Promislow 1992). Several other methods to examine costs that have received less attention, including: examination of costs inter-specifically across taxa that vary in their expression (Promislow 1992); ontogenetic examination of how the trait affects the bearer as it develops, similar to studies of the ontogeny of sexual size dimorphism (Badyaev 2002); and examination of

how certain sexually selected traits (e.g., exaggerated morphological structures) alter the functional relationships of the bearer (Ribak, Egge & Swallow 2009). Finally, costs of sexually selected traits have more recently been examined from an integrative perspective (Irschick *et al.* 2007, Oufiero & Garland 2007, Irschick *et al.* 2008).

Although sexually selected traits may reduce survival, they do not evolve in isolation from the rest of the organism; other 'compensatory' traits may evolve in concert with the sexually selected trait because they partially offset its cost. For example, Balmford, Jones & Thomas (1994) showed that male birds with longer tail feathers (presumed caused by sexual selection) also tended to have longer wings. They suggested that longer wings would increase the thrust, counterbalancing the drag associated with elongated tail feathers. Although several other authors have noted the potential importance of compensatory traits (e.g., (Kirkpatrick 1987, Møller 1996), few have incorporated them into studies examining the cost of sexually selected traits. Ignoring such traits may mask the potential negative effect of the sexually selected trait on other aspects of the organism's phenotype (Oufiero & Garland 2007), and potential impact on survival.

The purpose of my dissertation was to examine the costs of one well known sexually selected trait from a phylogenetic and integrative perspective, incorporating both evolutionary relationships and potential compensatory traits. To do this, I studied the *Xiphophorus* group of poeciliid fish because of the sexually selected elaboration of the caudal fin – the sword. This trait evolved in part because of a pre-existing female bias, in which females prefer males with longer swords (Basolo 1995, 1996). Relative sword length shows considerable variation among extant species within the group (Fig. 0.1).

The sword offers several unique opportunities to examine the costs of a sexually selected trait. First, the natural variation in the expression of the trait allows for comparisons of males with and without the sword, without any experimental manipulation that may alter functional relationships in 'unnatural' ways. Second, existing phylogenetic hypotheses for the group (Marcus & McCune 1999, Morris, de Queiroz & Morizot 2001) allow for the incorporation of phylogenetic information in statistical analyses (e.g., Garland, Bennett & Rezende 2005). Third, this trait has the potential to directly impact locomotor performance, which may indirectly affect foraging success, survival, and therefore fitness (Arnold 1983, Garland & Carter 1994, Garland & Losos 1994), as it is linked to the thrust-producing system (the caudal peduncle and fin). Few sexually selected morphological structures are directly linked to the thrust producing system (e.g, elongated tail feathers as opposed to wing feathers of birds); therefore few offer the opportunity to examine the potential effects of drag and physiological burden. Finally, previous studies provide mixed support for the costliness of the sword (Ryan 1998, Basolo & Alcaraz 2003, Royle *et al.* 2006, Kruesi & Alcaraz 2007).

In the first chapter of my dissertation, I review and discuss how costs of sexually selected traits have been evaluated. Using the literature, I show how costs of sexually selected traits have been approached in the past, with examples from various studies. I focus on the locomotor performance costs that may be imposed by sexually selected traits. I also introduce an integrative approach, incorporating compensatory traits and path analyses, to detect potential locomotor costs of sexually selected traits. Motivated by a published dataset (Pyron 1996), I simulate data to model the effect of the sexually selected sword on swimming endurance, taking into account heart ventricle mass as a compensatory trait. I show that ignoring the compensatory trait leads to a positive effect

of sword length on endurance. This result is consistent whether comparing males alone or using a male-female dimorphism index. In this chapter, I highlight the importance of an integrative approach to the study of sexual selection, and alternative ways to examine locomotor costs.

The second chapter of my dissertation focuses on the various swimming performance measures used for fish and their repeatability. To effectively determine if the sexually selected trait is a cost to swimming abilities in fish, the repeatability of the measures should be known. If measurement of a trait is not repeatable, then selection may not be able to act on it, the trait may not be heritable, and there may be no detectable relation between the sexually selected trait and locomotor performance. Therefore, using 20 male Trinidadian guppies (*Poecilia reticulata*), I examine the repeatability of critical swimming speed (U_{crit}), maximum speed (U_{max}) and the velocity attained during a c-start escape response. I examine the repeatability of these traits over varying time scales, from several days to more than a year later. The results show that most swimming performance measures are repeatable on short and medium time scales, but only U_{max} is consistent over a year later. Most traits decreased over a year, as would be expected from normal aging processes, but U_{max} significantly increased. Finally, analyses of performance traits and body size reveal very few significant correlations; no swimming performance trait was significantly correlated to size or a shape index.

In the third chapter, I test whether the sexually selected sword is a cost to an aerobic locomotor performance, critical swimming speed (U_{crit}), by comparing mean values for 19 species of *Xiphophorus* and *Priapella* (Fig. 0.2). Using a total of 579 male and female fish from these 19 species, I examine whether species with longer swords

have a reduced U_{crit} through statistical analyses that incorporate some potentially compensatory traits and independently derived phylogenetic information. Several compensatory traits were measured, including maximum oxygen consumption (VO_{2max}), heart mass, gill mass, citrate synthase activity and lactate dehydrogenase activity in tail muscle, and various measurements of body size and shape (Fig. 0.3). I also examine the effect of reproductive allocation by the females on their critical swimming speed to determine if species that allocate more energy to reproduction show a reduction in critical swimming speed. Both whole-organismal and residual (from regressions on standard length) trait correlations are examined. Several multiple regression models are compared using various combinations of predictors to determine if sword length has a negative effect on critical swimming speed.

Using both conventional and phylogenetic statistical methods, I find that sword length does not reduce U_{crit} among males. In fact, using phylogenetically independent contrasts, sword length has a significant positive effect on U_{crit} when heart mass is also included in the model. This suggests that sword length is not a locomotor handicap; moreover, it may serve as an indication to the female of the male's swimming abilities (one indicator of male 'quality'). Furthermore, I find that an index of reproductive allocation (regressor dry mass) has a significant negative effect on female U_{crit} under non-phylogenetic models. This suggests that females incur a locomotor cost as they allocate energy to producing eggs. The results from this dissertation show that the exaggeration of a 'classic' sexually selected trait does not necessarily result in a locomotor cost.

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Figure 0.1. Phylogenetic distribution of sword length among 19 species of *Xiphophorus* and *Priapella*. Colors mapped on phylogeny represent sword length (done by mapping a continous trait onto the phylogeny in Mesquite). Points on scatterplot represent species mean sword length ± standard error. Example sword lengths given along the bottom of the x-axis, sword size in the pictures is approximately equal to value along the x-axis and corresponds to a species with that sword length.

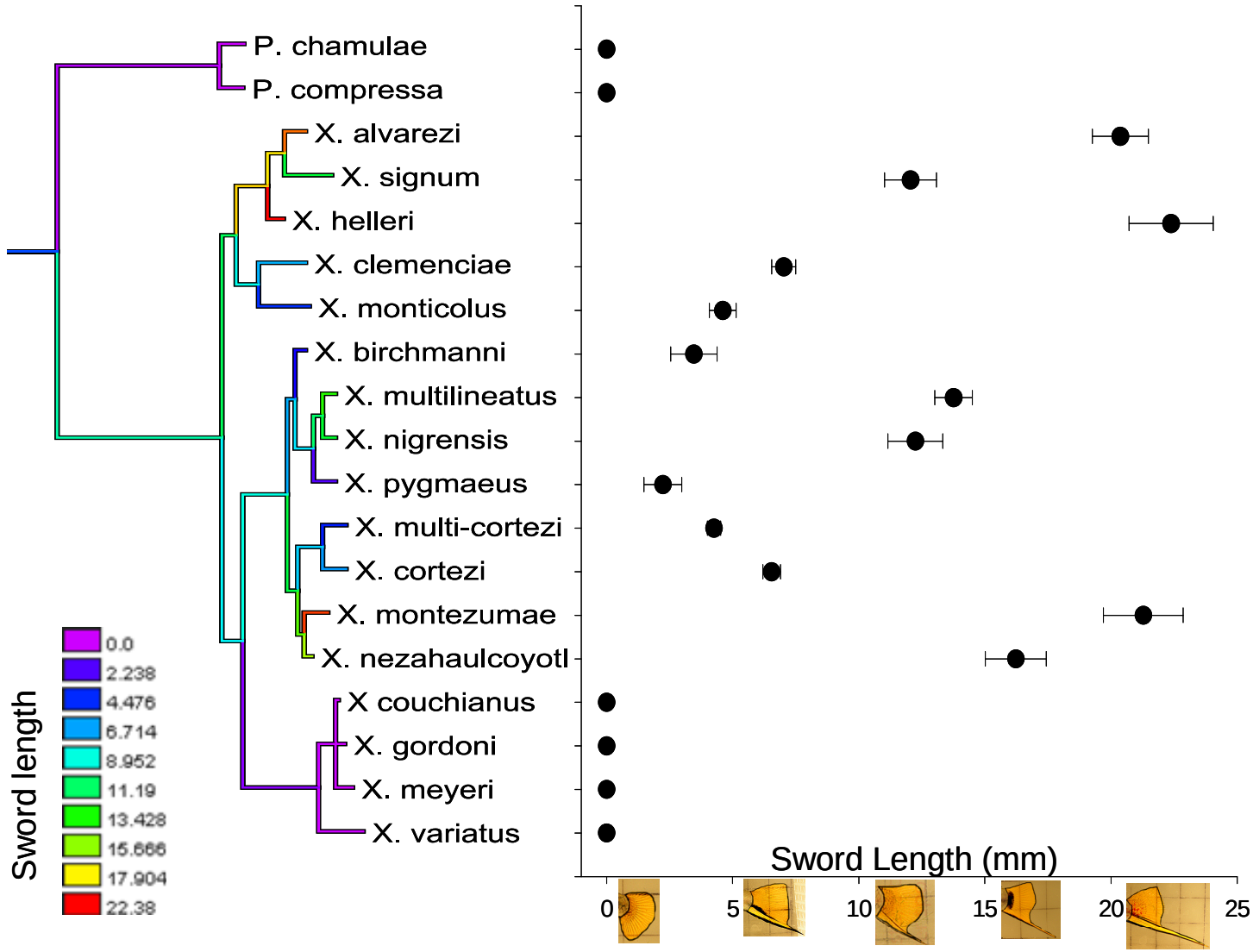

P. chamulae
P. compressa
X. alvarezi
X. signum
X. helleri
X. clemenciae
X. monticolus
X. birchmanni
X. multilineatus
X. nigrensis
X. pygmaeus
X. multi-cortezi
X. cortezi
X. montezumae
X. nezahaulcoyotl
X couchianus
X. gordoni
X. meyeri
X. variatus
Sword length
0.0
2.238
4.476
6.714
8.952
11.19
13.428
15.666
17.904
22.38
Sword Length (mm)
0
5
10
15
20
25

Figure 0.1

Figure 0.2. Distribution of sword length mapped onto a phylogenetic hypothesis for the group. Bar plot represents sample sizes for males and females tested among the 19 species. Black bars represent number of males for each species; white bars represent number of females used for each species.

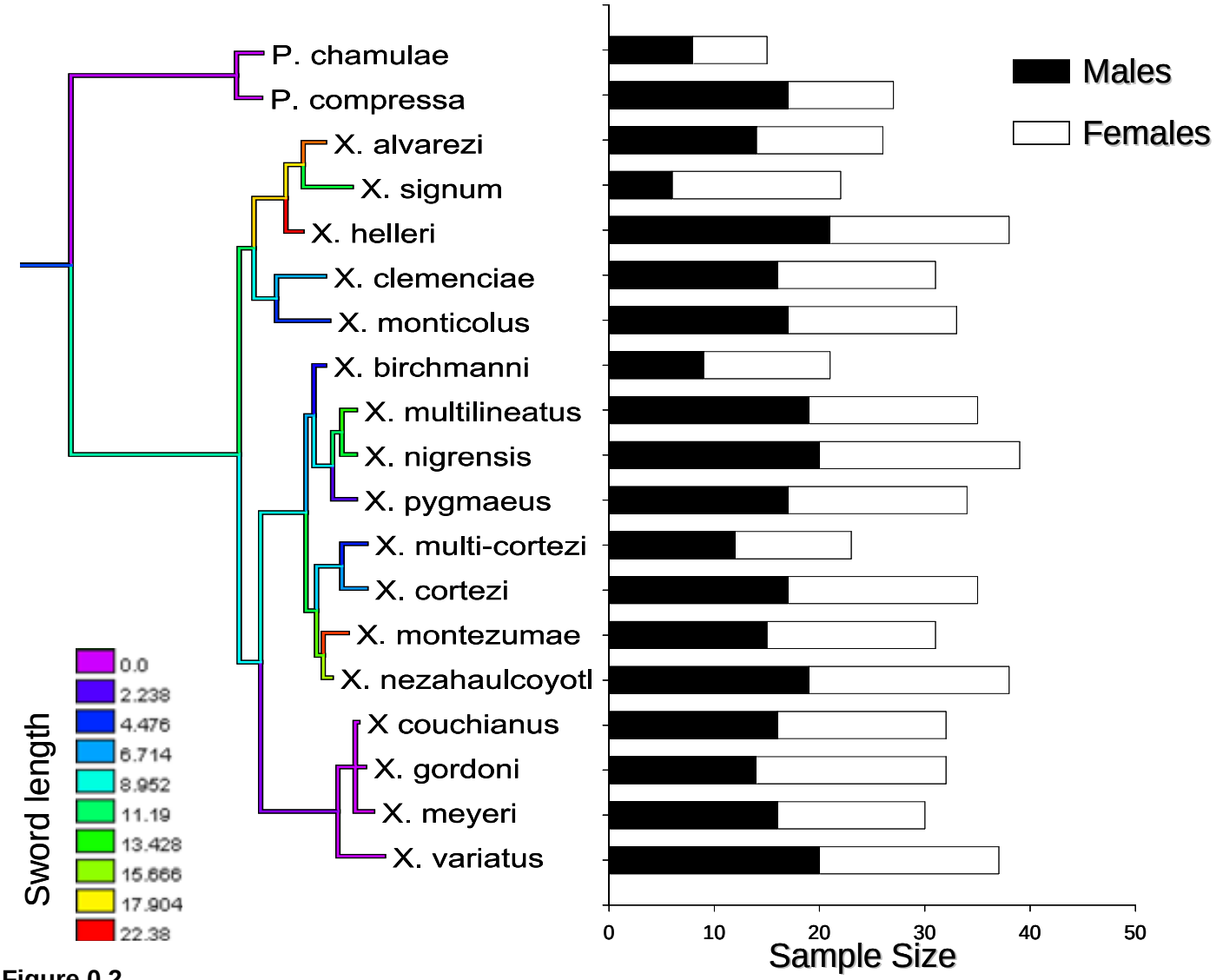
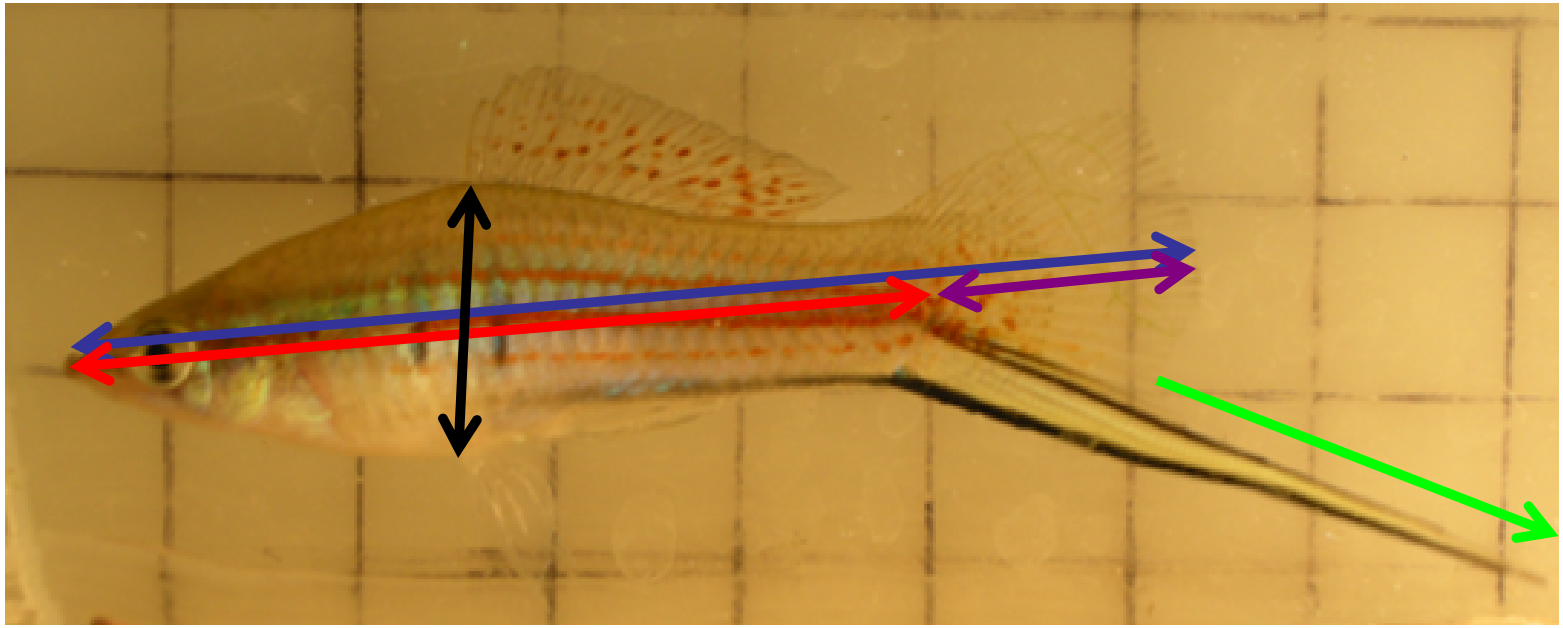

P. chamulae
P. compressa
X. alvarezi
X. signum
X. helleri
X. clemenciae
X. monticolus
X. birchmanni
X. multilineatus
X. nigrensis
X. pygmaeus
X. multi-cortezi
X. cortezi
X. montezumae
X. nezahaulcoyotl
X couchianus
X. gordoni
X. meyeri
X. variatus
Males
Females
0
10
20
30
40
50
Sample Size
Sword length
0.0
2.238
4.476
6.714
8.952
11.19
13.428
15.666
17.904
22.38

Figure 0.2

Figure 0.3. Picture depicting the various size measurements used in Chapters 2 and 3. Total length is the length of the fish from the most anterior portion to the edge of the caudal fin, standard length is the length of the fish from the most anterior portion to the insertion (base) of the caudal fin, body depth is the depth of the fish from the anterior insertion of the dorsal fin to the anterior insertion of the anal fin, caudal fin length was the difference in total length and standard length, sword length is the length of the sword (extension of the lower margin of the caudal fin) from the edge of the caudal fin to its most distal point. Also measure were body mass and tail mass (fish were dissected along the body depth line and tail muscle weighed.



Total Length (TL)
Standard Length (SL)
Body Depth (BD)
Sword Length

Caudal Fin length = TL – SL
Body Mass
Tail Mass

Figure 0.3

Chapter 1

Evaluating performance costs of sexually selected traits

1.1 Summary

1. Aspects of locomotor performance are thought to be important in a variety of natural contexts, including foraging and escaping from predators. The role of locomotor abilities in sexual selection is less well documented, but is probably of wide importance.

2. Sexual selection may exaggerate traits beyond their optimum with respect to biomechanical or physiological function, thus imposing "costs" of various types. Locomotor performance, in conjunction with its lower-level determinants and its behavioural and ecological consequences, constitutes an ideal set of phenotypes with which to study such costs.

3. Organisms may possess traits that compensate for the negative effects of sexually selected traits on performance abilities. Ignoring these may lead to erroneous conclusions about the cost of a putatively sexually selected trait. At the same time, ignoring the effects of sexual selection on functionally important traits may lead one to underestimate their functional significance.

4. Many organismal properties are affected by, or at least correlate with, overall body size, so it must be considered in any study of adaptation or sexual selection. For inter-specific or inter-population comparative studies, phylogeny must also be considered in statistical analyses.

5. We present a hypothetical data set that illustrates the perils of ignoring potential

compensatory mechanisms when studying the costs of a sexually selected trait. We also discuss different ways of studying costs, including analyses of a single sex and of the differences between the sexes.

1.2 Introduction

Since Charles Darwin, sexual selection has been recognized as a factor that often seems to oppose adaptation via natural selection, and that can lead to the evolution of elaborate and even bizarre secondary sexual characteristics. Darwin (1859) coined the term sexual selection and wrote that it "depends, not on a struggle for existence, but on a struggle between the males for possession of the females; the result is not death to the unsuccessful competitor, but few or no offspring." In 1871 he wrote "sexual selection depends on the success of certain individuals over others of the same sex, in relation to the propagation of the species; while natural selection depends on the success of both sexes, at all ages, in relation to the general conditions of life." Although some researchers do not draw a distinction between natural and sexual selection, Darwin's conceptualization has nonetheless proven to be a very powerful paradigm for subsequent and current research (e.g. Kirkpatrick 1987, Kotiaho 2001, Badyaev 2002, Stearns & Hoekstra 2005).

Elaborated secondary sexual characters result from two main types of sexual selection, intrasexual (e.g. male-male combat) and intersexual (or epigamic, e.g. female choice of males with particular characteristics). The opportunity for sexual selection is generally thought to be greater in males than in females because the reproductive success of males is limited by access to females (because sperm production is typically cheap), whereas that of a female is limited by the number of offspring she can carry or raise, or by food, shelter or other such resources required for provisioning and care of offspring, either in utero or after hatching or birth (Trivers 1972, Stearns & Hoekstra 2005). Therefore, in general, we expect to find sexually selected traits in males more often than in females, and this expectation is supported by evidence for most groups of

animals (reviewed by Bradbury & Andersson 1987, Andersson 1994). Birds provide many familiar examples of traits thought to have evolved via sexual selection, including complex songs, bright coloration and elaborate plumage morphologies, such as the tail of male peacocks (Bradbury & Andersson 1987). Such traits are generally presumed to increase the male's reproductive success (Stearns & Hoekstra 2005) and have indeed been shown to do so in various empirical studies [e.g. Andersson (1986) on widowbirds]. However, such traits as greatly elongated tail feathers may impose costs for instance in terms of ability to forage and escape from predators. Obviously, any trait subject to sexual selection will also be subject to natural selection, and hence will not evolve without limits.

Beginning with Fisher (1930 and earlier), several theories and models have been proposed for the evolutionary origin and subsequent maintenance or elaboration of putatively sexually selected traits (reviewed by Kirkpatrick 1987, Ryan & Keddy-Hector 1992, Schall & Staats 1997, Meade & Arnold 2004; see also Stearns & Hoekstra 2005). Despite many differences, all of these ideas have at least one feature in common: the presence of elaborate sexually selected secondary characteristics, which appear to increase the reproductive success of the male (typically) while possibly compromising the bearer's survival or increasing its energy requirements. In principle, costs may be associated with any sexually selected trait (Møller 1989, Nicoletto 1991, Evans & Hatchwell 1992, Barbosa & Møller 1999, Park et al. 2000, Kotiaho 2001, Basolo & Alcaraz 2003).

One cost that has been proposed is lower ability to escape predation (Møller 1996), potentially through a reduction in locomotor performance. More generally, the morphology of an organism can affect its performance abilities, and thus aspects of its

Darwinian fitness. Arnold (1983) introduced a paradigm that simultaneously highlighted the effects of subordinate phenotypes (e.g. morphological and biochemical variation) on organismal performance abilities (e.g. in locomotion) and, in turn, the effects of organismal performance abilities on components of Darwinian fitness. A diagram motivated by his paradigm is depicted in Figure 1.1. Arnold's (1983) "centrality of organismal performance paradigm" (Garland & Carter 1994) has been much-discussed and elaborated since its introduction, and considerable theoretical and experimental work has examined effects of morphology on performance (Garland & Losos 1994, Plaut 2000, Ojanguren & Brana 2003, Billman & Pyron 2005, Blake *et al.* 2005). However, only a few studies have considered the costs of sexually selected traits in this framework (Ryan *et al.* 1982, Ryan 1988, Møller 1989, Nicoletto 1991, Evans and Hatchwell 1992, Kotiaho 2001, Basolo and Alcaraz 2003, Evans 2004, Karino *et al.* 2006). For instance, birds (Thomas 1993) and fish (Beamish 1978, Webb 1984, Weihs 1989) exhibit sexually selected elongated tails and fins/feathers, along with an increase in body depth in some species of fish (Ptacek 1998), that may decrease the efficiency of locomotion because they increase drag (see also Pettersson & Hedenström 2000).

Barbosa and Møller (1999) used theoretical calculations to argue that longer tail feathers in male barn swallows (*Hirundo rustica*) increased flight drag and decreased foraging efficiency, yet they did not measure actual flight performance as has been done in some bird biomechanical studies [e.g. Buchanan & Evans 2002, see Irschick and Garland (2001) for a review]. Similarly, several studies have examined the costs of sexually selected traits in poeciliid fish. For example, Nicoletto (1991) examined the relationship between critical swimming speed and tail morphology in a feral population of guppies (*Poecilia reticulata*) from New Mexico. Although he did not find any relationship

between tail shape and critical swimming speed, he also did not find a significant difference in the areas of the three tail types he examined. In a study of swordtails (*Xiphophorus montezumae*), another member of the Poeciliidae, Basolo and Alcaraz (2003) found a higher rate of oxygen consumption, both routinely and during courtship, in males with intact swords as compared with those that had their swords surgically removed. Finally, a recent study of variation among six populations of *Gambusia* found that C-start performance (Domenici & Blake 1997) was positively associated with standard length and negatively associated with gonopodium length in a multiple regression, leading the authors to conclude that "relatively large gonopodia seem to incur a cost of reduced burst-swimming speed" (Langerhans et al. 2005). Therefore, some evidence indicates that sexually selected traits actually can impose a performance cost. On the other hand, Royle *et al*. (2006) found that male *Xiphophorus helleri* with the relatively longest swords had the highest C-start performance, which suggests the possibility of one or more compensatory traits that may have covaried with sword length (see below and Fig. 1.1).

Because whole-organism performance is not solely determined by any one trait, natural selection may cause alterations of other traits to counteract the negative effects of a sexually selected trait, and such traits can be termed “compensatory traits” (Fig. 1.1, Kirkpatrick 1987, Møller 1996, Jennions et al. 2001). According to Kirkpatrick (1987, pp. 49-50), “Compensatory modifications of the males to cope with the demands of the preference, such as increased aerobic scope and enlarged musculature, will often evolve.” For example, *Gambusia affinis* from populations with predators appear to have evolved body shapes that increase their maximum burst-swimming speed (Langerhans *et al.* 2004).

1.3 Locomotor performance in relation to costs imposed by sexually selected traits

From the perspective of Figure 1.1, locomotor performance comprises an excellent set of phenotypes to study the costs of sexual selection. First, most behaviours involve locomotion (Garland 1994a,b, Garland & Losos 1994, Blake 2004), including escaping predators, capturing prey and fighting (Briffa & Sneddon 2007); therefore, a decrease in locomotor ability (performance capacity) is likely to have fairly direct effects on the Darwinian fitness of the individual. This assumes that animals actually use their maximal abilities in some important situations (review in Irschick & Garland 2001). Indeed, several studies have now provided empirical evidence of natural selection acting on locomotor abilities (e.g., Miles *et al.* 2000, Sinervo *et al.* 2000, Le Galliard *et al.* 2004, Husak 2006, references therein). Second, for many organisms, some aspects of locomotor performance are relatively easy to quantify in the laboratory (e.g. for fish, see Beamish 1978, Webb 1984, Domenici & Blake 1997, Plaut 2001, Nelson *et al.* 2002, Blake 2004, Lauder & Drucker 2004). Third, aspects of morphology and physiology can have direct effects on performance abilities (Beamish 1978, Arnold 1983, Garland & Losos 1994, Plaut 2000, Blake *et al.* 2005), and sexual selection can exaggerate a trait beyond its optimum with respect to its effect on locomotor function, thus causing a decrease in performance ability. Fourth, given our fairly good understanding of the mechanisms underlying variation in locomotor performance, at least for some groups of animals, it is possible to imagine various traits that might compensate for the adverse effects of a sexually selected trait (e.g. see Evans & Thomas 1992, Balmford *et al.* 1994, Møller 1996). Finally, it is possible that individual variation in locomotor abilities may

reflect overall health or vigour, and hence be a subject of sexual selection (see Introduction, Garland *et al.* 1990b, Chappell *et al.* 1997, Robson & Miles 2000, Husak *et al.* 2006, Kotiaho and Puurtinen 2007).

In principle, sexual selection could give rise to potential handicaps related to locomotor abilities in any group of organisms. Hummingbirds, for example, rely on flying ability to a great extent in their daily activities, including foraging and territorial interactions. Sexual dimorphism exists in many species, including male plumage characteristics that are thought to have been sexually selected. Sex differences in morphology and flight performance have been documented (e.g. Chai *et al.* 1996), but we are not aware of any studies that have claimed that the sexually selected traits impose a penalty in terms of flight performance or energetic costs.

Among the vertebrates, fish and lizards have received the greatest attention in terms of measuring locomotor performance. Here, we focus on fish, with particular reference to the genus *Xiphophorus* (swordtails and platyfish in the family Poeciliidae), which includes about 20 livebearing, freshwater species. This emphasis reflects our ongoing studies with this group and also the fact that it has been relatively well-studied in terms of sexual selection, morphology and performance. Several modes of swimming in fish have the potential to be affected by a sexually selected trait. These swimming modes capture an array of locomotor capabilities, and informative performance traits can be measured for each. A common measure of a fish's swimming performance is its "escape response," often characterized by a "C-start," in response to an external stimulus, such as dropping an object into its tank (Domenici & Blake 1997). This mode of swimming is important for evading predators (Walker *et al.* 2005) and is characterized as an "unsteady" swimming state. Therefore, if exaggerated morphologies hinder a fish's

ability to escape, such as by increasing the drag, then it may have direct effects on its survival. More recently, studies have measured a fish's sprinting abilities down a "dragstrip," similar to studies of sprinting in terrestrial vertebrates (Nelson *et al.* 2002). This mode of swimming would come after the escape response as the fish continues to evade the predator after the initial attack (or when pursuing prey). Similarly, exaggerated morphologies may hinder this performance, leading to a decrease in survival or foraging success. A third type of performance often measured as an indicator of a fish's health and aerobic capacity is critical swimming speed (U_{crit}) (Kolok 1999, Reidy *et al.* 2000, Plaut 2001). This performance can be classified as "steady" swimming, is similar to endurance measures in terrestrial vertebrates, and is characterized by more aerobic respiration (Reidy *et al.* 2000). Exaggerated morphologies may reduce swimming efficiency and increase cost, and thus decrease U_{crit}. In addition to $U_{crit,}$ the endurance of a fish can also be examined. This mode of swimming is similar to endurance trials in terrestrial vertebrates (e.g. see Garland 1984, 1993, 1994a,b; Djawdan 1993, Garland & Losos 1994; Cullum 1998, Perry *et al.* 2004) and can be defined as the time a fish can swim continually against a prescribed velocity (Beamish 1978, Plaut 2001). Similar to its effect on U_{crit}, sexually selected traits have the potential to reduce swimming efficiency or increase the cost, and thus reduce endurance at a given swimming speed.

In addition to the various modes of swimming in fish, maximum oxygen consumption (VO_{2max}) can be measured during steady-state swimming of gradually increasing intensity to directly determine whole-animal aerobic capacity (Gordon *et al.* 1989, Plaut & Gordon 1994, Chappell & Odell 2004). All else being equal, a higher VO_{2max} will correlate directly with a higher U_{crit}. Therefore, if an exaggerated morphology caused by sexual selection had an adverse effect on swimming efficiency, and hence

lowered U_{crit}, then an increase in VO_{2max} would be one way that a fish might compensate, i.e. increase U_{crit} up to "normal" levels.

Some aspects of locomotor performance may trade-off due to biomechanical, biochemical or physiological relations (Vanhooydonck & Van Damme 2001, Vanhooydonck *et al.* 2001, Van Damme *et al.* 2002, Blake 2004). For example, many studies and reviews of locomotor performance have recognized that an individual usually cannot perform well under both aerobic and anaerobic modes (Garland & Losos 1994, Reidy *et al.* 2000, Ojanguren & Brana 2003, but see Garland 1988, Chappell & Odell 2004). This is due to the amount of different muscle fibre types within the individual. If an individual is a good sprinter, then it will typically have relatively more white muscle at the cost of red muscle, making it relatively poor at endurance (Mosse & Hudson 1977, Jayne & Lauder 1994, Gibb & Dickson 2002, Syme *et al.* 2005). Therefore, it is best to examine an array of performance measures to obtain a comprehensive picture of the effect that a sexually selected trait may have on performance (cf. Fig. 1.1).

It is also important to acknowledge that different aspects of locomotor performance may be favoured in different ecological contexts. For example, many members of the Poeciliidae inhabit areas both with and without predators (Reznick *et al.* 1996, Basolo & Wagner 2004, Langerhans *et al.* 2004, Langerhans *et al.* 2005), and recent evidence suggests that the fish from high-predator environments may be evading predators through an increase in C-start (see Langerhans *et al.* 2005 and Walker *et al.* 2005; but see Chappell & Odell 2004). If only one performance were measured, such as critical swimming speed (e.g. Ryan 1988, Nicoletto 1991), then a significant effect might not be found because this might not be the aspect of performance that is most relevant with respect to predation. Instead, selection might be acting on another swimming mode,

such as C-start. On the other hand, recent studies have shown that interpopulation variation in U_{crit} correlates with stream velocity or habitat type (stream vs. lake) (McGuigan *et al.* 2003, Nelson *et al.* 2003), although C-start was not measured in these studies. Thus, it is theoretically possible that a sexually selected trait might impose a cost on some aspect of performance that was not important from the standpoint of natural selection in a given set of ecological conditions. In the wild, it is also possible that behavioural mechanisms can compensate for performance deficiencies (Garland *et al.* 1990a, Garland 1994a; Garland & Carter 1994, Garland & Losos 1994, Jennions *et al.* 2001), thus shielding performance abilities from natural selection, but this "behaviour as a filter" hypothesis is beyond the scope of the present paper (see also Clobert *et al.* 2000, Robson & Miles 2000, Husak *et al.* 2006).

Below, we develop a hypothetical multi-species data set with which to illustrate some ways to analyze the costs of a sexually selected trait. We use the fish genus *Xiphophorus* as a model, and we simulate the effects that the sexually selected “sword” has on endurance. We also include ventricle (heart) mass as a potential compensatory trait. Finally, we analyze the data two ways, 1) by comparing males across species and 2) by comparing the difference between males and females across species. For our example, the two analyses lead to similar results and demonstrate that when an important compensatory trait is left out of the model the sexually selected trait may seem to have a positive effect on performance.

1.4 Study system that motivated our hypothetical example

Within the genus *Xiphophorus* the predominant sexually selected trait is an elongation of the lower margin of the caudal fin (the “sword”), which is exhibited only by males. Recent phylogenies indicate that the sword has evolved more than once in *Xiphophorus* (the sister lineage, *Priapella*, lacks swords) (Marcus & McCune 1999, Morris *et al.* 2001). Apparently, the sword has evolved in relation to a pre-existing female preference (Basolo 1990, 1995), but is also used during male-male interactions (see Bisazza 1993). Evidence indicates that females prefer males with swords, even within species that lack swords. Within the genus, there is a mixture of mating tactics including forced copulation (gonopodial thrusting) and courtship displays. Species exhibiting the sword will display in front of the female with unpaired fins spread and show a stereotyped swimming display that varies among species (Bisazza 1993). Therefore, it seems clear that the sword is a sexually selected trait and that it may impose a cost.

Preliminary work on the costs of swords has yielded mixed results. Ryan (1988) examined swimming endurance of male *X. nigrensis* and found no effect of natural variation in sword length in wild-caught and F1 lab-reared individuals. Moreover, he did not include measures of other traits, such as heart mass, gill area or tissue aerobic capacity that might vary and override (at least partially) any effect of sword length. Basolo and Wagner (2004) found that *X. helleri* from habitats with predators had significantly different body morphology to conspecifics from predator-free environments, including shorter swords, which suggests that swords may adversely affect escape abilities. However, recent evidence on *X. helleri* suggests that sword length may have a positive effect on C-start performance (Royle *et al*. 2006). Finally, in *X. montezumae*, Basolo and Alcaraz (2003) demonstrated a cost in routine oxygen consumption of males

with swords as compared with both males that had their swords surgically removed and females.

1.5 Creation and analysis of comparative data set

As an example of the left half of Figure 1.1, we model the effect of an elongation of the lower margin of the caudal fin ("sword") of *Xiphophorus* individuals on endurance. We performed path analyses using Amos version 5 (http://amosdevelopment.com/). We include body size in the analyses because of its expected positive effect on endurance capabilities, because heart (ventricle) size should covary strongly with body size, and because some sexually selected traits may also covary with body size. Ventricle mass (e.g. see Odell *et al.* 2003) is included as a compensatory mechanism to counteract the negative effects of the sword on endurance. Although we have argued above that it is best to examine an array of locomotor capabilities, as well as multiple compensatory mechanisms, this simplified model serves to illustrate the potential effects of a sexually selected trait on performance, as well as compensatory mechanisms that might have coadapted to overcome the cost.

As a frame of reference, we began with the data on male and female standard length for 61 species of North American minnows from Pyron (1996). We then excluded species with a length < 20 mm or > 190 mm to make the overall size range more similar to that found within *Xiphophorus*, leaving 57 species. As a group, Pyron's (1996) minnows are not sexually dimorphic in standard length, so we multiplied female standard lengths by 1.2 to mimic the situation in *Xiphophorus* (most species of poeciliids are sexually dimorphic, with males being the smaller sex [Bisazza 1993]). To create swords, we computed a random variable with an exponential distribution (SPSS shape

parameter = 3). From this, we set values smaller than 0.1 to zero (because some *Xiphophorus* have no swords) and values larger than 1.15 to 1.15 (because some swordtails have swords that are slightly longer than standard length). We then computed sword length as the resulting value multiplied by male standard length. A plot of sword vs. body length is shown in Figure 1.2. We then $\log_{10}$-transformed both female and male standard length, and sword length was transformed as $\log_{10}$(sword + 1) because females lack swords.

To model a trait that might compensate for the negative effects of sword length on swimming endurance, we computed $\log_{10}$ ventricle mass (e.g. see Odell et al. 2003) as -4 + (3 * $\log_{10}$ standard length) + (0.8 * $\log_{10}$ sword length). We then added random noise (separately by sex) to this value by drawing values from a normal distribution with mean zero and standard deviation of 0.2. Figure 1.3 shows a plot of the ventricle mass data.

Log_{10} swimming endurance was computed as:

-0.5 + (1 * $\log_{10}$ standard length) + (0.5 * $\log_{10}$ ventricle mass) + Noise

where Noise was a random normal variable with standard deviation of 0.15 (separately by sex). Finally, for males, we modeled a detrimental effect of sword length by adding the term -0.3 * ($\log_{10}$ (sword length + 1)). The relation between endurance and standard length is shown in Figure 1.4.

Figure 1.5 shows the hypothetical data for $\log_{10}$ swimming endurance (minutes) of males and females. The values are somewhat positively correlated (Pearson's r = 0.258, 2-tailed P = 0.053), but the main pattern in the data is that females generally have substantially greater endurance (paired t = 9.56, P = $2 * 10^{-13}$), with the mean $\log_{10}$ endurances being 1.2916 (females) vs. 0.9420 (males), or 19.6 vs. 8.7 minutes on the

arithmetic scale. Given that many species of males have swords, one might be tempted to infer that the lower average endurance of males is caused by the handicapping effect of those swords. However, males are also smaller in log standard length, and that alone might cause the difference in endurance. Although the overall comparison of males with females is ambiguous, analyses of differences between the sexes can be used (see below).

If we focus only on males, as only they possess swords in this example, then the following path analyses for $\log_{10}$ endurance demonstrate the problem of not considering a compensatory trait, i.e. ventricle size. If only body size and sword size are included in the model, then the path analysis (Fig. 1.6, top right) indicates that swords actually have a statistically significant *positive* effect on endurance. However, when ventricle size is included, the negative effect of sword length becomes clear (Fig. 1.6, top left). As judged by the magnitude of the standardized partial regression coefficients (Beta = a path coefficient), ventricle mass is a stronger predictor of log endurance than is log sword length. Note, however, that ventricle mass and sword length are strongly correlated (r = 0.811: Fig. 1.6, top), which could cause problems of multicollinearity (e.g. see Slinker and Glantz 1985). Another possible indication of multicollinearity in the present data set is the fact that the full path models (left side of Fig. 1.6) indicate a negative effect of standard length on endurance, whereas the effect built into the simulations was actually positive. (This may also be partly an effect of the complicated relationship between sword length and standard length, as shown in Fig. 1.2.) Several statistical approaches can be used to deal with such strong intercorrelations between independent variables (e.g. see Garland 1984, Slinker & Glantz 1985). If possible, however, the best strategy is to sample additional species whose multivariate phenotype

tends to reduce the strength of intercorrelations.

As discussed above, another way to analyze this type of data is by computing differences between the sexes for each species. The rationale for this is that females may be nearer to the optimum for any particular trait, as dictated by natural selection. Thus, male characteristics, such as performance or a trait that might handicap performance, can be compared with females, which would often lack the putative handicap, or at least express it at a reduced magnitude. As in Promislow *et al.* (1992), we computed differences in log-transformed values, i.e. log male - log female endurance, log male - log female standard length, and log male - log female ventricle mass. For sword length, we can simply use log (male value + 1) becaue all females lacked swords in this hypothetical data set. A path model (Fig. 1.6, bottom left) indicates that the *difference* in endurance between males and females is significantly positively related to the difference in ventricle size, and negatively related to the difference in sword size. And, as above, if we do not include the difference in ventricle size, then we get the misleading result that the difference in sword length is a *positive* predictor of the difference in endurance (Fig. 1.6, bottom right).

1.6 Discussion

Our simulated data set demonstrates the interplay among various traits that have the potential to influence locomotor abilities, as well as demonstrating the use of different types of analyses to examine costs. It also illustrates that compensatory traits can mask the negative effects (costs) of a sexually selected trait. This can occur when one analyzes only the sex that possesses the putatively costly trait (e.g. swords in males of the fish genus *Xiphophorus*), as well as when considering the difference in traits

between the sexes (e.g. log male value minus log female value). Indeed, our hypothetical data set illustrates that, in either type of analysis, the putatively costly trait can even appear to have a positive effect on organismal performance if a compensatory trait is not included in the analysis (Fig. 1.6).

Here we have presented a simple case considering a single performance measure, a single sexually selected trait that handicaps that performance, a single compensatory trait (ventricle mass), and body size. However, multiple performance measures, handicaps and compensatory traits can be included in path analyses (e.g. see Fig. 10.5 in Garland & Losos 1994, Bauwens *et al.* 1995, Petraitis *et al.* 1996, Miles *et al.* 2000, Armbruster *et al.* 2002, Caumul & Polly 2005, Angilletta *et al.* 2006). If this were a real data set, then phylogenetically based statistical methods would need to be used (e.g. Abouheif & Fairbairn 1997, Prum 1997, Ryan & Rand 1999, Armbruster *et al.* 2002, Aparicio *et al.* 2003, Blomberg *et al.* 2003, Cox *et al.* 2003, Billman & Pyron 2005, Caumul & Polly 2005, Garland *et al.* 2005, Ives *et al.* 2007).

1.6.1 EXISTING STUDIES OF COMPENSATORY TRAITS

Previous studies examining the cost of a secondary sexual characteristic have produced mixed results. Although some have documented a clear cost to components of Darwinian fitness (e.g. Ryan *et al.* 1982, Garcia *et al.* 1994), others have found no effect of the trait (e.g. Ryan 1988, Chappell *et al.* 1995) or even a positive effect of the trait on performance (Royle *et al.* 2006) or on components of Darwinian fitness (Petrie 1994). Although these studies examine an array of potential costs, the discrepancies may arise because compensatory mechanisms were not taken into account.

The idea of compensatory mechanisms decreasing the costs of sexually selected

traits is not a novel one. Møller (1996) suggested several types of “cost-reducing” traits in relation to the production and maintenance of a sexually selected character, including mechanisms that are “produced in advance of or simultaneously with the sex trait which reduce the cost of the character.” Balmford et al's. (1994) comparative study of birds suggests that greater wingspans have evolved to reduce the cost of tails elongated by sexual selection. Similarly, Pettersson and Hedenström (2000) demonstrated theoretically that, in fish, adjustments in standard metabolic rate (SMR) can compensate for hydrodynamical disadvantages (e.g. high drag from elongated tails) under certain food conditions.

Some evidence suggests that sexual selection may favour males with higher locomotor abilities and/or aerobic capacities, which would affect endurance. This has been suggested for lizards in general (see Garland 1993, Cullum 1998), for *Varanus gilleni* (Bickler and Anderson 1986) and for helodermatid lizards, in which males have higher body size-adjusted maximal oxygen consumption (Beck *et al.* 1995). It was also suggested for junglefowl (Chappell *et al*. 1996), but empirical studies have yielded ambiguous results (Chappell *et al.* 1997, 1999). Because body size often correlates positively with locomotor endurance (e.g. Garland 1994b), increases in body size might result from sexual selection, if such selection favours high endurance. Thus, larger males in some species may indicate that sexual selection has favoured high endurance. Of course, so many traits correlate with body size that it is tenuous to single out endurance in the absence of direct evidence that sexual selection is acting on endurance (see also Anderson & Vitt 1990). Stronger evidence would be provided by evidence that males show higher endurance than females after adjusting statistically for correlations with body size. Existing empirical studies are mixed regarding this possibility

in lizards (Garland & Else 1987, Cullum 1998, Dohm et *al.* 1998). Three correlational studies of lizards have examined individual variation in laboratory-measured locomotor performance in relation to social dominance in experimental arenas, and all found evidence to support a relationship. In *Sceloporus occidentalis*, dominance was positively related to speed but not stamina (Garland *et al.* 1990b). In *Urosaurus ornatus*, dominance was positively related to both speed and stamina (Robson & Miles 2000). In *Anolis cristatellus*, dominance was positively related to endurance and to the rate of assertion displays in the field, but not to speed (Perry *et al.* 2004). Evidence suggesting that endurance may be subject to sexual selection in lizards also comes from field studies of *Uta stansburiana* (see Miles *et al.* 2000, Sinervo *et al*. 2000, and references therein). Most recently, Husak *et al.* (2006) found that maximal sprint speed of territorial, adult male collared lizards (measured in the laboratory) was a positive predictor of the number of offspring sired in the field.

1.6.2 ALTERNATIVE WAYS TO STUDY COSTS OF SEXUALLY SELECTED TRAITS

The assumed costs of sexually selected traits have been examined in various ways. Kotiaho (2001, p. 366) stated "to count as an evolutionarily significant cost, an increase in the magnitude of sexual traits has to either increase mortality or decrease reproductive success." We agree with that point, but would add, consistent with Arnold (1983; and see Fig. 1.1), that it can also be interesting and informative to study costs of sexually selected traits on organismal performance abilities (performance gradients), whether or not the performance ability has an important effect on Darwinian fitness.

In any case, Kotiaho's (2001) definition does not specify any particular "baseline" for comparison. One possibility is to compare individuals of the same sex and

population. Individuals with higher values for the putatively costly trait (independent variable) should show lower values for performance traits and/or for components of Darwinian fitness. For example, some studies have demonstrated that males within a species with higher values for the putatively sexually selected trait have a decrease in overnight body mass (Thomas 2002), locomotor abilities (Langerhans *et al.* 2005, Karino *et al.* 2006) and survival (Ryan *et al.* 1982, Garcia *et al.* 1994, but see Petrie 1994) (and see Kotiaho 2001 for a review of such correlational studies). Additionally, some studies of individual variation have involved experimental manipulations, such as lengthening or shortening of tails. These studies have further demonstrated costs within the same sex and species (foraging efficiency: Møller 1989, Evans & Thomas 1992; oxygen consumption: Basolo & Alcaraz 2003; number of matings: Andersson 1986; survival: Møller & de Lope 1994, Kotiaho 2000).

Among populations (or among species), a similar approach can be taken. For example, mean values can be computed for the sexually selected trait and either locomotor performance or actual fitness components, such as average survivorship or litter size. For instance, Promislow and colleagues examined the survival cost of sexual size dimorphism among species of mammals (Promislow 1992) and birds (Promislow *et al.* 1992). They found that the degree of male-biased adult mortality was positively correlated with the degree of sexual size dimorphism. Additionally, this approach can be used to investigate compensatory mechanisms among species. For example, Balmford *et al.* (1994) found that, among 57 species of birds, those that exhibited longer tail feathers, potentially through the result of sexual selection, also exhibited greater wingspans, which they suggested had evolved to reduce the “cost” of the secondary sexual character. As noted above, such comparative studies require phylogenetically

based statistical methods.

Alternatively, the effects of an assumed costly trait in males could be compared with its effects in females of the same species, with the general perspective that values of the trait in females are nearer to the optimum set by prevailing natural selection (e.g. see Lande 1980). Most morphometric traits have strong positive genetic correlations between the sexes (Lande 1980, Falconer & Mackay 1996, Møller 1996, Badyaev 2002). Therefore, the absence of a putatively sexually selected trait in females suggests that natural selection is acting against it in that sex. Some sexually selected traits are sex-limited, females lacking them entirely, as in some bird songs or the swords of *Xiphophorus* species. In that case, one obviously cannot study the effects of the trait in females within a given population. However, one can study several populations and/or species that vary in the degree of the trait in the male, compute mean values for each population or species, and then determine whether the difference in the sexually selected trait (mean male value - mean female value) is associated with the difference in locomotor performance or survival. Note that sexual dimorphism in body size is most commonly studied as a ratio of male/female size (e.g. Balmford *et al.* 1994, recent discussion in Cox *et al.* 2003), but that cannot be done for a trait that is absent in females. In our hypothetical example, analysis of differences between male and female traits yielded similar results. In both cases, the sword has a positive effect when ventricle mass is not included in the model, but the negative effect of the sword is observed when ventricle mass is included (Fig. 1.6).

The above-mentioned approaches are complicated by several biological and statistical issues. Morphology, performance and components of Darwinian fitness are often correlated with body size, and body size is often sexually dimorphic. Therefore,

body size will generally need to be considered in any study of a sexually selected trait. The causes of body size dimorphism are numerous (see reviews in Fairbairn 1997, Cox *et al.* 2003), and body size itself is often thought to be the subject of fairly intense sexual selection. One classic example is pinnipeds, in which interspecific variation in male/female size is strongly positively correlated with harem size (Alexander *et al.* 1979; other examples of comparative analyses of size dimorphism in vertebrates include Dewsbury *et al.* 1980 [a rare common-garden study], Oakes 1992, Emerson 1994, Pyron 1996, Cox *et al.* 2003). Given that body size affects almost all organismal properties, including visibility and fighting ability (Briffa & Sneddon 2007), it is often difficult to know whether selection has acted on size per se or on some correlated trait. Moreover, one "easy" way for selection to change a trait, such as fecundity or locomotor endurance (Garland 1994b), may be just to change body size.

However, the study of sexual dimorphism in body size itself as an indicator of sexual selection is problematic because the appropriate null model is not necessarily that the sexes should be equal in size. Male and female size are likely to be strongly positively genetically correlated within populations (see review in Badyaev 2002), which should tend to inhibit sexual divergence in body size (Lande 1980), but both empirical and theoretical studies suggest that this will not always be a strong constraint on the evolution of sexual dimorphism (Reeve & Fairbairn 1996, 2001). In addition, assuming that the positive genetic correlation between male and female size is not too strong a constraint, sex differences in size would be expected for three obvious reasons. First, given their very different roles in reproduction and associated behavioural ecology one might generally expect different sex-specific optima from the standpoint of natural selection alone. Second, differences in reproductive activities are likely to lead to

differences in energy expenditure, which would in turn affect growth rate and thus adult body size (e.g. see Anderson & Vitt 1990). Third, differences in the hormonal milieu may lead to sex differences in growth and differentiation of various organs, behaviour (including general activity levels), energy expenditure and partitioning, and hence body size at most ages (e.g. Cox *et al.* 2005). Thus, any difference in body size cannot be taken as clear evidence of past sexual selection. This line of reasoning is similar to arguments that have been used to point out limitations of comparative studies that involve only two species [Garland & Adolph 1994]. As reviewed by Badyaev (2002), an important area for future research is detailed studies of sex-specific regulatory mechanisms that allow sex-specific expression of genes that are shared between the sexes. Most genes that underlie sexual size dimorphism are not on the sex chromosomes, so sex-specific regulatory mechanisms must account for most size dimorphism.

The study of secondary sexual characters (genetically determined sex characteristics that are not directly connected with the act of reproduction and/or characteristics that have been shaped by sexual selection) as indicators of sexual selection suffers less from such problems, for several reasons. First, differences in energy expenditure would not lead as directly to sex differences in secondary sexual characters as compared with growth rate and hence body size. Second, the significance of many secondary sex characteristics (e.g. human male facial hair) with respect to natural selection seems far lower than for body size, which affects or at least is correlated with innumerable other traits. Therefore, by default, sex differences in secondary sexual characters are more likely to be the result of sexual selection. Third, the evolution of sex differences in secondary characters should be relatively easy

because most are controlled primarily by the gonadal steroids, via tissue-specific differences in responsiveness.

Measures of organismal performance, such as locomotor endurance, may be affected by both body size and secondary sexual characters (Fig. 1.1), as well as the hormonal milieu (e.g. Sinervo *et al.* 2000). All else being equal, body size is likely to have a positive effect on endurance-type activities (e.g. Garland 1984, 1994b), but a negative effect on acceleration and manoeuverability. Indeed, it has been suggested that the "reversed" sexual size dimorphism (males smaller than females) in some groups of birds likely evolved in response to female choice for acrobatic display elements (Prum 1997). Similarly, habitat height is negatively correlated with male/female size in spiders, consistent with the hypothesis that smaller males are favoured in species in which males must climb to reach females located in high habitats (Moya-Laraño *et al*. 2002). As discussed above, many secondary sexual characters (e.g. elongated tail feathers of birds) would be expected to have a negative effect on most measures of locomotor performance. However, such effects may not be apparent if organisms have evolved compensatory mechanisms to overcome the handicap (e.g. Balmford *et al.* 1994, Møller, 1996). Thus, locomotor performance poses an interesting topic of study for those interested in sexual selection and its consequences. If a putatively sexually selected trait covaries with body size, then this correlation needs to be accommodated (Fig. 1.6). If the trait under consideration scales isometrically with body size, then a simple ratio of trait/size can be used (e.g., Balmford *et al.* 1994).

1.6.3 CONCLUDING REMARKS

We encourage future studies to take an integrative, multi-level, and functionally informed approach to the study of sexual selection, physiology and performance. We also encourage those who work on sexual selection to take a more pluralistic view and acknowledge in their research programs that all traits may also be subject to natural selection (e.g. see King 1989 on snake tail lengths), even some of the classic examples of sexually selected traits, such as bird tail feathers (e.g. see Buchanan & Evans 2000; Aparicio *et al.* 2003). At the same time, we would caution adaptationists that even our most cherished examples may have been shaped by sexual selection to a greater extent than we have imagined. For example, Simmons and Scheepers (1996) have argued that the elongated necks of giraffe evolved primarily in response to sexual selection, rather than natural selection related to feeding at high levels, as is traditionally presented in text books. Much work remains to be done in sorting out the relative importance of natural selection, sexual selection and genetic drift; phenotypic plasticity; how pleiotropic gene action may constrain or facilitate the rate and direction of multivariate, hierarchical evolution; and how fundamental biochemical and biophysical properties may constrain the possible "morphospace" into which organisms can evolve. Equilibria set by the balance between natural and sexual selection -- within the confines determined by genetic architecture, developmental and physiological mechanisms -- will be dynamic as environmental and demographic conditions change, thus causing temporal changes in the selective regime, and evolution of the genetic architecture itself.

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Figure 1.1. Path model discussed in the text. Arrows indicate direction of putative causality (for simplicity, terms to represent unexplained variation are omitted). Solid lines represent causal relationships that are almost certain to occur, whereas dashed lines represent those that are less certain to occur and/or may vary in sign. Correlations between traits can be positive or negative and are represented by double-headed arrows (some possible correlations are omitted for simplicity). ***Compensatory Trait*** refers to such subordinate traits as heart size, gill area or muscle fiber type that may have evolved to counter the detrimental effects of a ***Sexually Selected Trait*** on organismal ***Performance*** (e.g., locomotor speed or stamina). Plus signs indicate positive effect, minus signs indicate negative effect; parentheses indicate that the sign of the relationship may differ from that shown. Sexually selected traits are assumed to have a negative effect on performance (see text), whereas compensatory traits have a positive effect by definition. Sexually selected traits evolve as a compromise between natural and sexual selection (and within the confines of genetic, developmental, and physical constraints). Therefore, while they will have a net positive effect on at least one component of Darwinian fitness, such as fecundity (e.g., number of females obtained by a male), they typically have negative effects on other fitness components, such as adult survivorship or age at first reproduction (e.g., because they adversely affect growth rate). Conversely, performance is likely to have positive effects on all fitness components by increasing the ability to acquire mates, forage, and avoid predation. Body size will often, but not always, be positively correlated with both sexually selected traits (e.g., tail length) and compensatory traits (e.g., heart size). Body size will often have a positive effect on some aspects of locomotor performance (e.g., stamina) but a negative effect on others (e.g., maneuverability). Here shown as a single box, performance actually comprises

many elements, some of which may trade-off because of biomechanical or physiological relations (e.g., speed vs. stamina: see text). Moreover, different aspects of performance may be selectively important in different ecological settings. This conceptualization assumes that animals can be motivated to exhibit a maximal level of performance that can, at least in principle, be measured with some degree of accuracy. Not shown in the path model is the role of behavior, which may often shield performance abilities from selection and/or allow compensation for low abilities (see Garland et al. 1990a, Garland 1994a, Garland and Carter 1994, Garland and Losos 1994). Following Arnold (1983), paths to performance can be termed performance gradients, whereas those to fitness can be termed fitness gradients.

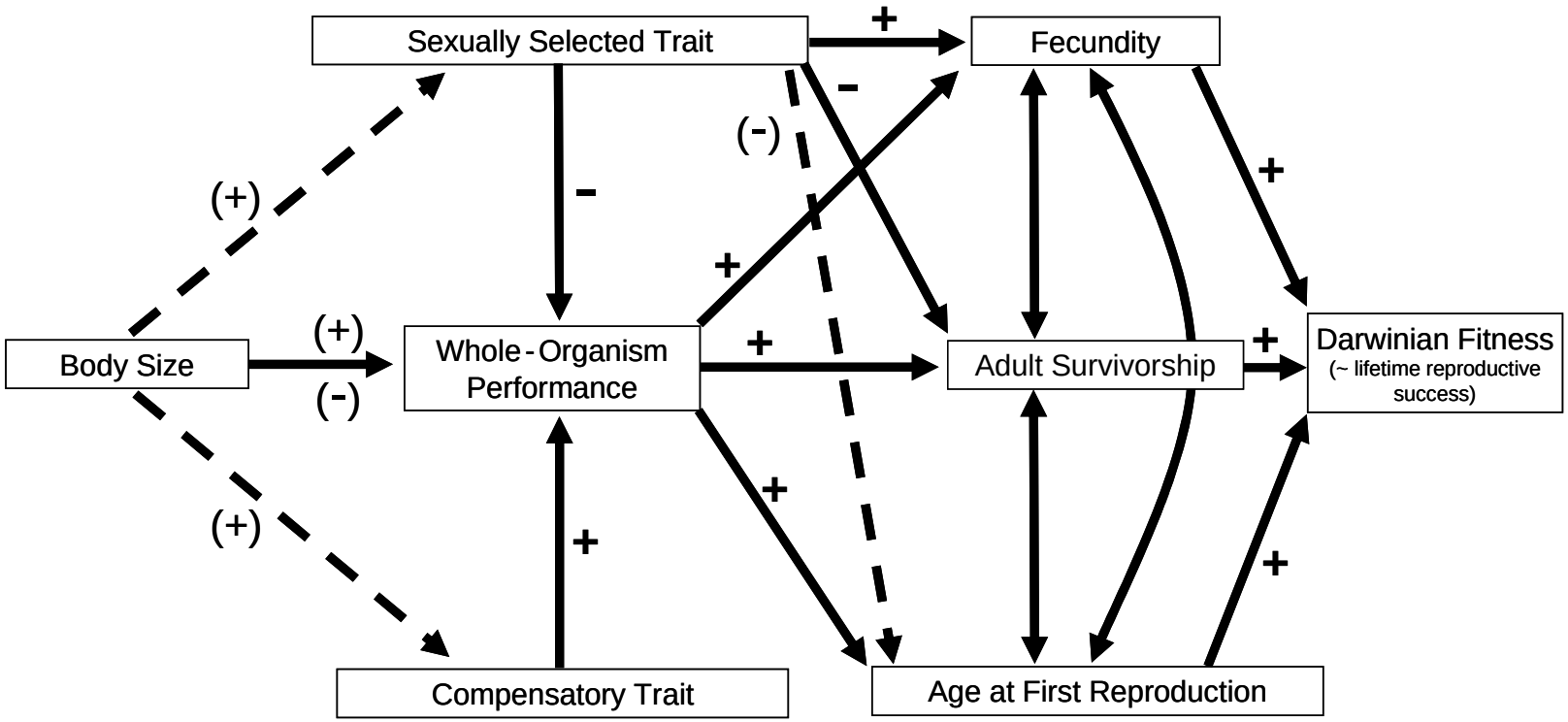

Sexually Selected Trait
Fecundity
Body Size
Whole-Organism Performance
Adult Survivorship
Darwinian Fitness
(~ lifetime reproductive success)
Compensatory Trait
Age at First Reproduction
(+)
(-)
+
-

Figure 1.1

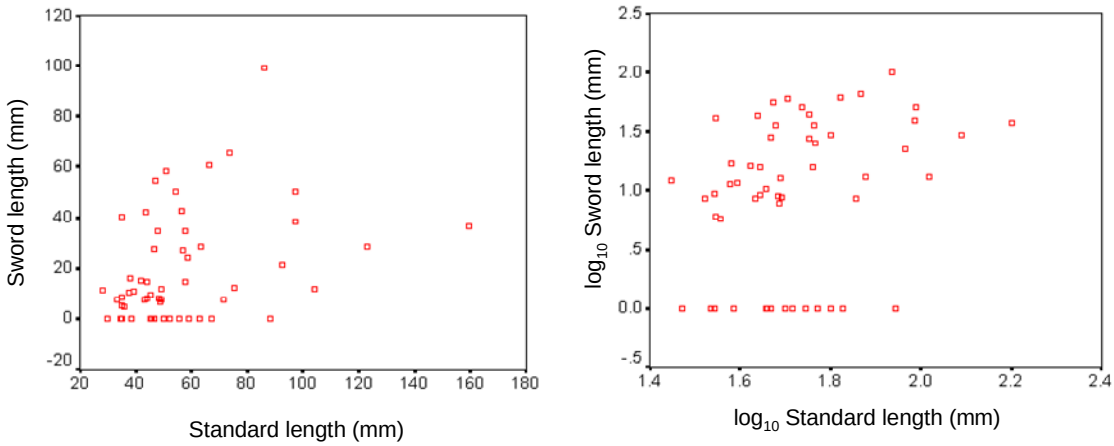


Figure 1.2. Hypothetical data for sword length (mm) in relation to standard length (mm) for males of 57 species of fish.

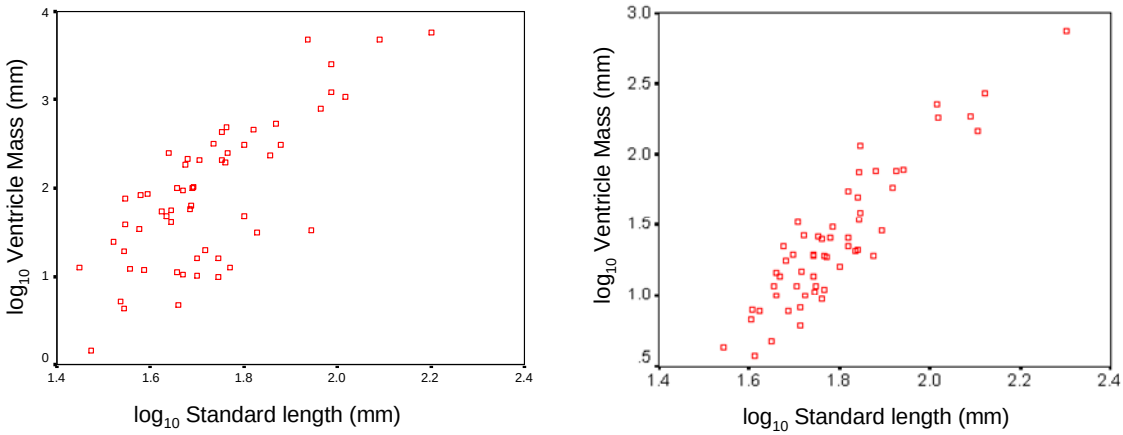


Figure 1.3. Hypothetical data for $\log_{10}$ ventricle mass (mg) in relation to $\log_{10}$ standard length (mm) for 57 species of fish. Note that males (left) show greater variation in ventricle mass because it was computed partly as a function of sword length, which only (some) males possess

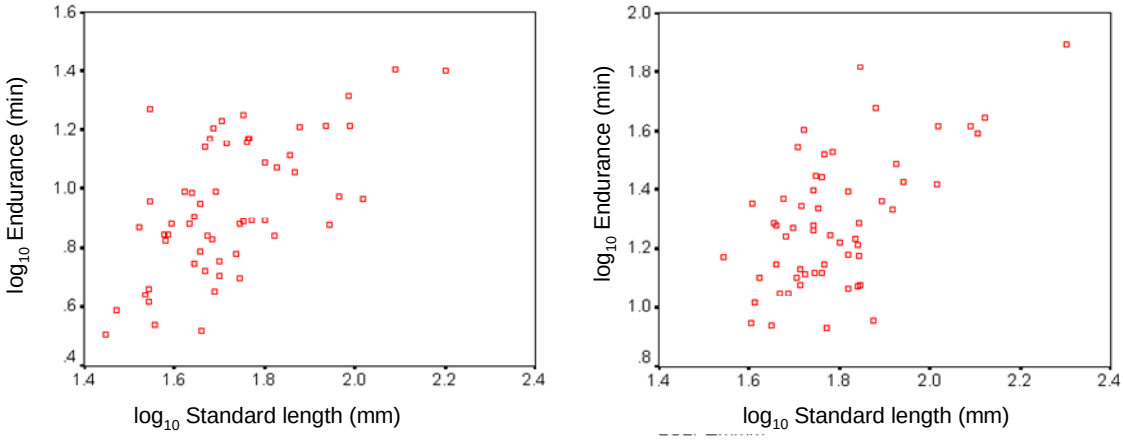


Figure 1.4. Hypothetical data for $\log_{10}$ swimming endurance (minutes) in relation to $\log_{10}$ standard length (mm) for 57 species of fish (males on left, females on right). Note different Y axis ranges; on average, males have lower endurance than females for a given body size (see also Fig. 1.5).

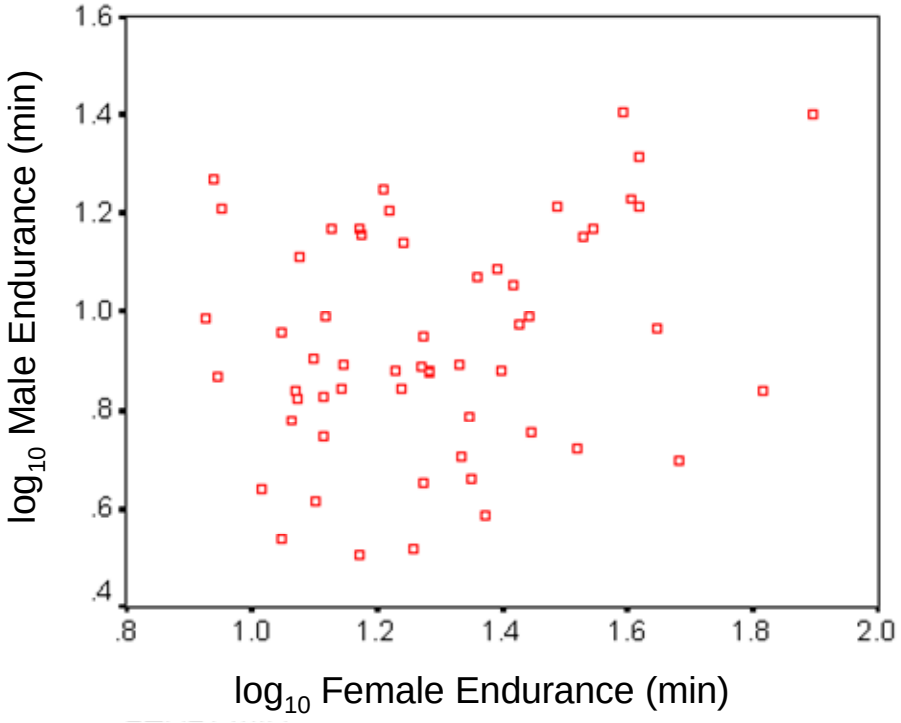
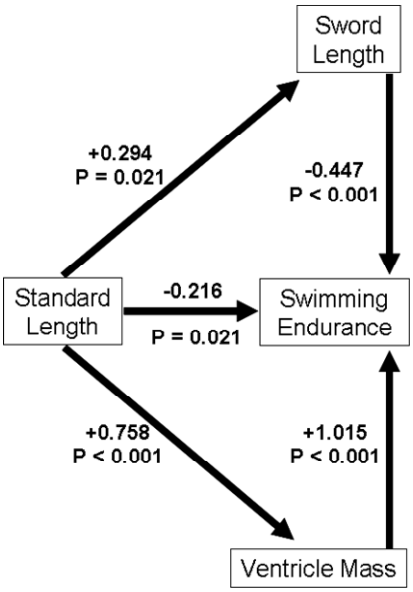
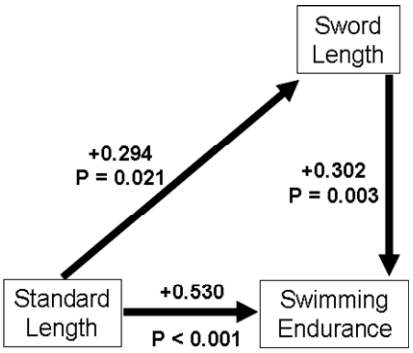


Figure 1.5. Hypothetical data for $\log_{10}$ swimming endurance (minutes) of males and females for 57 species of fish. Dashed line indicates equal endurance; thus, on average, females have greater endurance, which reflects their larger body size and absence of swords, which negatively affect endurance in males that possess them (see text).

Figure 1.6. Path models examining the relationships of body size, sword length (a sexually selected trait), ventricle mass (a compensatory trait), and swimming endurance in males only (top panel) and using the male - female difference for each trait (bottom panel). Data analyzed are those shown in Figures 2-5. Also included are path models with ventricle mass removed. Tables of pairwise Pearson correlations are given for each analysis (* indicates 2-tailed $P < 0.05$, ** $P < 0.01$). Both analyses show that when the compensatory trait is excluded, sword length appears (erroneously) to have a statistically significant positive effect on swimming endurance. This result is also obtained when examining the simple correlation tables. However, when the full models that include ventricle mass are analyzed sword length appears (correctly, given how the data set was created -- see text) to have a negative effect on swimming endurance.

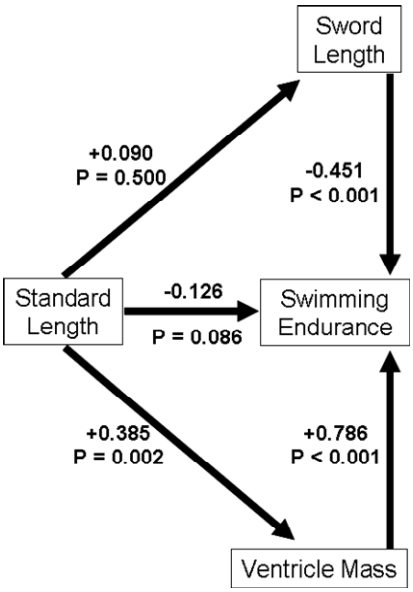
Male

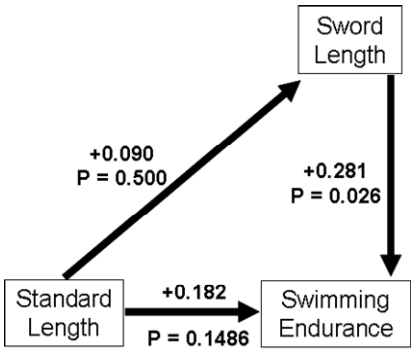




	Ventr.	Sword	Endur.
Length	0.758**	0.294*	0.619**
Ventr.		0.811**	0.716**
Sword			0.458**

Male - Female





	Ventr.	Sword	Endur.
Length	0.385*	0.090	0.207
Ventr.		0.837**	0.547**
Sword			0.296*

Figure 1.6

Chapter 2

Repeatability and correlation of swimming performances and size over varying time scales in the guppy (*Poecilia reticulata*)

2.1 Summary

1. Although the repeatability of a trait is of interest for several reasons, few studies have critically examined the repeatability or correlation of metrics of swimming performance. We quantified repeatability of three swimming performances (burst speed during a c-start escape response, critical swimming speed [U_{crit}], maximum speed [U_{max}]) and size/shape measures over short (within-day), medium (days and weeks), and long (more than a year) time scales in a small poeciliid fish, the Trinidadian guppy (*Poecilia reticulata*).

2. We found that individual differences in most swimming performance metrics were repeatable on various time scales. Burst speed during c-start was repeatable within a day, as well as over days and weeks, but it was not repeatable over the span of a year. However, the medium term repeatability was only statistically significant when accounting for the variation of individuals within a day and correcting for attenuation. U_{crit},U_{max} and size were repeatable on medium time scales, but only size and U_{max} were repeatable over the span of a year. Our index of shape was not statistically repeatable over any time scales.

3. When examining the correlations of performances and size, only a few traits were related to each other. U_{crit} and U_{max} were significantly positively related to each other for trial 1 only, but not any of the others. U_{max} and the mean c-start burst speed were correlated on trial 2 only. No other traits were significantly related to each other, and no performance traits were related to size or shape. U_{crit} and U_{max} also did not differ in their overall mean values, suggesting that U_{max}, a more recently described assessment of fish swimming performance, may be a reliable alternative.

4. All traits, except our index of shape, shifted in their mean value over the span of a year. U_{crit} and c-start burst speed decreased significantly, consistent with previous findings on the effects of senescence on neuromuscular systems. However, U_{max} and size increased significantly over a year.

5. The measures of swimming performance used in this study are becoming widely used in ecological and evolutionary studies. Our results suggest that individual differences in these measures are relatively stable on various time scales. We found no significant relation of these performance metrics with body size or a simple index of shape, and few relations among the performance measures, suggesting that they measure different aspects of locomotor physiology and/or motivation under the test conditions used. Finally, while U_{crit} and c-start burst speed decreased over the span of the year, consistent with theories of senescence, U_{max} significantly increased over a year, suggesting that further studies of this recently described performance metric are needed.

2.2 Introduction

The locomotor performance of organisms has many important ecological and evolutionary implications, with relevance for finding food, finding mates, avoiding predators, and dispersal (e.g., Irschick & Garland 2001, Perry *et al.* 2004, Oufiero & Garland 2007, Irschick *et al.* 2008, refs. therein). In fish, swimming performance has been categorized into three main modes; sustained, prolonged, and burst (Beamish 1978). Within these three modes of swimming, prolonged and burst have been studied extensively, including aspects of their physiology, biomechanics, and neurobiology. More recently, studies have been focusing on the ecological and evolutionary implications of variation in swimming performance (Nicoletto 1991, O'Steen, Cullum & Bennett 2002, Ghalambor, Reznick & Walker 2004, Blake *et al.* 2005, Langerhans, Layman & DeWitt 2005, Karino *et al.* 2006, Kruesi & Alcaraz 2007). Although many of these studies examine differences among populations, species or groups (McGuigan *et al.* 2003, Nelson, Gotwalt & Snodgrass 2003, Odell, Chappell & Dickson 2003, Chappell & Odell 2004, Langerhans *et al.* 2004), more are beginning to examine differences at the individual level (Kolok 1999). However, a key to studies at the individual level is assessing the amount of intraindividual variation, or repeatability, for the trait of interest.

The repeatability of locomotor performance has been well documented in terrestrial vertebrates, with much attention in squamate reptiles (e.g., Sorci *et al.* 1995, Perry *et al.* 2004, and reviewed in Kolok 1999). However, the repeatability of swimming performances in teleost fish has received less attention and has only recently begun to be evaluated critically (Nelson & Claireaux 2005, Claireaux *et al.* 2007). The repeatability of a trait is important for several reasons (Bennett 1987, Boake 1989). First, and most simply, it indexes the reliability and reproducibility of the protocol by which a trait is

measured (Losos, Creer & Shulte II 2002). Second, significant repeatability sets the stage for investigations of the proximate (mechanistic) factors that contribute to variation in the trait (Bennett 1987, Bennett, Garland & Else 1989). Third, repeatability generally sets the upper limit of heritability for the trait (e.g., Brodie & Garland 1993, Boake 1989, but see Dohm 2002). Finally, repeatability also sets a general upper limit to the intensity of selection that can be applied to a trait, which is important both in laboratory experiments (Garland & Rose 2009) and in field studies that attempt to quantify selection in nature (e.g., Clobert *et al*. 2000; Irschick *et al*. 2008). In other words, if a trait has low repeatability, then it is "harder" for selection to act on it, i.e., separate the favored from disfavored individuals.

The repeatability of swimming performance in fish has been examined in several species, for several traits, and over varying time scales (Table 2.1). Most of the research has focused on the critical swimming speed (U_{crit}), an endurance measure that is often used as an indicator of health in fisheries science (Plaut 2001). More recent work has examined the repeatability of sprint speed in fish, similar to sprint-speed measures of squamate reptiles (Nelson *et al.* 2002). These studies have generally focused on larger-bodied, commercially important species. However, smaller species (mainly members of the Poeciliidae) are becoming model organisms in evolutionary and ecological research, with more studies examining swimming performances. Yet, the only studies of repeatability for poeciliid fish yet published are short-term and involve the c-start (Chappell & Odell 2004, Langerhans *et al.* 2004, Royle *et al.* 2006), which gauges burst-swimming capabilities (Domenici & Blake 1997).

The repeatability of a performance measure will depend on the consistency of both psychological factors (e.g., motivation) and the physiological and morphological

subordinate traits that govern physical performance abilities (Bennett 1987, Harris & Steudel 2002, Belke & Garland 2007). Obviously, the morphology of an organism can have strong effects on its performance capabilities (Arnold 1983, Garland & Losos 1994, Boily & Magnan 2002, Blake 2004, Billman & Pyron 2005). Additionally, different performance measures may trade-off with one another. For example, it has been proposed -- and shown in some, but not all, cases -- that endurance performance trades-off with sprinting performances, in part because of the different muscle fiber types associated with each performance (Garland & Losos 1994, Vanhooydonck, Van Damme & Aerts 2001). Few studies have examined this in fish; those that have often find no relation at the level of individual variation (e.g., Chappell and Odell 2004, Claireaux *et al.* 2007, but see Reidy *et al.* 2000). Those that have shown a trade-off often demonstrate it at the population or species level (Langerhans 2006); however, as Bennett (1987) notes, "If two factors are functionally related, they should be significantly correlated among individuals at the species levels" (presuming, of course, that the repeatability of the traits in question is high enough).

The purpose of the present study was to quantify the repeatability of several performance measures over varying time scales in a small poeciliid fish, the Trinidadian guppy (*Poecilia reticulata*, Figure 2.1). We examine the repeatability of burst speed during a c-start escape response (as measured as the maximum 2 millisecond (ms) velocity attained during an escape response) on a short (minutes), medium (days and weeks), and long time scale (more than a year). We also examine the repeatability of critical swimming speed (U_{crit}), as has been done in larger fish species, on a medium (weeks) and long (more than a year) time scale. Thirdly, because guppies are generally too small to break photocell infrared beams used in sprint-performance measures, we

use a measure of maximum or "burst" speed in a flow tunnel, as described in Reidy *et al.* (2000) and more recently in Farrell (2008). We classify this measure as maximum speed (U_{max}), and measure its repeatability on a medium (weeks) and long (more than a year) time scale. Finally, we also test for relations between the performance measures and with size traits.

Few studies have measured long-term repeatability of locomotor performance in vertebrates (Huey & Dunham 1987, van Berkum *et al.* 1989, Huey *et al.* 1990, Robson [2000] cited in Angilletta, Niewiarowski & Navas 2002, Miles 2004). The longest measure in fish has been six months for critical swimming speed and sprint speed in European sea bass (Claireaux *et al.* 2007). Long-term repeatability has additional implications, including the effects of senescence on locomotor performance (Huey *et al.* 1990, Reznick *et al.* 2004).

2.3 Methods and Materials

2.3.1 ANIMAL HUSBANDRY

Male Trinidadian guppies originating from the Quare river drainage of Trinidad (*Poeciliia reticulata*, N = 24, Figure 2.1) were used to assess the amount of individual variation associated with several swimming performance metrics in fish. The breeding stock was originally collected in 2002, and our sample was from approximately the 10th generation of captive breeding. For the present study, beginning at approximately 90 days of age, fish were individually maintained in 2 ½ gallon aquaria, with a 12L:12D light cycle. Water was pretreated (e.g., with UV sterilization and chemicals to maintain pH and rid water of chlorines and chloramines), and partial water changes were done approximately every

two weeks. Fish were fed flake food, live brine shrimp, liver paste, and freeze-dried blood worms *ad libitum*. Prior to any of the performance measures, individuals were fasted for at least 24 hours.

2.3.2 PERFORMANCE MEASURES

Burst speed was the first performance metric tested on all fish by eliciting an escape response often characterized by a c-start (Domenici & Blake 1997), and was measured similar to methods outlines in Chappell and Odell (2004). Burst speed was measured by placing individuals in a small arena (30cm (L) X 30cm (W) X 20.5cm (H)) filled with approximately 4-5 cm of water to minimize vertical movement. A plastic cylinder (16.5 cm D X 20.5 cm H) was placed in the center of the tank above a 10 cm grid. This plastic cylinder kept the fish in view of the high-speed video camera, and the grid allowed for distance calibration. Additionally, opaque plastic surrounded the plastic cylinder to reduce any outside visual distraction and stress levels of the fish being tested. A digital thermometer was placed inside the arena and temperature was recorded for each trial.

A mechanical stimulus (metal weights) weighing 51.92g (~ 4cm D X 1.5cm H) was dropped from a height of 85 cm, outside of the fish's view, to elicit a c-start escape response. The stimulus was released after a 15-minute acclimation, and responses were captured using a Red Lake Imaging high-speed video camera (Red Lake Motion Scope M1) at 500 frames per second. To obtain proper lighting, the arena was lit with two 120 W reflector bulbs placed ~85 cm above the testing arena. Because these lights tended to increase the temperature of the water, ice was added periodically outside of the testing arena, and the water was changed every couple of trials to try and maintain

temperature at ~23°C, similar to room and housing temperatures.

Three trials were recorded for each fish within approximately 30 minutes. If a fish did not respond, or responded poorly after the first stimulus, it remained in the tank for 2 minutes until the stimulus was released again; this procedure was repeated one additional time. If no successful trials were obtained from the fish, then the individual was removed from the study. If a fish responded well, it also remained in the arena for 2 minutes for an additional measurement; this procedure was repeated to obtain 3 successful measures of the individual's escape response (no more than 5 trials were conducted if 3 successful trials were not obtained initially).

The escape responses were converted to digital (.avi) files and the center of mass of the fish was digitized from 10 ms prior to initiation of the c-start to 50 ms after the c-start began using custom Matlab software to obtain x,y-coordinates (Mathworks Inc., Natick, MA, USA). The velocity was obtained from a first order derivative of the displacement of the center of mass from the x,y-coordinates. C-starts generally last only a few tenths of a second (Domenici and Blake 1997), so analysis up to 50 ms should capture the initial velocity during the c-start; a similar protocol has been used previously in guppies (Chappell and Odell 2004). The velocity data were also smoothed using a 5 point moving average because there is often noise associated with estimating this parameter when they are calculated from the x,y coordinates (Walker 1998). We then found the maximum velocity over a 2 ms period for each trial and day and used this as our measure of burst speed during a c-start escape response. Additionally, we estimated the time to attain the maximum velocity for each trial to determine whether it was occurring during or after the c-start escape response. Previous studies have estimated the time to the end of stage 1 in guppies to be ~12ms (Walker et al. 2005); therefore, we

compared the time to maximum velocity to the time at the end of stage 1. The end of stage 1 was determined as the first decrease in velocity after the initiation of the c-start, even if it lasted for only one frame (2 ms). This allowed us to determine which stage of the c-start the maximum 2 ms velocity was reached.

The second performance metric measured was maximum speed (U_{max}), similar to Reidy *et al.* (2000) and Farrell (2008). Fish were acclimated to a flow tunnel for 15 minutes at low velocity (~3 cm s^{-1}) to orient the fish to flow direction. The flow tunnel holds approximately 55 L of water and is 119.5 cm (L) X 15.3 cm (W) X 18.3 cm (H). The area where the fish was tested is 12 cm (L) X 15.3 cm (W) X 11.5 cm (H). This smaller area was obtained by placing grating throughout the working area of the flow tunnel, which also served to attain laminar flow. After the 15-minute acclimation, flow was increased at approximately 0.30 cm s^{-2} until the fish could no longer maintain position in the flow and was pushed against the back grating or sides of the flow tunnel. The final speed for which the fish was able to maintain position was considered its maximum speed (U_{max}).

Finally, critical swimming speed (U_{crit}) was measured following methods outlined in Brett (1964), Beamish (1978), and subsequent studies (Kolok 1999, Plaut 2001). Similar to U_{max} measures, fish were acclimated to the flow tunnel for 15 minutes at a low velocity. After the 15-minute acclimation period, flow was increased 4.5 cms^{-1}/5 minutes. Each fish was tested until it could no longer maintain position, and could not remove itself from the back grating after three taps on the side of the tunnel. Both U_{crit} and U_{max} were measured at room temperature (~23 C).

For all swimming performance metrics we assume that motivation of the individuals was maximal or near-maximal. Additionally, we did not observe any fish that

seemed to refuse to swim for any of the trials.

2.3.3 SIZE AND SHAPE

We also examined repeatability of an index of size and shape, and correlated them with the performance measures. During testing, fish were anaesthetized using MS-222, and body mass (to the nearest 0.001 g), total length (TL: from tip of snout to end of the caudal fin, to the nearest 0.01 mm), standard length (SL: from the most anterior portion to the insertion [base] of the caudal fin, to the nearest 0.01 mm), and body depth (BD: at the insertion of the dorsal fin, to the nearest 0.01 mm) were measured. We used TL as our index of size since it had the lowest coefficient of variation (mass: coefficient of variation [CV] = 13.16%, SL: CV = 6.92%, TL: CV = 3.61%, BD: CV = 6.91%). For shape we used body depth/total length. These measurements were recorded a total of three times over a span of months and days, using digital calipers under a dissecting scope. After all performance measures had been taken, the fish were euthanized with a lethal dose of MS-222 and again measured. Results from a principal components analysis of the measures were similar and so are not reported.

2.3.4 EXPERIMENTAL DESIGN

We assessed the repeatability of these performance measures several ways and over varying time scales (Figure 2.2). Because the time between measures varied for each performance metric we refer to each day as a trial, Figure 2.2 outlines the timing between each trial for the 3 performance metrics and size measures with the day given that each trait was measured. We measured size first and used this as Day 1. For burst speed during c-start, we examined short, medium, and long-term repeatability. Short-

term repeatability was measured similar to previous studies, where the time between trials was minutes, making it a within-day comparison (Figure 2.2). Medium-term repeatability was assessed over a month (trial 2: day 36), and again over a week (trial 3: day 42). Finally, long-term repeatability was measured by testing the fish 16 months later (trial 4: day 510). Thus, we have a total of four trials, each with 1-3 c-start escape responses in each. For $U_{max,}$ we assess only medium- and long-term repeatability over a period of days and weeks (trial 1: day 55, trial 2: day 61, trial 3: day 75) and over a year (trial 4: day 520). Similarly, U_{crit} was assessed over a period of weeks (trial 1: day 83, trial 2: day 103, trial 3: day 131) and over a year (trial 4: day 525). Finally, the repeatability of size and shape was assessed over a period of months (trial 1: day 1, trial 2: day 150), days (trial 3: day 156), and 11 months later (trial 4: day 535). The time scale was chosen based on the time it took to measure each swimming performance metric three times: each performance metric had the first three trials measured before beginning a new performance measure. Due to the difference in time to measure all individuals for the trait, this produced the various time scales of days, weeks or months.

2.3.5 STATISTICAL ANALYSIS

Repeatability of the various trials (see Fig. 2.2) was analyzed in several ways. Because we obtained approximately 12 c-start trials per fish, over short, medium and long time periods, the repeatability was determined depending on which time frame we were assessing. For short-term repeatability of c-start burst speed, we used the intraclass correlation coefficient (I_{cc}) (Lessells & Boag 1987). This test was used because we had up to three trials per fish, and the mean value of the within-day trials were not expected to change. The I_{cc} has been used in other studies examining within-day repeatability of

escape response burst speed (Langerhans *et al.* 2004, Royle *et al.* 2006). For the medium- and long-term repeatability, we used Pearson's product-moment correlation coefficient. This test was used because the means might change over this longer time period (which would artificially reduce the magnitude of the I_{cc}), and because we were interested in the relationship between each specific set of days (i.e., trials over a week versus over a month or over a year).

Studies often use the maximum velocity of the fish on a given day as its value of c-start burst speed (Chappell & Odell 2004, Langerhans *et al.* 2004); however, at least one study has demonstrated in amphibians that the mean velocity on a given day might also be important (Austin & Shaffer 1992). Therefore, we tested for repeatability of the single highest velocity obtained on a given day among the four trials (i.e., the highest 2 ms burst speed velocity attained among the three within day c-start trials). We also averaged the highest velocity for each of the three within-day measures and compared them among trials, and also included a comparison of the means while correcting for attenuation (Adolph and Hardin 2007). For the long-term repeatability of c-start burst speed, we compared trial 3 to trial 4 by examining the single highest maximum for the respective day, as well as the mean for the day and the mean corrected for attenuation. Repeatability for U_{crit}, U_{max}, size, and shape is not as complicated due to the lack of repeated measures within a day. Therefore, we used Pearson's correlation coefficient to test for repeatability among the 4 trials. For size, we examined the repeatability of total length, for shape we examined the repeatability of body depth/total length. Similar to c-start velocity, when testing for long-term repeatability in U_{max}, U_{crit}, and size/shape we again compared trial 3 to trial 4 using Person's correlation coefficient to be consistent with our measure of repeatability. We also looked for differences in mean values over

the span of a year using a paired t-test for all traits (including all size measures) to compare the mean value for trial 3 to the mean value of trial 4.

Finally, we examined relations between the performance metrics and between performance metrics and size/shape using Pearson correlations for values taken at a given trial. For our measure of size, we again used total length of the fish because it had the least amount of variance compared to the other size measures. We also correlated the performance traits to our index of shape, body depth/total length. For all of these analyses, we judged statistical significance at $P < 0.01$ (2-tailed) to reduce problems of multiple comparisons.

The temperature during U_{max} and U_{crit} was maintained at room temperature and did not vary substantially. However; the temperature did vary for c-start measurements because of the lights used to illuminate the arena. Therefore, to test if temperature was a predictor of velocity during c-start, we regressed velocity against the temperature for the trial.

2.4 Results

2.4.1 SIZE AND SHAPE

Mean values for all size and shape measurements and all trials are summarized in Appendix 2.1. Total length (Fig. 2.3A, Table 2.2) was repeatable across all trials (trial 1 vs. 2: $r = 0.7111$, $p < 0.001$; trial 2 vs. 3: $r = 0.8452$, $p < 0.001$; trial 3 vs. 4: $r = 0.4600$, $p = 0.031$). In contrast, our index of shape, body depth/total length was not repeatable among any of the trials (trial 1 vs. 2: $r = 0.1179$, $p = 0.592$; trial 2 vs. 3: $r = 0.3632$, $p = 0.081$; trial 3 vs. 4: $r = 0.2111$, $p = 0.346$, Table 2.2). All size measures increased

significantly over the span of a year (Table 2.3); however, body depth/total length did not change over a year.

2.4.2 BURST SPEED DURING C-START

Burst speed during a c-start escape response was not significantly related to temperature for any trial. Therefore, we did not include temperature in any further analyses. The average time it took fish to reach maximum velocity was 18.297 ± 3.223 ms, and in 86.18% of the trials maximum velocity was achieved at the end of stage 1; in the remaining trials maximum velocity was achieved during stage 2. Within-day repeatability for velocity was not statistically significant for trial 1 (intraclass correlation coefficient [I_{cc}] = 0.1395, p = 0.1622), but was for trials 2 (I_{cc} = 0.2482, p = 0.0272) and 3 (I_{cc} = 0.3803, p = 0.0027). When the fish were tested again more than a year later, repeatability was marginally non-significant (I_{cc} = 0.2713, p = 0.0604).

Mean burst speed was significantly repeatable between trials 2 and 3, and after correction for attenuation was also repeatable between trials 1 and 2 (Table 2.2, see also Figure 2.3B). After a year, the mean and maximum velocity attained during c-start decreased significantly (Table 2.3, Figure 2.3B), and neither was repeatable, even after correcting the mean velocity for attenuation (Table 2.2).

2.4.3 MAXIMUM SPEED (U_{MAX}) AND CRITICAL SWIMMING SPEED (U_{CRIT})

The maximum speed the fish maintained in the flow tunnel ($U_{max,}$ Fig. 2.3C) was significantly repeatable over a time period of weeks (Table 2.2, trial 2 & 3) and more than 14 months (trial 3 & 4). Surprisingly, U_{max} increased significantly over the span of 14 months (Figure 2.3C, Table 2.3, t = -4.491, p < 0.001).

Critical swimming speed (U_{crit}) was repeatable over weeks, but not over a year (Table 2.2). Similar to c-start burst speed, U_{crit} declined significantly over the span of a year (Figure 2.3D, Table 2.3).

2.4.4 RELATIONS BETWEEN PERFORMANCE METRICS AND MORPHOLOGY

Relations of performance metrics and morphology are summarized in Table 2.4. Critical swimming speed and U_{max} were consistently positively related, and significantly so at the first trial. Mean c-start burst speed significantly positively correlated with U_{max} at trial 2, but this appears to be an anomalous result considering the lack of correlation at other trials. None of the performance measures was significantly related to body size or shape.

2.5 Discussion

Our results demonstrate that individual differences in three swimming performance metrics, which characterize two different modes of swimming in teleost fish, as well as body size, are repeatable on various time scales, including long-term repeatability more than a year later for at least one performance metric (U_{max}) and size (TL). This relative stability of individual differences is important for two general ecological/evolutionary reasons. First, if individual differences are not consistent over time, then natural or sexual selection cannot "target" particular phenotypes with any degree of accuracy. Second, repeatability generally sets an upper limit to heritability (but see Dohm 2002), and hence the rate of evolutionary (genetic) response to selection of a given intensity.

We found that most traits change in an expected fashion over a time span of a year or more, as fish progress through middle-to-old age. For instance, it is predicted

that performance metrics will decrease during later ages due to the effects of senescence on the neuromusculature system (Reznick *et al.* 2004). As expected, we found that both U_{crit} and burst speed during a c-start escape response decreased significantly after a year (Table 2.3, Figure 2.3). However, U_{max} surprisingly increased significantly (Table 2.3). Not surprisingly, body size increased significantly (Table 2.3), although the increase was less than 5%, which is typical for mature male poeciliids (Bisazza 1993).

The escape response of a fish, as characterized by the c-start, has been well studied from many disciplines (Domenici & Blake 1997, Domenici 2002); however, only a few studies have examined its repeatability. We examined the velocity attained during a c-start escape response and found short-term repeatabilities (Table 2.2, and see Results) within the range of those reported previously (Garenc *et al.* 1999, Chappell & Odell 2004, Langerhans *et al.* 2004, Royle *et al.* 2006). We did not find significant long-term repeatability of c-start performance, and no previous study has reported comparable values.

Our results for c-start mean burst speed suggest that using the mean value of several measures separated by short time intervals, and correcting for attenuation (Adolph & Hardin 2007), increases repeatability (Table 2.2, Figure 2.3B). Few other studies have examined medium- and long-term repeatability of speed during a c-start escape response. For example, the longest study on burst speed in fish found significant repeatability over two days, on six individual *Gasterostereus aculeatus* (Garenc *et al.* 1999). Although many studies of terrestrial vertebrates have found repeatability of maximum speeds (e.g., Huey & Dunham 1987, Djawdan & Garland 1988, Huey *et al.* 1990, Brodie & Garland 1993, van Berkum *et al*. 1989, Garland & Losos 1994, Chappell

et al. 2007, but see Losos *et al.* 2002), our results suggest that in future studies it may be beneficial to obtain multiple trials for an individual fish and use the mean velocity during a c-start escape response instead of the single maximum velocity (Table 2.2).

The c-start escape response of fish has been proposed to be important in predator/prey interactions, and recent studies have begun to highlight the importance of various aspects of this response. Walker *et al.* (2005) examined several aspects of c-start escape response in relation to successful escapes during staged encounters between guppies (*P. reticulata*) and a natural fish predator (*Crencichla alta*), and recorded the trials with a high-speed video camera. Successful escapes were associated with long approach distances, low predator strike velocities, increased initial flight angles, increased accelerations, and increased stage 1 turning velocities (Walker *et al.* 2005). Because we only measured velocity during a c-start escape response, more studies are warranted on the repeatability of other aspects of this performance.

Our results for repeatability of critical swimming speed (U_{crit}) are consistent with previous studies, which have reported repeatabilities of 0.44 to 0.86 (Table 2.1), and are similar to endurance repeatabilities in squamate reptiles (Kolok 1999, Perry *et al.* 2004). Although no studies have yet demonstrated the direct fitness benefits of an increased in U_{crit} or endurance in fish, some studies have begun to explore its relationship with environmental characteristics, such as water velocity (McGuigan *et al.* 2003, Nelson *et al.* 2003).

Maximum swimming speed (U_{max}) is a recently described performance trait and has not been evaluated for adaptive significance or repeatability. We found its repeatability to be similar to that of U_{crit}, at least on a medium time scale (Table 2.2). U_{crit} and U_{max} tended to be positively correlated, and significantly so for at least the first trial

(Table 2.4). Surprisingly, their mean values were not significantly different for the first three trials (trial 1: $t = 0.788$, $p = 0.439$; trial 2: $t = 0.301$, $p = 0.766$, trial 3: $t = 0.478$, $p = 0.637$, and see Appendix). However, there was a difference in their means for the fourth trial ($t = 9.204$, $p < 0.001$), with fish having a significantly higher U_{max}. These results suggest that the measure of U_{max} may be an equally reliable trait to measure in fish performance, but see also Farrell (2008). Because the ramping velocity is much faster in U_{max}, the trials are much shorter (~95 seconds in our study) than U_{crit} trials (~22 minutes in our study) (Reidy *et al.* 2000, Farrell 2008). However, caution must be taken because our results demonstrate a significant increase in U_{max}, but a decrease in U_{crit}, causing a significant difference in their means on a long time scale. Therefore, more definitive tests of U_{max} are needed to determine its relationship to U_{crit} (and see Farrell 2008).

The reason for the increase in U_{max} between trials 3 and 4 is unknown, but may be related to the simultaneous increase in body size (Table 2.3, Fig. 2.3C, Appendix). Yet, U_{max} is not significantly related to size for any of the trials (Table 2.4). Additionally, U_{crit} decreased over a year, and it is positively correlated with U_{max} on the 4th trial ($r = 0.525$, 2-tailed $p = 0.012$). The increase in U_{max} was also not a function of training affects, as the fish were not intentionally exercised for a year. Gibert, Huey & Gilchrist (2001) found that the walking speed of fruit flies increased with age when reared at cold temperatures, but decreased when flies were reared at warm temperatures. However, all fish in our study were reared under similar conditions, so temperature acclimation cannot explain the increase in U_{max}. In our study, fish were mature prior to trial 1, so the increase in U_{max} is not likely a function of developmental effects on the neuromusculature system. However, there is an example of post-maturational age-related increase in stamina in natural populations of *Sceloporus merriami* (Huey *et al.*

1990). In any case, further studies are needed to determine why this performance metric increased with age.

A negative relationship may exist between burst speed and endurance because they are affected by many of the same subordinate traits, such as muscle fiber type composition (Webb 1984, Weihs 1989, Garland & Losos 1994, Vanhooydonck *et al.* 2001, Bonine, Gleeson & Garland Jr 2005). This relationship has been investigated at the individual level in one previous study of guppies. Chappell and Odell (2004) examined the relationship between burst speed during an escape response and maximum oxygen consumption (a likely component of endurance capacity) in male and female Trinidadian guppies, and for both sexes found no evidence of a trade-off. Claireaux et al. (2007) also examined this relationship at the individual level in the European sea bass (*Dicentrarchus labrax*) by measuring U_{crit} and sprint speed in a group of marked fish, over a period of weeks and months. They found no relationship between U_{crit} and sprint speed in any of their analyses (e.g., the entire cohort, survivors over 6 months, and non-survivors). They suggest that while these two modes of swimming, burst and prolonged, rely on overlapping morphological and physiological systems, they are still distinct for each mode of swimming. For instance, prolonged is powered by aerobic respiration and may be limited by fuel or delivery of oxygen to the muscle, whereas burst is powered by anaerobic means and may be limited by neuromusculature factors. Therefore, they conclude, that "This disconnection may account for the fact that U_{crit} and sprint speeds respond differently to environmental constraints, and it introduces the possibility of independent selection trajectories for the two performance metrics (Claireaux *et al.* 2007)." Our results (Table 2.4) also indicate no relationship between burst (escape response) and prolonged (U_{crit}) swimming, whether examining the mean c-

start burst speed or the maximum. More studies are needed to tease apart this relationship between sprint and endurance performance in fish; both at the interspecific and intraspecific levels, corrections for "measurement error" may reveal new and interesting results (see Adolph & Hardin 2007; Ives, Midford & Garland 2007).

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Table 2.1. Repeatability values reported in the literature for a variety of fish and performance measures, sorted from shortest to longest interval between replicate trials for each type of performance.

Species	N	Performance	Time Period	Repeatability	Type	Source
Poecilia reticulata	86	C-start (speed)	2 minutes	0.805	P	Chappell & Odell 2004
Xiphophorus helleri	28	C-start (speed)	minutes	0.23	I	Royle et al. 2006
Gambusia affins	18	Burst speed	Within a day	0.89	I	Langerhans et al. 2004
Gasterosteus aculeatus	6	Burst speed	2 days	0.943	S	Garenc et al. 1999
Dicentrarchus labrax	32	Sprint	4 Weeks	0.43	S	Claireaux et al. 2007
Gadus morhua	23	Sprint	3 months	0.756	P	Reidy et al. 2000
Dicentrarchus labrax	52	Sprint	6 months	0.23 n.s.	S	Claireaux et al. 2007
Micropterus salmoides	7 9	U_{crit}	4 days	0.86 (11°C) 0.77 (22°C)	S	Kolok 1992
Oncorhynchus mykiss	8	U_{crit}	8 days	0.857	S	Gregory & Woods 1998
Pimephales promelas	10	U_{crit}	10-14 days	0.83	S	Kolok et al. 1998
Rhinichthys atratulus	14	U_{crit}	1 month	0.6	K	Nelson et al. 2003
Gadus morhua	12	U_{crit}	3 months	0.771	P	Reidy et al. 2000
Dicentrarchus labrax	52	U_{crit}	6 months	0.44	S	Claireaux et al. 2007

I = Intraclass correlation coefficient, P = Pearson product-moment correlation, S = Spearman rank correlation, K = Kendall's τ.
n.s. = not significant

Table 2.2. Repeatabilities of performance measures comparing trials 1 with 2, 2 with 3, and 3 with 4. Pearson correlation coefficient reported, except for C-start Burst Speed (mean), where we also report r values corrected for attenuation (Adolph & Hardin 2007; see "Statistical Analysis" in Methods). Bold indicates statistically significant correlations based on 2-tailed tests.

	Trials 1 vs. 2	Trials 2 vs. 3	Trial 3 vs. 4
U_{max}	r = 0.3529 p = 0.091	**r = 0.5410** p = 0.006	**r = 0.4252** p = 0.048
U_{crit}	**r = 0.7365** p < 0.001	**r = 0.5782** p = 0.003	r = 0.3427 p = 0.118
C-start Burst Speed (maximum)	r = 0.1396 p = 0.515	r = 0.1636 p = 0.445	r = 0.0778 p = 0.744
C-start Burst Speed (mean)	r = 0.1841 p = 0.389	**r = 0.4301** p = 0.036	r = 0.1565 p = 0.510
C-start Burst Speed (mean, corrected for attenuation)	**r_{corr} = 0.4563** p = 0.0328	**r_{corr} = 0.7574** p < 0.0001	r_{corr} = 0.2677 p = 0.4002
Total Length	**r = 0.7111** p < 0.001	**r = 0.8452** p < 0.001	**r = 0.4600** p = 0.031
Body Depth/TL	r = 0.1179 p = 0.592	r = 0.3632 p = 0.081	r = 0.2111 p = 0.346

Table 2.3. Mean values (± standard deviations) for trial 3 and 4 in all fish. All traits, except the ratio of body depth to total length, changed significantly over the span of a year, as indicated by a paired t-test.

	N	Trial 3	Trial 4	T	df	2-tailed p
U_{max} (cm s^{-1})	22	29.13 ± 3.65	33.13 ± 4.12	-4.491	21	< 0.001
U_{crit} (cm s^{-1})	22	29.26 ± 4.02	25.43 ± 3.93	2.966	21	= 0.007
C-start maximum burst speed (cm s^{-1})	20	95.65 ± 22.89	66.73 ± 16.18	4.793	19	< 0.001
C-start mean burst speed (cm s^{-1})	20	93.76 ± 12.85	63.52 ± 16.93	6.904	19	< 0.001
Mass (g)	22	0.105 ± 0.015	0.136 ± 0.021	-7.433	21	< 0.001
Standard length (mm)	22	16.26 ± 0.83	17.43 ± 1.19	-5.049	21	< 0.001
Total length (mm)	22	21.94 ± 0.86	23.61 ± 1.20	-7.12	21	< 0.001
Body depth (mm)	22	4.10 ± 0.28	4.31 ± 0.33	-3.146	21	= 0.005
Body depth/Total length	22	0.187 ± 0.009	0.183 ± 0.012	1.439	21	= 0.165

Table 2.4. Pearson correlations for the 4 swimming performance traits, total length as a measure of body size, and body depth/total length as an index of shape. r_1 = trial 1, etc. Significant correlations ($p < 0.01$) based on 2-tailed tests and not corrected for multiple comparisons are marked in bold and with *

.

	U_{crit}	C-start Burst Speed (maximum)	C-start Burst Speed (mean)	Total Length	Body Depth/TL
U_{max}	**r_1 = 0.550*** r_2 = 0.380 r_3 = 0.221 r_4 = 0.525	r_1 = -0.243 r_2 = 0.261 r_3 = -0.131 r_4 = -0.060	r_1 = -0.505 **r_2 = 0.616*** r_3 = 0.048 r_4= -0.258	r_1 = 0.378 r_2 = -0.080 r_3 = 0.145 r_4 = 0.277	r_1 = 0.098 r_2 = -0.063 r_3 = 0.151 r_4 = 0.074
U_{crit}		r_1 = 0.010 r_2 = 0.208 r_3 = 0.173 r_4 = 0.193	r_1 = -0.143 r_2 = 0.253 r_3 = 0.067 r_4 = -0.069	r_1 = -0.117 r_2 = 0.173 r_3 = 0.209 r_4 = 0.361	r_1 = 0.141 r_2 = 0.147 r_3 = 0.138 r_4 = 0.197
C-start Burst Speed (maximum)			**r_1= 0.779*** r_2 = 0.457 r_3 = 0.410 r_4 = 0.439	r_1 = -0.398 r_2 = 0.068 r_3 = -0.204 r_4 = 0.328	r_1 = 0.256 r_2 = 0.095 r_3 = -0.414 r_4 = 0.434
C-start Burst Speed (mean)				r_1 = -0.474 r_2 = -0.036 r_3 = -0.346 r_4 = -0.299	r_1 = 0.128 r_2 = -0.110 r_3 = -0.375 r_4 = 0.031
Total Length					r_1 = 0.191 r_2 = 0.142 r_3 = 0.310 r_4 = -0.138

* $p < 0.01$

Table 2.4

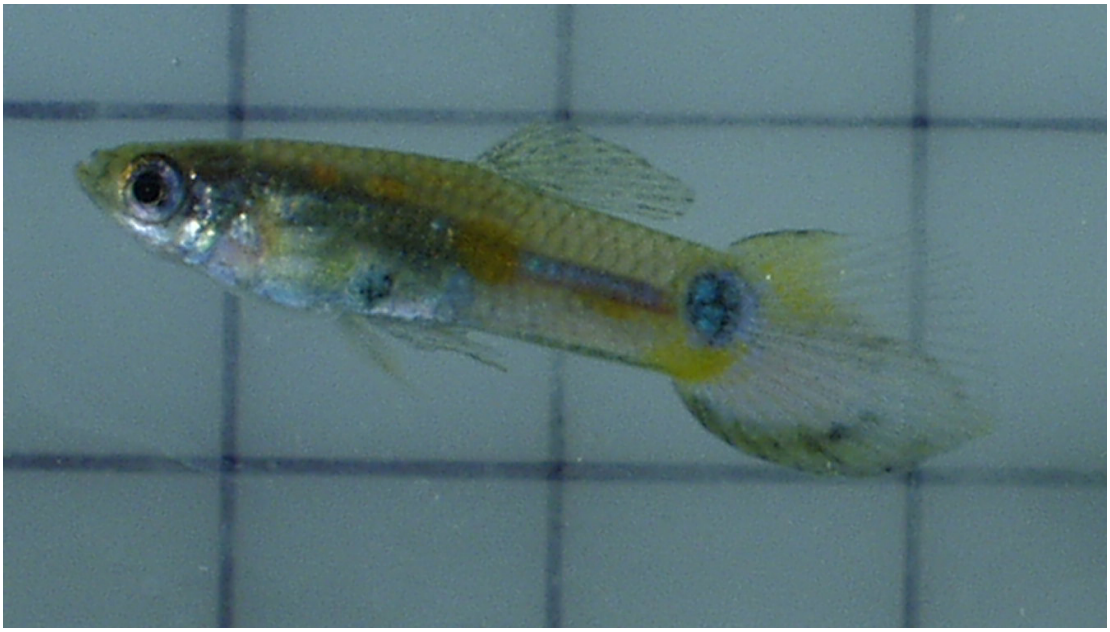

Figure 2.1. Male Trinidadian guppy (*Poecilia reticulata*) originating from the Quare river drainage. Grid represents 1 cm scale.

Figure 2.2. Timeline for locomotor and size measures. Because the interval between measurements varied, we simplified the measures by referring to each measurement day as a trial; however, we also report the actual day each trait was measured with size being measured on Day 1. Additionally, there are three measures of within-trial repeatability for c-start only.

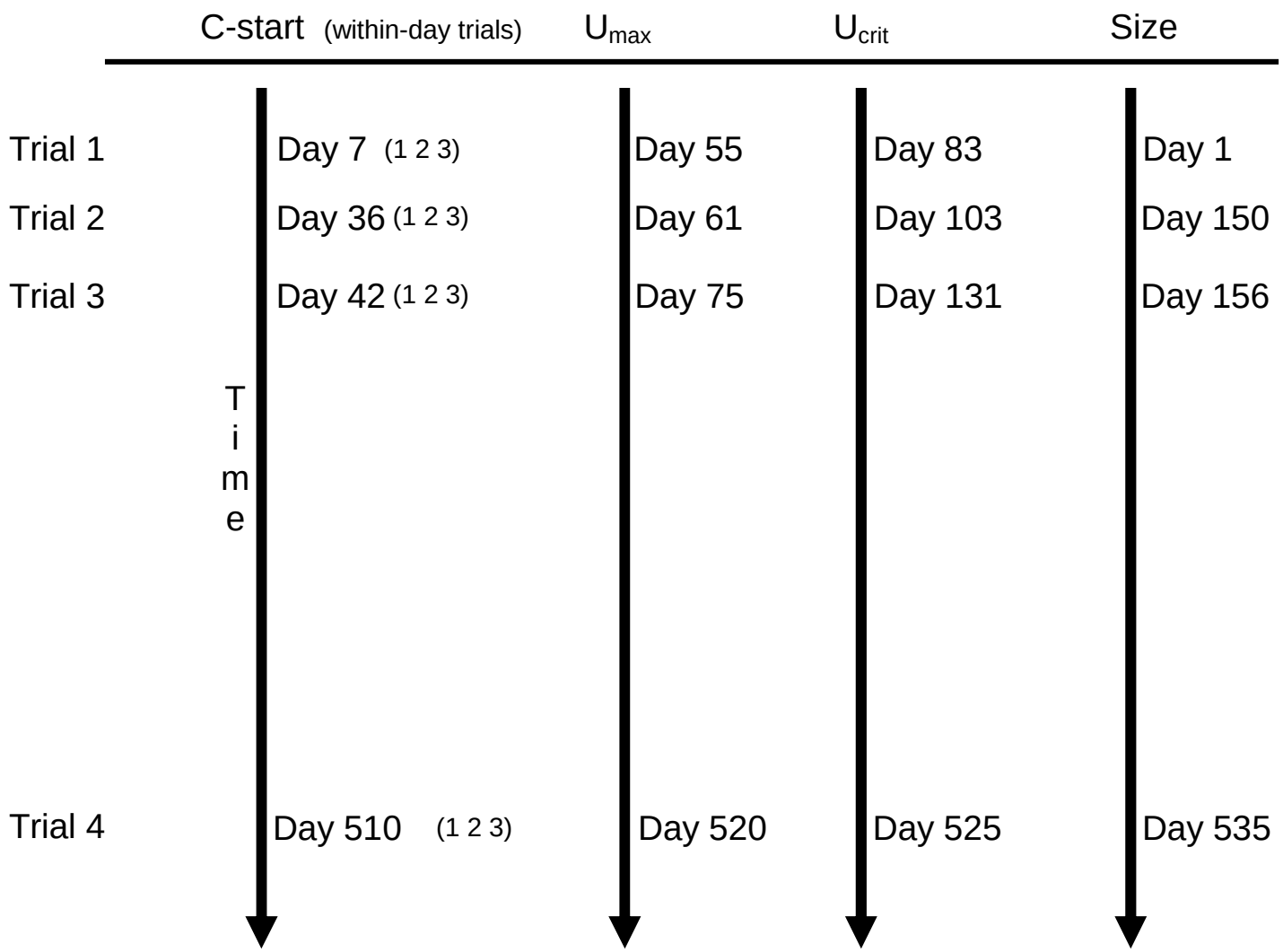
C-start (within-day trials)
U_{max}
U_{crit}
Size
Trial 1
Day 7 (1 2 3)
Day 55
Day 83
Day 1
Trial 2
Day 36 (1 2 3)
Day 61
Day 103
Day 150
Trial 3
Day 42 (1 2 3)
Day 75
Day 131
Day 156
Time
Trial 4
Day 510 (1 2 3)
Day 520
Day 525
Day 535

Figure 2.2

Figure 2.3. Plots of individual values for total length (A) mean c-start burst speed (B), maximum speed (U_{max}, C), and, critical swimming speed (U_{crit}, D). Each point represents an individual's value for that day. Lines connect an individual's value among the four days.

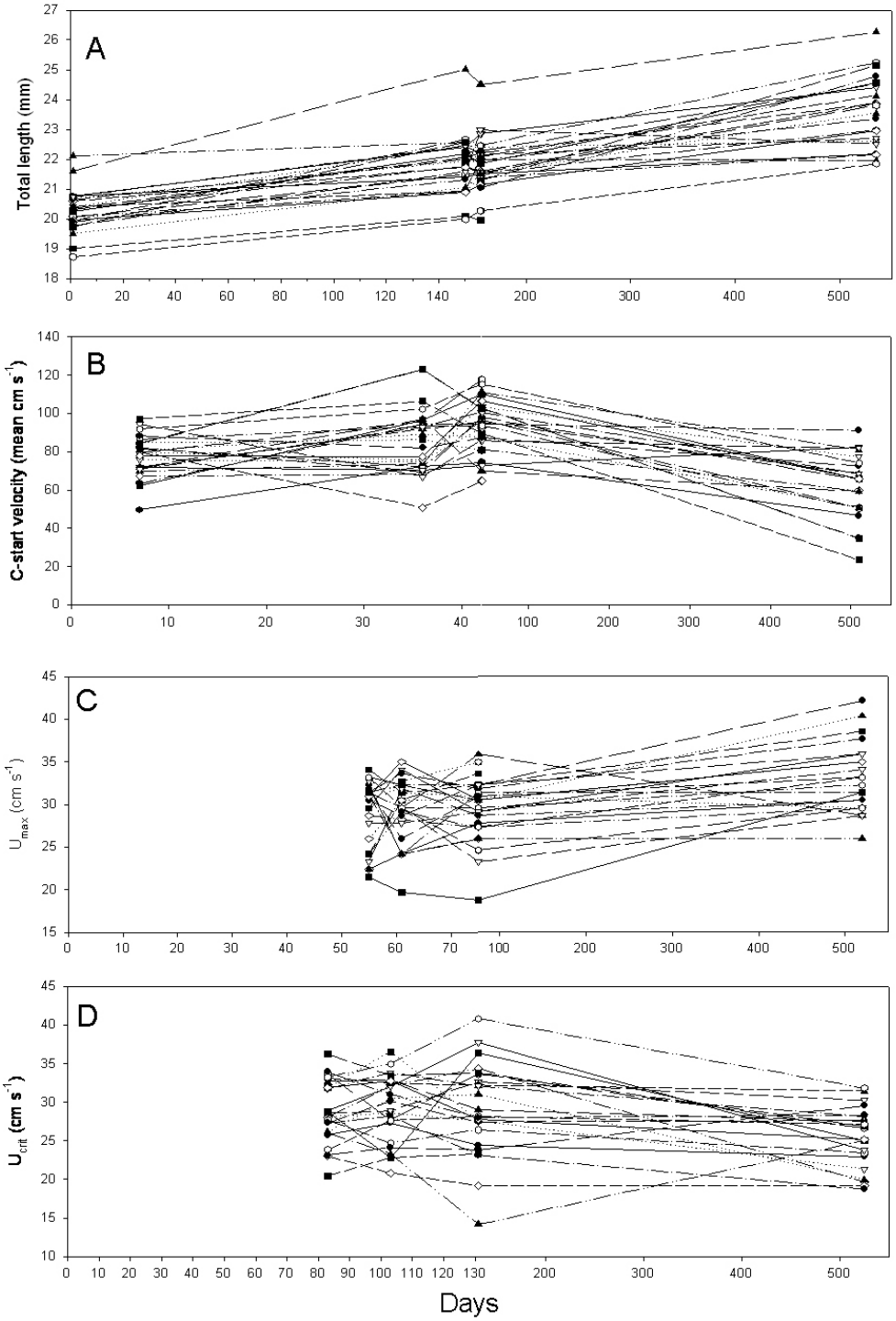
A
Total length (mm)
B
C-start velocity (mean cm s-1)
C
Umax (cm s-1)
D
Ucrit (cm s-1)
Days

Figure 2.3.

2.7 Appendix

Descriptive statistics for all traits measured for all trials. Sample sizes (N), means, and standard deviations reported.

Trait	N	Mean	Standard Deviation
Maximum speed (U_{max})			
Trial 1	24	29.41	3.797
Trail 2	24	29.41	3.653
Trail 3	24	29.56	3.785
Trial 4	22	33.13	4.121
Critical swimming speed (U_{crit})			
Trial 1	24	28.81	4.063
Trail 2	24	29.13	4.327
Trail 3	24	28.94	6.002
Trial 4	22	25.43	3.930
C-start burst speed (maximum)			
Trial 1	24	83.32	10.228
Trail 2	24	96.03	11.248
Trail 3	24	95.96	22.564
Trial 4	20	66.73	16.181
C-start burst speed (mean)			
Trial 1	24	77.62	10.944
Trail 2	24	84.27	15.851
Trail 3	24	92.94	14.313
Trial 4	20	63.52	16.931
Mass			
Trial 1	23	0.0805	0.01059
Trail 2	24	0.1048	0.01683
Trail 3	24	0.1037	0.01627
Trial 4	22	0.1361	0.02051
Standard length (SL)			
Trial 1	23	14.89	1.030
Trail 2	24	16.14	0.880
Trail 3	24	16.17	0.876
Trial 4	22	17.43	1.195
Total length (TL)			
Trial 1	23	20.28	0.733
Trail 2	24	21.82	1.014
Trail 3	24	21.86	0.906
Trial 4	22	23.61	1.198

Trait	N	Mean	Standard Deviation
Body Depth (BD)			
Trial 1	23	3.796	0.2621
Trail 2	24	4.218	0.3217
Trail 3	24	4.093	0.2973
Trial 4	22	4.314	0.3309
Body depth/total length			
Trial 1	23	0.1872	0.0099
Trail 2	24	0.1932	0.0104
Trail 3	24	0.1871	0.0088
Trial 4	22	0.1828	0.0120

Chapter 3

The cost of bearing a sword: endurance costs and sub-organismal compensations among species of *Xiphophorus*

3.1 Summary

1. Sexually selected traits are assumed to incur a cost to the bearer, which can limit their expression. For example, morphological traits can evolve that are potentially beyond the functional optimum for locomotor abilities, resulting in a cost to locomotion (in terms of ability and/or energetics) and potentially reducing components of Darwinian fitness (e.g., survival).

2. We examined the relation between the sexually selected 'sword' and one measure of aerobic locomotor performance, critical swimming speed (U_{crit}), among species of *Xiphophorus* fish. We also included traits that may compensate for the adverse effects of bearing a longer sword: maximum oxygen consumption, heart and gill mass, tail mass, citrate synthase and lactate dehydrogenase activity in tail muscle, and caudal fin length. An increase in any one (or combination) of these traits in sworded species could potentially reduce the locomotor cost of the sword. In addition, we measured an index of reproductive allocation in the females to determine if allocating more to offspring has a negative effect on locomotor performance.

3. We examined an average of ~14 males and 14 females from each of 17 species of *Xiphophorus* and two species of *Priapella*. We computed sex-specific mean values for

each species, and then applied phylogenetically informed statistical methods to determine if the sword is a negative predictor of critical swimming speed, taking into account potentially compensatory traits.

4. Mean U_{crit} showed considerable variation among species, ranging from 14.5 to 35.6 cm s^{-1} for males and from 14.8 to 37.2 cm s^{-1} for females. Phylogenetic statistical analyses indicated that little of this variation was explainable by variation in body size.

5. Considering the 13 of 19 species that exhibited a sword in males, relative sword length ranged from 7% to 55% of standard length. Bivariate scatterplots suggested a generally positive relation between sword length and standard length, but these plots are misleading because "grade shifts" have occurred, i.e., only some lineages exhibit swords, and within those lineages the relation with body size is weak.

6. After adjusting for relations with standard length, most traits exhibited statistically significant phylogenetic signal, as did body size itself.

7. Using both conventional and phylogenetic allometric relations with standard length, we obtained residuals to examine the effect of candidate predictors on critical swimming speed. Multiple regressions of phylogenetic residuals revealed that residual $\log_{10}$ sword length and residual $\log_{10}$ heart mass both had a significant positive effect on residual $\log_{10}$ critical swimming speed of males, but only residual $\log_{10}$ body depth was a significant (positive) predictor in non-phylogenetic analyses. For females, in the non-phylogenetic model residual $\log_{10}$ body depth and sword length were significant positive

predictor of residual $\log_{10}$ critical swimming speed, while residual $\log_{10}$ tail mass and reproductive allocation were significant negative predictors. However, phylogenetic analyses indicated no significant predictors of residual U_{crit} for females. Based on analysis of residuals, there were no significant predictors of VO_{2max} in either sex. Interestingly, residual sword length was significantly negatively correlated with both residual heart and gill mass, both non-phylogenetically and phylogenetically in males, suggesting a trade-off in resource allocation to these structures

8. Our results suggest that as the sword evolved it did not reduce aerobic swimming ability. The positive effect of the sword on critical swimming speed in males may be related to unmeasured biomechanical effects or not including other important compensatory traits, such as body or fin shape, in our analyses. Future studies should explore whether the sword has a negative impact on other aspects of swimming performance.

3.2 Introduction

Traits that evolve in response to sexual selection are some of the most compelling for study by evolutionary biologists (Darwin 1859, 1871, Andersson 1994). The interest in these traits stems from the seemingly 'un-natural' expression of phenotypes (e.g., tails of male peacock, eye extensions of stalk-eyed flies, antlers of and Irish elk) that are believed to evolve to increase reproductive fitness (i.e., sexual selection) while also meeting the demands of natural selection. Despite the origin or maintenance of these traits (e.g., (Fisher 1930; Grafen 1990; Hamilton and Zuk 1982; Kirkpatrick 1982, 1987; Meade and Arnold 2004), they are all assumed to impose a fitness cost to their bearer (typically, reduced survivorship), thereby limiting their expression.

Commonly, sexually selected traits are in the form of a behavior, difference in coloration or an exaggeration of a morphological characteristic (Andersson 1994), all of which may incur costs. These costs can be direct effects on survival (e.g., increased susceptibility to predation) or indirect (e.g., increased energy expenditure, see Kotiaho 2001), and have been examined for a variety of traits in a variety of taxa. For example, Ryan *et al.* (1982) demonstrated the direct costs of male Tungara frogs calling, where predatory bats cued in on the calls. Males that advertised to attract females also made themselves more apparent to predators. Indirect costs can come in the form of increased physiological burden, such as an increase in oxygen consumption (indicating increased energy expenditure) with the presence of the trait (e.g., Basolo and Alcaraz 2003).

The morphology (form) of an organism often evolves to optimize its functioning in its environment (Dickson *et al.* 2000, but see Garland & Huey 1987, Dudley & Gans 1991); yet through sexual selection, morphologies can evolve that are beyond the

functional optimum, thus reducing Darwinian fitness in the context of natural selection (Garland 1988, Oufiero & Garland 2007). As one example, sexually selected traits may adversely affect the locomotor abilities of an organism, either through an increase in drag and consequent reduction in speed or maneuverability, a greater energy cost, or both. Many bird species appear to have evolved elongated tail feathers through sexual selection, and several studies have examined the potential costs (Evans & Thomas 1992, Barbosa & Møller 1999, Clark & Dudley 2009, but see Petrie 1992, 1994). Similarly, many fish species have evolved elongated fins, apparently as a result of sexual selection, and these may hinder locomotor abilities (e.g., Poeciliids (Bisazza, Grapputo & Nigro 1997)). Yet, other mechanisms may evolve to offset such costs.

Although potential costs of sexually selected traits have been well studied compensatory traits have often been overlooked. A compensatory trait can be defined as any trait that evolves to offset the cost of the sexually selected trait (Kirkpatrick 1987, Møller 1996, Oufiero & Garland 2007). For example, Balmford *et al.* (1994) demonstrated an increase in sexual dimorphism in wing length with an increase in sexual dimorphism in tail length among species of birds. The authors suggest that as tail ornaments evolve via sexual selection in males, they also evolve a longer wingspan to increase their thrust, thus counterbalancing the drag of the longer tail. Of course, compensatory traits may themselves incur a cost, e.g., related to costs of synthesis and maintenance. Ignoring compensatory traits can lead to erroneous results regarding the effects of a 'costly' sexually selected trait (Oufiero and Garland 2007).

The purpose of the present study was to determine the effect of a sexually selected trait on an important aspect of aerobic performance, accounting for some potential compensatory traits. To do this, we used the *Xiphophorus* genus of fish as a

model system. *Xiphophorus* are one of the examples of a group expressing a sexually selected trait listed by Darwin (1871) and are useful to examine the effect of a sexually selected trait on performance for several reasons. First, expression of the sword varies widely among the ~26 species in the genus (Fig. 3.1). Second, the sword is directly, physically linked to the main thrust-producing system of the fish, the caudal fin and caudal peduncle. These fish primarily use their caudal peduncle and fin for propulsion during intense natural exercise, and the sword is an extension of the caudal fin along the lower margin. Therefore, this structure may not only increase drag, but also increase energy costs during intense swimming. Conversely, the sword may also increase thrust due to an increase in surface area. Third, there is an intrinsic female bias for the sword (Basolo 1990; Basolo 1995; Basolo 1996; but see Rosenthal *et al.* 2002). In other words, females from species without swords, including the outgroup genus *Priapella*, prefer males with longer swords. Finally, phylogenetic hypotheses are available for the group (Marcus & McCune 1999, Morris *et al.* 2001), which allow for phylogenetically based statistical methods (Garland *et al.* 2005).

The effect of the sword on swimming performance has been previously examined with mixed results (Ryan 1988, Basolo & Alcaraz 2003, Royle *et al.* 2006, Kruesi & Alcaraz 2007, Oufiero & Garland 2007). Each of these studies used a single species, did not account for compensatory traits, examined different performance traits, and differed in their methods (e.g., some examined natural variation, some surgically excised the sword). We build upon these studies by using natural variation of the sword to determine the correlated evolution of sword length and aerobic performance. Specifically, using 17 species of *Xiphophorus* and 2 species of *Priapella* (their sister lineage) we examined the effect of the sword on critical swimming speed (U_{crit}), a

standard measure of a fish's aerobic endurance capacity (Brett 1964, Beamish 1978, Plaut 2001). To account for compensatory traits that may have evolved to offset the cost of the sword, we examined several traits that may contribute to aerobic performance: heart and gill mass, maximum oxygen consumption, and muscle citrate synthase (CS) activity. We also measured lactate dehydrogenase activity (LDH), an indicator of anaerobic metabolic capacity, because the fish often exhibit burst/coast performance when they are reaching fatigue near the end of critical swimming speed trials. We hypothesized that species with enlarged swords may have evolved an increase in any one of these traits (or a combination) to offset the locomotor cost of the sword. Ignoring these traits may mask the costliness of the sword on locomotor abilities (Oufiero & Garland 2007). Finally, we examined the relationships of these traits in females, including reproductive traits that may have a negative impact on locomotor performance (Plaut 2002, Ghalambor *et al.* 2004).

3.3 Methods and Materials

3.3.1 ANIMAL CARE AND HUSBANDRY

We used a total of 579 male and female fish in this study (Fig. 3.1, Appendix 3.1). Several adult mating pairs for each species were obtained from various laboratory populations (e.g., The *Xiphophorus* Genetic Stock Center, http://www.xiphophorus.txstate.edu/, see Appendix 3.1) and bred to obtain F1s and F2s for analyses. Species were reared separately under common garden conditions (Garland & Adolph 1991). When born, babies were isolated from parent fish (to avoid cannibalism) and reared in 19, 38 or 151 liter aquaria (depending on the size of the fish). F1 and F2 fish were fed brine shrimp, liver paste, freeze-dried bloodworms, and flake

food *ad libitum* throughout development. At the first sign of maturation (gravid spot in females and the development of the gonopodium in males) the sexes were separated. During testing, fish were housed individually in 9 liter aquaria and fed *ad libitum*. Approximately 14 male and 14 female fish, chosen at random, were tested from each species (Appendix 3.1). All fish from a species were tested at various times, and often several species were tested at once to minimize any effects of time of year. Fish were allowed to grow after maturation for up to six months, to reach larger sizes and (for the males) to adequately grow the sword. When males mature they develop their gonopodium first, after which the sword grows over a span of several weeks/months (Marcus & McCune 1999). Although it is not known if the sword grows indeterminately, most species exhibited fully developed swords within the six months. Prior to critical swimming speed and VO_{2max} measures, fish were starved 24 hours.

When fish had matured, and males grown their sword adequately, they were tested in the same order for performance measures. Sprint speed was tested first, followed by critical swimming speed several days later, maximum oxygen consumption was tested after an additional several days, and c-start was measured last. Fish were tested in batches of 20-30 individuals of mixed sex and species. After all individuals in a given batch were measured for performance, a live, digital photo was taken of them. The following day the fish were euthanized and size traits measured (see below and Fig. 0.3 in the Introduction to this dissertation).

3.3.2 SPRINT SPEED

Sprint speed was measured using the experimental apparatus and methods outlined in Oufiero *et al.* (2010). Fish were chased along a ‘racetrack’ lined with photocells linked to a computer that recorded maximum speed. Fish were acclimated for

15 min. to the entire racetrack, which measured 91cm (L) X 5.5cm (W) X 15.8cm (H). Twelve pairs of photocells (LS10 light screen, Banner Engineering Corp., Minneapolis, MN USA), each 5 cm apart, spanned the mid 60 cm of the racetrack beginning 18 cm from the starting side to allow the fish room to accelerate. Aquatic plants and pieces of clay pots were placed opposite the start side to offer a refuge towards which the fish would sprint. After the acclimation, fish were gently herded towards the start end of the racetrack, and then startled by hitting a small net directly behind the fish against the bottom of the track (the net did not make contact with the fish). If a fish did not sprint all the way to the refuge, then the net was lifted from the water (to avoid triggering the photocells) and again hit against the bottom of the track behind the fish. The entire procedure was repeated 3-4 times per individual, with ~30 seconds between trials. As the fish sprinted along the track it broke the infrared light beams emitted from the photocells and the timing of the break of the beams was recorded by custom computer software (Serrace.exe, T. Garland and P. E. Midford). The program calculated maximum sprint speed. We used the single highest speed attained over 10 cm (over a total of 3 consecutive photocells) from any one of the 3-4 trials as a measure of a fish's maximum sprint speed. Sprint speed was measured at room temperature and recorded for each trial. The average temperature was 23.0758 ± 1.03 S.D. with a range of 20.3 – 26.1 C. Mean values for sprint speed by sex and species are presented below for completeness, but are not analyzed in this chapter.

3.3.3 CRITICAL SWIMMING SPEED (U_{CRIT})

Critical swimming speed is defined as the maximum velocity a fish maintains for a specific period of time, ending in fatigue (Brett 1964, Beamish 1978). This measure of

swimming is similar to endurance measures often performed with terrestrial vertebrates (Garland 2010) and is characterized by a longer swimming period (i.e., > 2 sec.) and more aerobic respiration compared to burst swimming (Brett 1964, Beamish 1978, Hammer 1995, Reidy *et al.* 2000, Plaut 2001). Critical swimming speed is measured by ramping the velocity of a flow tunnel by a certain speed increment over a pre-determined time interval (e.g., 10 cms^{-1}/5min.). It is calculated using the following formula:

$$U_{crit} = U_i + [U_{ii} (T_i/T_{ii})],$$

where U_i is the highest velocity maintained for the whole interval (cms^{-1}), U_{ii} is the velocity increment (cms^{-1}), T_i is the time elapsed at fatigue velocity (min.), and T_{ii} is the prescribed time interval (min.) (Beamish 1978, Plaut 2001).

The setup and methods used for critical swimming speed measures were the same as those used in Oufiero and Garland (2009) and Oufiero *et al.* (2010). Fish were acclimated to a flow tunnel for 15 minutes at a low flow velocity (~3 cm s^{-1}) to orient them to the direction of flow. The flow tunnel holds approximately 55 L of water and the working area is 119.5 cm (L) X 15.3 cm (W) X 18.3 cm (H). The fish was tested in a smaller area [12 cm (L) X 15.3 cm (W) X 11.5 cm (H)] within grating, which also served to attain laminar flow. The velocity of the flow tunnel was measured and calibrated by filming a piece of cotton (a neutrally buoyant particle) at 500 frames per second three times at each speed on the controller. The velocity of the cotton was averaged for each speed on the controller; a regression was then used to predict the speed of the flow. Every five increments on the flow controller resulted in a water velocity increase of 4.5 cm s^{-1}. After the 15-minute acclimation the flow velocity was increased 4.5 cm s^{-1} every 5 minutes until the fish could no longer maintain position and was pressed up against the back grating. A fish was considered exhausted when it did not remove itself from the

grating after tapping on the sides of the tunnel and gently prodding the fish with a net. Critical swimming speed (Appendices 3.3 & 3.4) was measured at room temperature, which was recorded for each trial and averaged 23.024^{o}C ± 0.873 S.D., range 20.0-25.3 ^{o}C. Temperature did not significantly affect U_{crit} after taking into account size, sex, and species of the fish, so it was not included in the final statistical analyses (Temperature: $F_{1, 544} = 3.008$, $p = 0.083$; Standard length: $F_{1, 544} = 0.998$, $p = 0.318$; Sex: $F_{1, 544} = 0.003$; Species: $F_{20,544} = 21.095$, $p < 0.001$).

3.3.4 MAXIMUM OXYGEN CONSUMPTION (VO_{2MAX})

Maximum oxygen consumption (VO_{2max}, Appendices 3.3 & 3.4)) was measured through forced exercise in a closed system (Figure 3.2), similar to methods outlined in Chappell and Odell (2004). Two systems were used depending on the size of the fish. Each had a cylindrical sealed respirometer inside a larger square container connected to a circulating water bath to maintain a constant temperature of 23° C. The larger system measured 19.2 X 19.2 cm with a 9.5 cm diameter x ~ 4 cm high cylindrical chamber in the center (250 ml volume). The smaller system measured 18.3 X 19 cm, with a 6.2 cm diameter by ~4 cm high cylindrical chamber (150 ml volume). The cylindrical respirometer chambers were constructed out of clear plexi-glass.

To force fish to swim, a stir bar was placed in the bottom of the circular chamber, with mesh grating above it to prevent the fish from making contact with the stirbar. The chamber was placed on a magnetic stirrer with adjustable rotation speed. A lid with an oxygen microelectrode in the center (model 1302, Strathkelvin Instruments, Glasgow, Scotland) kept the fish out of the center of the chamber and allowed the microelectrode to measure well-mixed water. The microelectrode was connected to an oxygen meter

(model 781b, Strathkelvin Instruments, Glasgow, Scotland) and oxygen saturation (as percent) was recorded throughout the trial using Warthog Systems (LabHelper, http://warthog.ucr.edu/). Between trials an aerated stone was placed inside the respirometer to saturate the water with oxygen. The electrode was calibrated daily to 100% saturated oxygen levels by allowing an air stone to run for ~30 minutes inside the respirometer chamber. After this time, the air stone was removed, the respirometer was sealed, and a blank run recorded to ensure the percent saturation of oxygen inside the respirometer was 100%. Every few weeks the electrode was calibrated to zero by adding sodium bisulfite, which removes oxygen from the water (Duxbury 1963).

Fish were acclimated for 15 minutes in the respirometer. After acclimation, the fish was sealed in the respiroemter for closed system measurements, and the trial began. For the smaller respirometer, the speed was increased 0.5 units on the stir bar speed dial every 3 minutes; the larger chamber was increased 1 unit every 3 minutes. The difference in these increments reflected the size of the fish being tested, and ensured the fish would reach fatigue. A fish was considered fatigued when it no longer maintained its position in the respirometer and was carried by the flow of the water at least three consecutive turns. To ensure that this method of maximum oxygen consumption was eliciting a repeatable value (e.g., see Oufiero & Garland 2009; Garland 2010), a subset of 20 male *X. variatus* were tested for their repeatability over the span of a day and a week using Pearson's correlation coefficient with a two-tailed significance level of $p < 0.05$. The maximum oxygen consumption of the fish was significantly repeatable over the span of a day (Pearson's $r = 0.772$, $p < 0.001$) and over the span of a week (Pearson's $r = 0.779$, $p < 0.001$).

The maximum oxygen consumption was obtained from the recorded trials using Warthog Analyst software (LabAnalyst, http://warthog.ucr.edu/). Each file was smoothed using a 5 sample and 6 cycle filter. The files were then inverted by subtracting from 100 (initial percentage of oxygen in the water) to obtain the percent oxygen consumed. The derivative of the change in oxygen saturation was taken to obtain the rate of oxygen consumption (VO_2; µl O_2 m^{-1}) throughout the trial, taking into account the temperature and volume of water in the respirometer. Once VO_2 had been obtained, the highest continuous 60 second average throughout the trial was found and used as an individual's maximum oxygen consumption (VO_{2max}).

3.3.5 BODY SIZE AND SUBORGANISMAL TRAITS

When U_{crit} and VO_{2max} trials were complete, fish were euthanized in a lethal dose of MS-222 (Tricaine Methanesulfonate) and measured for several size traits (see Appendices 3.5 & 3.6): Body mass (to the nearest 0.001 g), total length (TL: from tip of snout to end of the caudal fin, to the nearest 0.01 mm), standard length (SL: from the most anterior portion to the insertion [base] of the caudal fin, to the nearest 0.01 mm), and body depth (BD: at the insertion of the dorsal fin, to the nearest 0.01 mm). Sword length was measured (to the nearest 0.01mm) from the most posterior edge of the caudal fin (the base of the sword) to the tip of the sword. Caudal fin length was calculated as TL-SL. The fish was then dissected from the anterior insertion of the anal fin to the anterior insertion of the dorsal fin to separate the caudal peduncle. The posterior section (caudal peduncle) was weighed to the nearest 0.001g, flash frozen in liquid nitrogen, and stored at -80°C until enzyme assays (see below). The anterior portion of the fish was preserved in 5% formalin. At a later date, the hearts and gills were dissected from all

fish, and reproductive organs from a subset of females (see Appenices 3.7 and 3.8). The entire heart was removed, blotted dry, and weighed to the nearest 0.01 mg; the gills were separated from surrounding cartilage, ensuring only gill tissue was weighed, blotted dry, and weighed to the nearest 0.01 mg. For females, the ovaries were removed, blotted dry, and weighed to the nearest 0.01 mg. Additionally, regressors (unfertilized eggs) were counted, dried for 24 hours, and weighed to the nearest 0.01 mg (Appendices 3.7 & 3.8). Almost all of the females used in the study were virgins, with a few exceptions (<5%). These three reproductive traits were all highly correlated with each other (see below); therefore, in final multiple regression analyses we only used regressor dry mass, as it is the closest approximation to how much the females are allocating to reproduction.

3.3.6 CITRATE SYNTHASE AND LACTATE DEHYDROGENASE

Citrate synthase (CS) and lactate dehydrogenase (LDH) activity were measured using a thermostatted SPECTRAmax Plus microplate spectrophotometer (Molecular Devices Corporation, Sunnyvale, CA) on a subsample of fish (~10 males and 10 females from each species). A kinetic assay was used for both, obtaining the V_{max} of enzymatic activity, following methods outlined in Odell, Chappell & Dickson (2003). The entire tail muscle for each fish was homogenized in a buffer solution (50mM Imidazole, 2mM EDTA [(Ethylenedinitrilo)tetraacetic acid], pH 6.6. at 20°C) equal to one part muscle mass to 9 parts homogenate buffer (1 in 10 dilution), using a tissue-tearor (Biospec Products, Bartlesville, OK). After homogenization, samples were centrifuged (Allied Fisher Scientific micro-centrifuge model 235C) for 10 minutes at 12,400 rpm in an incubator set at 1.5^{o}C (Precision low temperature illuminated incubator model 818).

After 10 minutes of centrifuging, 1 ml of the supernatant was removed and used for assays.

A total of 40 microplate wells (out of 96) were run at a time for both CS and LDH assays. Each sample was measured in triplicate with a control. This allowed for the measurement of 10 samples at once. Samples were sorted by size of the tail muscle; therefore, each microplate contained an approximately random assortment of species and sexes. Citrate synthase activity was measured at a wavelength of 412 nm, with 10 µl of tissue supernatant added to 100 µl of assay mixture. The assay mixture was made up of 0.8 µl of 400 mM $MgCl_2$, 8 µl of 2.0 mM DTNB, 10 µl 1.6 mM acetyl CoA, and 81.2 µl of 80 mM Tris Buffer (pH 8.0 at 20°C); all chemicals were mixed in 80 mM Tris buffer. After the assay mixture and tissue supernatants had been added to each well, 50 µl of 1.6 mM oxaloacetate was added to each (for the control wells, 50 µl of 80 mM Tris buffer was added), resulting in 160 µl in each well. This resulted in final concentrations of 2.0 mM $MgCl_2$, 0.1 mM DTNB, 0.1 mM acetyl CoA, and 80 mM Tris buffer. After the oxaloacetate had been added, the absorbance was recorded at 23°C (to match the temperature of the performance trials). The spectrophotometer cycled through reading 8 wells of the microplate at a time for 7 minutes, with a 4 second interval between each read at a wavelength of 412 nm. V_{max} was recorded for each sample, obtained as the slope of the line of time versus optical density for the first ten points of the line and was expressed as milli-units min^{-1}.

Similar methods were used to measure LDH activity. Because LDH is a faster enzymatic reaction, the tissue supernatant was further diluted to a 1 in 100 dilution in homogenate buffer solution. Ten µl of diluted supernatant was added to 100 µl of assay mixture containing 15 µl of 3.2 mM NADH, 32 µl of 500 mM KCl (both dissolved in 50

mM Imidazole, pH7.0 at 20°C), and 53 µl of 50 mM Imidazole. Fifty µl of 3.2 mM pyruvate was added to the mixture as the substrate, initiating the reaction. This resulted in final concentrations of 0.32 mM NADH, 100 mM KCL, 50 mM imidazole and 1.0 mM pyruvate in each well. The reactions were measured at 23°C for seven minutes, with 4 second interval between readings, at a wavelength of 340 nm. V_{max} was obtained as described above.

For citrate synthase and lactate dehydrogenase V_{max}, we corrected for the control and the assay batch (Appendix 3.3). Two batches were run on a given day; because chemicals were mixed fresh each day, there may have been slight differences in their amounts, leading to batch effects. We first obtained the mean and coefficient of variation (CV) for the triplicates. If the CV was greater than 10%, then we removed the V_{max} that was the most different. If all three were different, we took the value in the middle (however, this was the case for < 4% of the LDH V_{max} values and < 1% of the CS V_{max} values). We then subtracted the control V_{max} from the mean for each sample. We then corrected for batch effect by obtaining least squares means from SAS v9.2 Procedure Mixed with sex, species, and the sex*species interaction as fixed factors and batch as a random factor. We used the least squares means for each sex from each species in final analyses comparing species. Because LDH V_{max} is negative (V_{max} for LDH is expressed negatively since the reaction changes from a colored to a colorless solution), we reversed the sign so the values are positive.

3.3.7 PHYLOGENY

Genomic DNA was extracted following Meredith *et al.* (2010). Portions of seven nuclear gene regions were amplified, cleaned, and sequenced following the protocols outlined in

Meredith *et al.* (2010): (1) 5 partial exons (8 and 10), all of exon 9, and two introns (8 and 9) of the tyrosine kinase gene (X-src); (2) exon 1 of myosin, heavy polypeptide 6 (myh6); (3) exon 2 of ectodermal-neural cortex 1 like protein (ENC1); (4) exon 2 of glycosyltransferase (Glyt); (5) exon 1 of SH3 and PX domain containing 3 (SH3PX3); (6) a portion of the 7 transmembrane receptor region of rhodopsin (Rh); and (7) exon 3 of recombination activating gene-1 (Rag1). These gene segments were combined with the nuclear markers D2, D8, D29, and T36 (Meyer, Salzburger & Schartl 2006) resulting in a concatenated alignment of 7872 bp (after removal of alignment ambiguous). The dataset was partitioned into 12 segments (one for coding sequences from each of the seven different genes, D2, D8, D29, T36, and one for X-src introns). RAxML, randomized accelerated maximum likelihoods (Stamatakis 2006), was used to estimate the phylogeny and branch lengths (GTRGAMMAI model, 500 bootstrapped replicates, randomized maximum parsimony starting trees (which are used to estimate the maximum likelihood trees), and the fast hill-climbing algorithm with all other free parameters estimated).

After obtaining the phylogenetic hypothesis of *Xiphophorus*, we modified the tree in two ways (Figure 3.1, Appendix 3.2). First, because our two populations of *X. corezi* had no detectable genetic differences between them, resulting in branch lengths of zero, we only used the population from the *Xiphophorus* stock center (Appendix 3.1), which had the larger sample size. Second, because one of our species is a potential hybrid (what we are calling *X. multi-cortezi*), we added this species as a sister to *X. cortezi*, because of its morphological similarity, with branch lengths equal to those of the *X. cortezi* lineage. Finally, because we only had one male and one female from *X. continens*, we did not include this species in the final analyses.

3.3.8 STATISTICAL ANALYSIS

We examined the effect of sword length and other potential predictors (standard length, VO_{2max}, heart mass, gill mass, CS V_{max}, LDH V_{max}, tail mass, caudal fin length, body depth; and regressor dry weight for females) of critical swimming speed in both males and females, non-phylogenetically and phylogenetically. We first obtained mean values and standard errors for each sex within a species (Appendices 3.3 - 3.8). We also obtained the mean and standard error of $\log_{10}$-transformed values for each sex within a species for all traits except CS and LDH. Because sword length was zero for some individuals and species, we used the $\log_{10}$ of sword length +1; because some females did not have reproductive traits we used $\log_{10}$(ovary mass + 0.0001), $\log_{10}$(regressor number +1), and the $\log_{10}$(regressor dry mass +0.0001). We used these mean values for tests of phylogenetic signal (Blomberg, Garland & Ives 2003) and correlations of whole-organism traits. Phylogenetically informed correlations were performed on standardized independent contrasts of the traits obtained from PDTREE (Garland, Midford & Ives 1999, Garland & Ives 2000), with the correlation computed through the origin (no intercept).

Because body size may affect most if not all traits used in this study, we examined allometric equations for each trait in both males and females. Although all of our size traits were significantly positively correlated, we used $\log_{10}$ standard length as an index of size. Body mass may have been more appropriate for allometric relationships with mass measures, but because we were interested in the effects of sword length, which is a linear measure, we used standard length for all traits. We used the Matlab Regressionv2.m program (Lavin *et al.* 2008) to obtain the slope, intercept, and 95% confidence intervals (from parametric bootstrapping) for each allometric

equation under three models of evolution: non-phylogenetic (Ordinary Least Squares or OLS; equivalent to using a star phylogeny), phylogenetic generalized least squares (PGLS) with our branch lengths (as shown in Fig. 3.1 and presented in Appendix 3.2), and a phylogenetic regression model that uses an Ornstein-Uhlenbeck transformation to model the residuals as subject to stabilizing selection (RegOU, with REML estimation of d and the regression parameters; see Lavin *et al.* 2008).

To correct the traits for associations with body size, we obtained residuals from regressions of the trait ($\log_{10}$-transformed for all traits except CS and LDH) on $\log_{10}$ standard length. This was first done with conventional OLS regressions, i.e., non-phylogenetically. Heart mass and gill mass residuals were obtained from a multiple regression with preservation time included as an additional independent variable. These residuals were used for bivariate correlations and multiple regression analyses.

To obtain phylogenetically corrected residuals (reviewed in Revell 2009), we used PDTREE (Garland *et al.* 1999, Garland & Ives 2000) to obtain phylogenetically independent contrasts (Felsenstein 1985, Garland, Harvey & Ives 1992). We regressed the standardized independent contrast for each trait on the standardized independent contrasts for standard length, through the origin (Garland *et al.* 1992), and obtained the residuals. Heart mass and gill mass were again regressed on both standard length and preservation time, with the values for preservation time contrasts computed on a star phylogeny that had been rescaled to have a height of unity. This was done because preservation time is a nuisance variable that is not phylogenetically inherited (Wolf, Garland Jr & Griffith 1998, Perry & Garland Jr 2002). These phylogenetically corrected residuals were then used to examine the correlations between pairs of traits (through the origin) and potential predictors of critical swimming speed in multiple regression

analyses (through the origin). We also examined size-corrected trait values for phylogenetic signal following Blomberg *et al.* (2003), by using the “residuals” obtained from the fifth column of the RSD file produced by PDTREE (for heart and gill mass, these computations do not account for possible associations with preservation time).

We examined several multiple regression models in males and females separately to determine the effect of the sword on critical swimming speed, while also accounting for possible effects of other potential predictors. All of these regressions used residuals, as described above. We first examined a ‘physiological’ model with sword length, VO_{2max}, heart mass, gill mass, CS, and LDH as predictors, both non-phylogenetically and phylogenetically. We next examined a ‘morphological’ model with sword length, body depth, tail mass, and caudal fin length as potential predictors of critical swimming speed. Thirdly, we examined a 'full' model with all traits included. Finally, we used backwards stepwise regression with the 'full' model. Therefore, in each model sword length is included to determine if it has a negative effect (two species of females had minimal swords therefore sword length is also included in female models), as well a set of candidate compensatory traits. Furthermore, for females, residual $\log_{10}$ regressor dry mass was included in all models to determine if amount of reproductive allocation had a negative impact on critical swimming speed. Multiple regression analyses with residual trait values are a similar approach to the path analysis in Oufiero and Garland (2007), where body size has a direct effect on all traits. All phylogenetic multiple regressions were calculated through the origin.

We also examined these same multiple regression models, but with VO_{2max} as the dependent variable, and the same set of independent variables. These multiple regressions were also performed on males and females separately, non-phylogenetically

and phylogenetically through the origin. All correlation and multiple regression analyses were performed with SPSS v11.5.

3.4 Results

Mean U_{crit} showed considerable variation among species, ranging from 14.5 to 35.6 cm s^{-1} for males and from 14.8 to 37.2 cm s^{-1} for females (Fig. 3.3A, Appendix 3.3). In males, this interspecific variation in U_{crit} (log-transformed) was only weakly positively related to $\log_{10}$ standard length (Fig. 3.4A). None of the allometric models showed a statistically significant slope (Table 3.7), and r^2 values were small (OLS 7.6%, PGLS 3.8%, RegOU 4.5%). In females (Figs. 3.5A, 3.6A), the OLS allometric model indicated a significant positive slope for U_{crit}, but the PGLS and RegOU models did not (Table 3.8; see also below 3.4.3 ALLOMETRIC EQUATIONS).

Considering the 13 of 19 species that exhibited a sword in males, relative sword length ranged from 7% of standard length in *X. pygmaeus* to 55% in *X. helleri* (Appendix 3.9). Bivariate scatterplots suggest a generally positive relation between sword length and standard length (Figs. 3.3C, 3.4C), but these plots are misleading. The apparently positive relation is driven by the fact that species lacking swords are mostly small in body size (see Appendix 3.5), and restricted to two lineages (clades), i.e., the two species of *Priapella* and the four *Xiphophorus* species at the bottom of the phylogeny shown in Figure 3.1. As a result of this "grade shift" (see also below 3.4.3 ALLOMETRIC EQUATIONS), the OLS allometric model erroneously suggests a positive relation between log sword length and log standard length, whereas the PGLS and RegOU models indicate no statistically significant relation (Table 3.7).

3.4.1 PHYLOGENETIC SIGNAL

Males

Body mass, standard length, and most size-corrected traits exhibited significant phylogenetic signal, with several exceptions (Table 3.1). Log_{10} maximum oxygen consumption and heart mass did not show phylogenetic signal, nor did their size-corrected values. Citrate synthase did not have significant phylogenetic signal, although LDH did for both raw and size-corrected values. The diagnostic r was not significant for size or for any size-corrected trait, suggesting that contrasts are adequately standardized. However, many of the correlations were in the range of -0.3 to -0.4, and statistical power to reject the null hypothesis is somewhat limited with only 19 species.

Females

For females, there were more traits that did not exhibit phylogenetic signal compared to the traits in males (Table 3.2). Similar to males, size-corrected heart mass and VO_{2max} did not exhibit phylogenetic signal (although their non-size-corrected values did: Table 3.2). Gill mass and both enzyme activities did not exhibit phylogenetic signal, either correcting for size or not. Finally, both body mass and tail mass exhibited significant phylogenetic signal for log_{10} transformed values, but not for size-corrected values.

3.4.2 WHOLE-ORGANISM CORRELATIONS

Males

Non-phylogenetic (Table 3.3) and phylogenetic (Table 3.4) correlations among traits for males were similar. Standard length was significantly correlated with most traits (Fig. 3.3 & 3.4), except U_{crit}, CS, and LDH non-phylogenetically (and sword phylogenetically).

Sword length was significantly positively correlated with heart mass, gill mass, and VO_{2max} non-phylogenetically, but was not correlated with any trait phylogenetically. Critical swimming speed was not correlated with any trait non-phylogenetically or phylogenetically. Most size traits were significantly positively correlated non-phylogenetically and phylogenetically. Finally, CS and LDH were not significantly correlated with any other trait except each other non-phylogenetically.

Females

For females, similar results were obtained when correlating whole-organismal traits non-phylogenetically and phylogenetically (Tables 3.5 & 3.6). Standard length was significantly correlated with most traits except gill mass, CS, LDH, and sword length non-phylogenetically; and U_{crit}, gill mass, CS, LDH and sword length phylogenetically (Fig. 3.5 & 3.6). Critical swimming speed was correlated with VO_{2max}, heart mass, body mass, tail mass, and body depth non-phylogenetically, but was not correlated with any trait phylogenetically. VO_{2max} was significantly correlated with all size traits non-phylogenetically and phylogenetically; most size traits were significantly correlated non-phylogenetically and phylogenetically, and CS and LDH were not correlated with any other traits either non-phylogenetically or phylogenetically.

The reproductive traits of the females were all highly correlated with each other non-phylogenetically ($\log_{10}$ ovary mass and $\log_{10}$ regressor number: $r = 0.913$, $p < 0.001$; $\log_{10}$ ovary mass and $\log_{10}$ regressor dry mass: $r = 0.977$, $p < 0.001$; $\log_{10}$ regressor number and $\log_{10}$ regressor dry mass: $r = 0.907$, $p < 0.001$). However, they were not correlated with any other traits. Phylogenetically, all three reproductive traits were again highly correlated with each other ($\log_{10}$ ovary mass and $\log_{10}$ regressor number: r =

0.958, $p < 0.001$; $\log_{10}$ ovary mass and $\log_{10}$ regressor dry mass: $r = 0.983$, $p < 0.001$; $\log_{10}$ regressor number and $\log_{10}$ regressor dry mass: $r = 0.941$, $p < 0.001$). However, they were not significantly correlated to any other trait ($p > 0.05$).

3.4.3 ALLOMETRIC EQUATIONS

Males

As can be seen by comparing scatterplots for raw values (Figure 3.3) with those for log-transformed values (Figure 3.4), the latter generally provided better homoscedasticity and linearity. Allometric equations were similar among the three models (OLS, PGLS, and RegOU) for all traits except sword length (Table 3.7). For sword length, the slope changed from 4.17 under the OLS model, where it was significantly different from the value of 1.00, to 0.83 and 1.12 under the PGLS and RegOU models, respectively, where it was not significantly different from 1.00. Further analyses indicated that the "grade shift" (e.g., see Garland *et al.* 2005) in sword length was responsible for the discrepancy between the OLS and phylogenetic models, i.e., some entire lineages have swords whereas others do not (Fig. 3.1). Adding a Clade variable (1 = platyfish, 2 = Northern swordtails, 3 = Southern swordtails, 4 = *Priapella*) significantly improved the OLS model (ln ML Likelihood increased from = -6.00 to 1.31, ln likelihood ratio test $chi^2 = 14.63$, d.f. = 3, P = 0.002; partial F for Clade = 5.41, d.f. = 3, 14, P = 0.011) and changed the slope to 1.21 (95% bootstrap C.I. = -1.38 to 3.86).

The linear measurements (caudal fin length and body depth) had slopes around 1 and mass measurements scaled with slopes around 3, consistent with isometry (Fig. 3.3 & 3.4). Based on an ln likelihood ratio test the RegOU model was significantly better than the OLS model for gill mass, LDH, body mass and body depth. In all the remaining

traits except sword length and U_{crit}, the RegOU model was significantly better than the PGLS. For sword length and U_{crit}, the RegOU model was not significantly better than the OLS or PGLS models. For most traits (except gill mass, body mass and body depth) the OLS and RegOU models had higher likelihoods than the PGLS model.

Females

Allometric equations for females were similar to those of the males (Table 3.8). Most traits had similar slopes and intercepts for all three statistical models, except for U_{crit}. Under the OLS model, the slope for U_{crit} was 0.88 and significantly different from zero, whereas the slope under the PGLS model was -0.12 and under the RegOU 0.16, both statistically non-significant. Furthermore, there was no significant difference between the RegOU model and the OLS or PGLS models. Addition of a Clade variable (see above) to the OLS model did not eliminate the discrepancy.

Linear measurements (caudal fin length and body depth) scaled with standard length with a slope around 1, similar to males, while most mass measurements scaled with standard length with a slope around 3 (Fig. 3.5 & 3.6). Interestingly, gill mass scaled with standard length with a slope closer to 1 than 3. The RegOU model was significantly better than the PGLS model for all traits except caudal fin length, where the RegOU model was significantly better than the OLS model.

3.4.4 RESIDUAL CORRELATIONS

Males

Results for the residual correlations were similar for the non-phylogenetic and phylogenetic analyses (Table 3.9 & 3.10). U_{crit} was only significantly correlated with

body depth non-phylogenetically, and was not significantly related to any other traits phylogenetically (Fig. 3.7). Sword length was significantly negatively related to heart and gill mass in both analyses, and was not related to any other trait. Heart and gill mass were significantly positively correlated to each other in both analyses, significantly positively related to body mass non-phylogenetically, and significantly positively related to body depth non-phylogenetically and phylogenetically. Finally, VO_{2max} was not significantly related to any other trait either non- or phylogenetically.

Females

Correlations of residual trait values for the females were similar non-phylogenetically and phylogenetically (Tables 3.11 & 3.12), and slightly different than male correlations of residual trait values. U_{crit} (Fig. 3.8) and VO_{2max} were not significantly related to any other trait under either analysis. Heart mass and gill mass were significantly positively related to each other (but not to any other traits), as were several of the size measurements, under both analyses. In the phylogenetic analysis, CS was significantly positively related to LDH and body mass, but was not significantly related to any other traits non-phylogenetically.

The residuals of the reproductive traits were all positively correlated to each other (residual $\log_{10}$ ovary mass and residual $\log_{10}$ regressor number $r = 0.923$, $p < 0.001$; residual $\log_{10}$ ovary mass and residual $\log_{10}$ regressor dry mass $r = 0.984$, $p < 0.001$; residual $\log_{10}$ regressor number and residual $\log_{10}$ regressor dry mass $r = 0.948$, $p < 0.001$). The only other significant correlation was a negative correlation between residual $\log_{10}$ ovary mass and residual $\log_{10}$ tail mass ($r = -0.487$, $p = 0.024$), but there were no other significant correlations non-phylogenetically. Phylogenetically, all three

traits were again significantly positively correlated with each other (residual $\log_{10}$ ovary mass and residual $\log_{10}$ regressor number $r = 0.972$, $p < 0.001$; residual $\log_{10}$ ovary mass and residual $\log_{10}$ regressor dry mass $r = 0.987$, $p < 0.001$; residual $\log_{10}$ regressor number and residual $\log_{10}$ regressor dry mass $r = 0.975$, $p < 0.001$), but were not significantly correlated to any other traits.

3.4.5 MULTIPLE REGRESSIONS: CRITICAL SWIMMING SPEED (U_{CRIT})

Males

Under non-phylogenetic analyses, the only trait that was a significant predictor of residual $\log_{10}$ U_{crit} was residual $\log_{10}$ body depth (Table 3.13). None of the physiological predictors were significant, and the backwards stepwise regression resulted in a model that included only body depth. Phylogenetic analyses produced different results (Table 3.14). There were no significant predictors in the physiological, morphological or full models. However, backwards stepwise regression revealed a model where residual $\log_{10}$ sword length and heart mass had significant positive effects on residual $\log_{10}$ U_{crit}. When the best model from the non-phylogenetic analysis was analyzed phylogenetically, body depth did not have a significant effect (Table 3.14). Similarly, when the best model from the phylogenetic analysis was analyzed non-phylogenetically, sword length and heart mass did not have significant effects (Table 3.13).

Females

Results for the non-phylogenetic female analyses revealed several significant predictors of residual $\log_{10}$ U_{crit} (Table 3.15). There were no significant predictors in the physiological model non-phylogenetically. In both the morphological and full model,

residual $\log_{10}$ body depth had a significant positive effect on residual $\log_{10}$ U_{crit}, whereas residual $\log_{10}$ tail mass had a significant negative effect. Residual $\log_{10}$ regressor dry mass was a significant negative predictor in the morphological model only. The significant model from the backwards stepwise regression is shown in Table 3.15. In this model regressor dry mass and tail mass had significant negative effects on residual $\log_{10}$ U_{crit}, whereas body depth and sword length had a significant positive effects.

Phylogenetic results for the females were similar to the non-phylogenetic female results (Table 3.16). The main difference in the phylogentic models was that regressor dry mass was not a significant predictor. There were no significant predictors in the physiological or full modes; however, body depth had a significant positive effect on U_{crit} and tail mass a significant negative effect in the morphological model (similar to non-phylogenetic analyses). Finally, the backwards stepwise regression revealed a significant model including significant effects of LDH V_{max}, tail mass and caudal fin length, and body depth as a non-significant predictor. When body depth was removed from the analysis, LDH and caudal fin length retained there significant positive effects on residual U_{crit}, but residual tail mass was no longer a significant predictor (Table 3.16). When residual tail mass is removed, the effect of LDH remains significant, but the effect of caudal fin length becomes slightly non-significant.

3.4.6 MULTIPLE REGRESSIONS: MAXIMUM OXYGEN CONSUMPTION (VO_{2MAX})

Results for both males and females, both non-phylogenetically and phylogenetically for the multiple regressions predicting residual VO_{2max} did not produce any significant models or predictors (Tables 3.17 - 3.20). Under all analyses there were no significant models from the backward stepwise regression. Neither the physiological,

morphological or full models produced significant predictors. Therefore, after size is taken into account, no traits predicted maximum oxygen consumption.

3.5 Discussion

An underlying assumption of sexual selection theory is that sexually selected traits will impose some sort of cost, reducing one or more components of fitness (e.g., survival), which will eventually limit the expression of the trait (Zahavi 1975, Smith 1976, Kirkpatrick 1982, Andersson 1994, Meade & Arnold 2004). It is generally difficult to measure fitness (e.g., see McGraw & Caswell 1996) or even major components of fitness, so studies typically focus on a cost that is presumed to adversely affect fitness (Kotiaho 2001, Oufiero & Garland 2007). Following this logic, we examined the critical swimming speed of swordtail fish, based on the presumption that a reduction in this aspect of locomotor performance would result in a reduction in Darwinian fitness (Clobert *et al.* 2000, Irschick & Garland 2001, Irschick *et al.* 2008). Critical swimming speed is a measure of physiological endurance capacity, initially used in fisheries sciences (Brett 1964, Beamish 1978). It has more recently been examined in ecological and evolutionary contexts (Claireaux *et al.* 2007, Handelsman, Claireaux & Nelson 2010), with studies demonstrating that U_{crit} is repeatable (Oufiero & Garland 2009) and can diverge among populations in relation to differing selective regimes, such as predation (Oufiero *et al.* 2010). However, the fitness benefit of an increased critical swimming speed has not been demonstrated rigorously. Nevertheless, it is reasonable to assume that an increased U_{crit} among *Xiphophorus* and some poeciliids in general may have fitness benefits. First, several Poeciliids, including *Xiphophorus* and *Priapella* are found in fast-moving streams (e.g., Gordon 1953) where an increased U_{crit} may be

beneficial. Second, many *Xiphophorus* males will actively court females with complex maneuvers, fin displays, and chasing (Bisazza 1993). An increased U_{crit} may give males an advantage by allowing them to exhibit increased courtship displays (Basolo & Alcaraz 2003). Finally, several species of *Xiphophorus* engage in male-male interactions (Morris, Batra & Ryan 1992), and an increased U_{crit} (or a related endurance component) may allow one male to outcompete another, although to date no studies of fish have examined if an increase in aerobic locomotor performance correlates with mating and/or intra-specific competitive success (cf. Chappell *et al.* 1997, Perry *et al.* 2004, and references therein).

Previous studies have suggested or hypothesized that elongated swords reduce locomotor performance in *Xiphophorus* (Ryan 1988, Royle *et al.* 2006, Kruesi & Alcaraz 2007). Our results suggest that sword length has a positive effect on critical swimming speed when phylogenetic relationships, body size, and some possible compensatory traits are taken into account (Table 3.14). Reconciling these results with classical sexual selection theory is somewhat challenging. Many of the original hypotheses for the evolution of sexually selected traits were formulated intraspecifically (e.g., how the trait evolves among males within a species). For example, the handicap principle (Zahavi 1975, Smith 1976) was developed to explain the benefits of mating with a male exhibiting a trait that potentially reduces aspects of fitness. Under this model the sexually selected trait provides information to the female about the male's quality, and is preferred. A male with an exaggerated trait must have good genes or be in good condition to survive with the handicap.

In our study, an interspecific comparison of mean values for males suggests that the sexually selected sword is not a locomotor handicap. In fact, species with longer

swords tended to have greater critical swimming speeds (Fig. 3.3, Table 3.14). If sword length is a positive predictor of critical swimming speed interspecifically among male *Xiphophorus*, then evolutionarily the sword may in fact be an honest signal (Vanhooydonck *et al.* 2007). Few studies of fish have examined sexually selected traits interspecifically. However, a recent intraspecific study in Pacific blue-eye fish (*Pseudomugil signifier*) demonstrated that the dorsal fin has a positive effect on attractiveness and maximum swimming velocity, while the anal fin had a negative effect on maximum swim velocity (Wilson *et al.* 2010). Therefore, the dorsal fin of *P. signifier* may be an indication to the female of the male's ability to swim; similar to the interspecific effects of the sword in *Xiphophorus*. There may also be sexual selection for increased critical swimming speeds (Andersson & Iwasa 1996, Irschick *et al.* 2007, Husak & Fox 2008). Species with the sword often engage in male-male interactions and courtship displays. Selection may favor an increased critical swimming speed to enhance either of these interactions. However, no study has examined the relation of a measure of endurance to male-male interactions or courtship displays in fish (for junglefowl, see Chappell *et al.* 1997; for lizards, see Perry *et al.* 2004, and references therein).

Why might species with longer swords not have reduced critical swimming speeds? One explanation is that physiologically based compensatory traits that may affect critical swimming speed were not taken into account in the present study, such as muscle fiber type composition. Although we included several potential compensatory traits that are presumably involved in aerobic respiration, there may be other modifications of sworded males that help them compensate. For example, the shape of fish has been proposed to correlate with performance, and recent studies on *Gambusia*

have found divergence in body shape and swimming performance among populations that have experienced different predator regimes (Langerhans *et al.* 2004, Langerhans 2006, 2009). Species with elongated swords may have evolved modified body shapes, perhaps more streamlined or with an increased caudal peduncle, to compensate for the effect of the sword on performance. In fact in both males and females, residual $\log_{10}$ body depth had a consistent positive effect on critical swimming speed (Tables 3.13, 3.15, 3.16). Although this is not a measure of shape per se, it is an indication that overall shape may influence critical swimming speed. However, caution should be taken when interpreting our body depth results because apparent differences in body depth may be affected by variation in the position of the dorsal fin. Nevertheless, exclusion of shape measures may mask a negative effect of the sword. Similarly, there may be differences in the kinematics of swimming among species with varying sword length. That is, species with swords might have increased tail beat frequencies or tail beat amplitudes to compensate (Bainbridge 1958). To our knowledge, no study has examined possible kinematic differences among species in relation to a sexually selected trait in fish (but see Ribak *et al.* 2009).

The discrepancy in the existing intraspecific studies examining the effect of sword length on swimming performance may be the result of a difference in methods. Previous results on the effects of natural variation in sword length on endurance in *X. nigrensis* showed no statistically significant effect of sword length (Ryan 1988). Using five fish from three different size classes (large, intermediate and small) Ryan swam fish at 3.92 cm s^{-1} until the fish could not right itself. He found that size was the best predictor of endurance, and sword length had no effect (Ryan 1988). Other studies have surgically removed the sword and indicated a cost on other performance measures intra-

specifically (Basolo & Alcaraz 2003, Kruesi & Alcaraz 2007). For example, Kruesi and Alcaraz (2007) surgically removed the sword and found a 21% increase in U_{crit} when the sword was removed. However, backwards stepwise regressions including sword measurements and body size measurements within each group (intact and excised sword) found that none of the sword measures explained variation in U_{crit}. Furthermore, analysis of residuals did not reveal any significant effect of the sword (Kruesi & Alcaraz 2007). Therefore, the lack of an effect of natural variation may represent the fact that males develop the sword during maturation, and therefore 'adapt' (phenotypic plasticity, e.g., see Garland and Kelly 2006) to the structure. If the sword is experimentally reduced, then the males may swim better because they are adapted to swimming with the elongated structure (e.g., increased tail beat frequency).

Although the sword may not be costly to aerobic locomotion interspecifically, it may be costly via increased conspicuousness to predators, thereby imposing a survival cost without a locomotor cost; males with the sexually selected trait may not only be more visible to females, but also to predators (Endler 1980, 1983, Rosenthal *et al.* 2001, Basolo & Wagner 2004). For example, Basolo and Wagner (2004) have demonstrated a reduction in size-corrected sword length in *X. helleri* in areas with increased predation. Therefore, the natural variation in sword length across species may be partly a result of reducing the conspicuousness of males, and not because sword length reduces critical swimming speed. Alternatively, critical swimming speed may not be as important as other aspects of locomotor performance for escaping from predators. Furthermore, one intraspecific study found a positive effect of sword length on fast-start escape response in *X. helleri* (Royle *et al.* 2006). However, no studies to date have examined differential survival costs in relation to sword length in *Xiphophorus*.

It has also been proposed that the sword evolved via sensory exploitation (Basolo 1995, 1996). If this is true, then the sword would not necessarily convey any information to the female. In this model, the sword would not necessarily be a handicap, and the observed variation in sword length among species would not be due to variation in the cost, but instead to some other factor, such as variation in female preferences for the trait (Rosenthal *et al.* 2002, Wong & Rosenthal 2006).

Previous results have suggested that compensatory traits may also evolve with the sexually selected trait to offset its cost (Kirkpatrick 1987). According to Kirkpatrick (1987, pp. 49-50), "Compensatory modifications of the males to cope with the demands of the preference, such as increased aerobic scope and enlarged musculature, will often evolve;" however he cited no empirical examples to support the conjecture. We included several traits in this study that were thought to be predictors of critical swimming speed and hence could be evolutionarily modified to compensate for sword costs. If these traits evolved to offset the cost of the sword, then they should potentially be correlated with sword length (Balmford *et al.* 1994). Sword length was significantly positively correlated with VO_{2max}, heart mass, and gill mass in the whole-organism trait correlations non-phylogenetically (Table 3.3). However, when phylogenetic relationships were accounted for, as well as body size, sword length was not significantly positively correlated with any of the potential compensatory traits (Tables 3.4, 3.9, & 3.10). In fact, sword length was significantly negatively correlated with both heart and gill mass when residuals were examined non-phylogenetically and phylogenetically (Tables 3.9 & 3.10), suggesting a potential trade-off in resource allocation to these structures. However, heart mass was a significant positive predictor of U_{crit} for males in the multiple regression analysis phylogenetically (Table 3.14). In females, more of the potential predictors of

critical swimming speed were significant, including a positive effect of body depth; but a significant negative effect of tail mass non-phylogenetically and phylogenentically (Tables 3.15 & 3.16).

We found that our index of reproductive allocation, regressor dry mass, had a significant negative effect on critical swimming speed, non-phylogenetically among females (Table 15). This is consistent with previous intraspecific results in other poeciliids (Plaut 2002, Ghalambor *et al.* 2004). These results suggest that species that allocate more to reproduction, as indicated by a greater dry mass of unfertilized eggs, have reduced critical swimming speeds. Therefore, among these species there is a greater locomotor cost due to reproduction compared to the sexually selected trait. However, this result was not supported in phylogenetic analyses (Table 16).

The sexually selected sword in *Xiphophorus* was one of the first described by Darwin (Darwin 1871), has been well studied from many disciplines, and has been assumed to impose a cost to locomotor abilities (Darwin 1871, Ryan 1988, Royle *et al.* 2006) with some empirical support (Basolo & Alcaraz 2003, Kruesi & Alcaraz 2007). Our results demonstrate that the sexually selected sword found in many (but not all) species of *Xiphophorus* is not costly for aerobic locomotor performance in males. These results suggest that the trait may, at least in some species, be an honest signal to the females of the male's ability to swim. There may also be selection on critical swimming speed to increase finding mates or matings (Andersson & Iwasa 1996). Although the sword does appear costly to aerobic locomotion, it may negatively affect anaerobic locomotion, such as sprint or burst capabilities (C.E. Oufiero unpublished data, and see Appendices 3.9 & 3.10). Future studies that examine potential costs of sexually selected traits should adopt a more integrative approach. Studies should examine several candidate traits that

may be affected by the sexually selected trait, potential traits that may help compensate, and the females as well (Oufiero & Garland 2007). This will allow a better understanding of how the trait that increases matings interacts with the rest of the organism during the evolutionary history of the species.

3.6 References

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Table 3.1. Phylogenetic signal results from PHYSIG_LL.m for males. K is the amount of phylogenetic signal for the trait using the phylogenetic hypothesis (as shown in Figure 3.1). The mean square error (MSE) is reported for both a star phylogeny and the starting phylogeny, as well as the associated ln maximum likelihoods. Significant diagnostic r for phylogenetically independent contrasts (Garland *et al.* 1992) are in bold (19 species yields 18 contrasts and hence 16 d.f. for a regression of the absolute values of the standardized contrasts on their standard deviations; the two-tailed critical value is $\pm$0.468 for p = 0.05). A significant diagnostic indicates that the contrasts are not adequately standardized from a statistical perspective, and also suggests deviation from a Brownian-motion like mode of evolution.

Males								
	PDI file	MSE tree	MSE Star	K	P phylogenetic signal	ln likelihood tree	ln likelihood Star	Diagnostic r
$\log_{10}$ Standard length	MSWsluc.PDI	0.0092	0.0095	0.3229	0.007*	18.08	17.74	-0.4457
$\log_{10}$ U_{crit}	MSWsluc.PDI	0.0101	0.0119	0.5764	0.001*	17.12	15.61	-0.0672
$\log_{10}$ s.c.$^{\&}$ U_{crit}	MSWsluc.RSD	0.0098	0.0111	0.6021	0.001*	17.49	16.28	-0.0844
$\log_{10}$ VO_{2max}	MSWslvo.PDI	0.0262	0.0191	0.2481	0.072	8.14	11.17	-0.3256
$\log_{10}$ s.c.$^{\&}$ VO_{2max}	MSWslvo.RSD	0.0064	0.0048	0.2378	0.057	21.58	24.37	-0.2843
$\log_{10}$ heart mass	MSWslhrt.PDI	0.1072	0.0924	0.2904	0.028*	-5.23	-3.82	**-0.4785**
$\log_{10}$ s.c.$^{\&}$ heart mass	MSWslhrt.RSD	0.033	0.024	0.2339	0.082	5.95	9.01	-0.4328
$\log_{10}$ gill mass	MSWslgil.PDI	0.1136	0.0887	0.3017	0.056	-5.78	-3.43	-0.4170
$\log_{10}$ s.c.$^{\&}$ gill mass	MSWslgil.RSD	0.0278	0.0427	0.5445	0.001*	7.59	3.52	-0.3461
CS V_{max}	MSWslcs.PDI	1356.59	916.85	0.1987	0.133	-94.97	-91.25	-0.2959
s.c.$^{\&}$ CS V_{max}	MSWslcs.RSD	1134.39	734.39	0.1928	0.184	-93.27	-89.14	-0.3167
LDH V_{max}	MSWslldh.PDI	789.05	760.04	0.3118	0.011*	-89.82	-89.46	-0.3124
s.c.$^{\&}$ LDH V_{max}	MSWslldh.RSD	714.18	860.56	0.4034	0.001*	-88.87	-90.64	-0.2327
$\log_{10}$ body mass	MSWslms.PDI	0.0893	0.0902	0.3294	0.006*	-3.50	-3.60	-0.3958
$\log_{10}$ s.c.$^{\&}$ body mass	MSWslms.RSD	0.0036	0.0039	0.3775	0.006*	26.88	26.03	-0.2679
sword length	Mrawslsw.PDI	47.799	63.133	0.4324	0.001*	-63.18	-65.83	0.0553
sword length/SL	Mrawswsl.PDI	0.0332	0.0383	0.3771	0.004*	5.91	4.55	0.0600
$\log_{10}$ sword length	MSWslsw.PDI	0.1233	0.2825	0.8371	<0.001*	-6.56	-14.44	0.2356
$\log_{10}$ s.c.$^{\&}$ sword length	MSWslsw.RSD	0.1169	0.2226	0.7070	<0.001*	-6.05	-12.17	0.1660
$\log_{10}$ tail mass	MSWsltms.PDI	0.0912	0.1106	0.3709	0.005*	-3.70	-5.53	-0.3018
$\log_{10}$ s.c.$^{\&}$ tail mass	MSWsltms.RDS	0.0061	0.0053	0.2576	0.025*	21.95	23.25	-0.2453
$\log_{10}$ caudal fin	MSWsltln.PDI	0.0109	0.0140	0.3890	<0.001*	16.47	14.11	-0.3578
$\log_{10}$ s.c.$^{\&}$ caudal fin	MSWsltln.RDS	0.0010	0.0008	0.2489	0.032*	39.64	41.37	-0.2550
$\log_{10}$ body depth	MSWslbd.PDI	0.0146	0.0122	0.2461	0.038*	13.69	15.42	**-0.5392**
$\log_{10}$ s.c.$^{\&}$ body depth	MSWslbd.RSD	0.0014	0.0021	0.5233	<0.001*	35.85	32.29	0.3305

$^{\&}$ size-corrected phylogenetic signal calculated from the "residuals" from PDTREE, column 5 in *.RSD file.

Table 3.1

Table 3.2. Phylogenetic signal results from PHYSIG_LL.m for females. K is the amount of phylogenetic signal for the trait using our phylogenetic hypothesis (as shown in Figure 3.1). The mean square error (MSE) is reported for both a star phylogeny and our starting phylogeny, as well as the ln likelihoods. Significant diagnostic r for phylogenetically independent contrasts (Garland *et al.* 1992) are in bold (19 species yields 18 contrasts and hence 16 d.f. for a regression of the absolute values of the standardized contrasts on their standard deviations; the two-tailed critical value is $\pm$0.468 for p = 0.05).

Females								
	PDI file	MSE tree	MSE Star	K	P phylogenetic signal	ln likelihood tree	ln likelihood Star	Diagnostic r
log_{10} Standard length	FSWsluc.PDI	0.0036	0.0052	0.5362	<0.001*	27.09	23.53	-0.2668
log_{10} U_{crit}	FSWsluc.PDI	0.0138	0.0159	0.5560	0.006*	14.27	12.88	-0.1615
log_{10} s.c.[&] U_{crit}	FSWsluc.RSD	0.0137	0.0171	0.6014	0.001*	14.31	12.22	-0.1594
log_{10} VO_{2max}	FSWslvo.PDI	0.0198	0.0233	0.4367	0.001*	10.83	9.26	-0.1558
log_{10} s.c.[&] VO_{2max}	FSWslvo.RSD	0.0117	0.0086	0.2316	0.094	15.84	18.67	-0.2232
log_{10} heart mass	FSWslhrt.PDI	0.0785	0.0656	0.3006	0.027*	-2.27	-0.57	-0.3365
log_{10} s.c.[&] heart mass	FSWslhrt.RSD	0.0579	0.0318	0.1674	0.326	0.63	6.31	-0.3333
log_{10} gill mass	FSWslgil.PDI	0.1045	0.0685	0.1942	0.181	-4.99	-0.98	-0.2012
log_{10} s.c.[&] gill mass	FSWslgil.RSD	0.1008	0.0594	0.1843	0.262	-4.64	0.38	-0.2443
CS V_{max}	FSWslcs.PDI	611.10	265.20	0.1359	0.576	-87.39	-79.46	**-0.5009**
s.c.[&] CS V_{max}	FSWslcs.RSD	610.19	259.09	0.1309	0.596	-87.38	-79.24	**-0.4988**
LDH V_{max}	FSWslldh.PDI	979.22	579.51	0.1754	0.245	-91.87	-86.89	-0.4101
s.c.[&] LDH V_{max}	FSWslldh.RSD	880.69	542.91	0.2040	0.167	-90.86	-86.27	-0.3728
log_{10} body mass	FSWslms.PDI	0.0348	0.0499	0.4909	0.001*	5.45	2.03	-0.2062
log_{10} s.c.[&] body mass	FSWslms.RSD	0.0049	0.0028	0.1835	0.312	24.14	29.27	-0.4263
log_{10} tail mass	FSWsltms.PDI	0.0486	0.0726	0.5086	<0.001*	2.27	-1.53	-0.1477
log_{10} s.c.[&] tail mass	FSWsltms.RDS	0.0071	0.0049	0.2109	0.113	20.49	24.13	-0.1003
log_{10} caudal fin	FSWsltln.PDI	0.0044	0.0097	0.7027	<0.001*	25.20	17.63	-0.1077
log_{10} s.c.[&] caudal fin	FSWsltln.RDS	0.0013	0.0021	0.4780	<0.001*	36.70	32.25	0.1571
log_{10} body depth	FSWslbd.PDI	0.0050	0.0083	0.6489	<0.001*	23.94	19.08	0.3579
log_{10} s.c.[&] body depth	FSWslbd.RSD	0.0015	0.0013	0.3134	0.046*	35.62	36.68	-0.0627
log_{10} ovary mass	FSWslom.PDI	0.3216	0.1778	0.1650	0.342	-15.67	-10.04	-0.3203
log_{10} s.c.[&] ovary	FSWslom.RSD	0.3210	0.1820	0.1707	0.284	-15.65	-10.26	-0.3222
log_{10} # regressors	FSWslrn.PDI	0.1291	0.0748	0.1758	0.225	-6.99	-1.81	-0.4197
log_{10} s.c.[&] regressors	FSWslrn.RSD	0.1283	0.0715	0.1736	0.315	-6.94	-1.38	-0.4011
log_{10} reg. dry mass	FSWslrd.PDI	0.2591	0.1383	0.1572	0.370	-13.62	-7.65	-0.3336
log_{10} s.c.[&]reg. dry	FSWslrd.RSD	0.2560	0.1467	0.1687	0.270	-13.50	-8.21	-0.3533

[&] size-corrected phylogenetic signal calculated from physig_ll the "residuals" from PDTREE, column 5 in *.RSD file.

Table 3.2

Table 3.3. Correlations of whole-organismal traits. Males, non-phylogenetic, two-tailed p. Pearson' r in upper diagonal, two-tailed p in lower diagonal. Correlations significant at $p < 0.05$ (unadjusted for multiple comparisons) are in **bold**.

	$\log_{10}$ SL	$\log_{10}$ U_{crit}	$\log_{10}$ VO_{2max}	$\log_{10}$ heart	$\log_{10}$ gill	CS V_{max}	LDH V_{max}	$\log_{10}$ sword	$\log_{10}$ body mass	$\log_{10}$ tail mass	$\log_{10}$ caudal fin	$\log_{10}$ BD
$\log_{10}$ SL		0.275	**0.881**	**0.862**	**0.759**	-0.449	0.047	**0.767**	**0.978**	**0.979**	**0.979**	**0.922**
$\log_{10}$ U_{crit}	0.254		0.076	0.335	0.298	-0.250	-0.107	0.028	0.268	0.300	0.257	0.440
$\log_{10}$ VO_{2max}	<0.001	0.757		**0.736**	**0.681**	-0.248	0.209	**0.666**	**0.878**	**0.861**	**0.850**	**0.784**
$\log_{10}$ heart	<0.001	0.162	<0.001		0.954	-0.408	0.019	**0.544**	**0.915**	**0.875**	**0.819**	**0.908**
$\log_{10}$ gill	<0.001	0.216	0.001	<0.001		-0.400	0.071	**0.461**	**0.844**	**0.776**	**0.698**	**0.842**
CS V_{max}	0.054	0.302	0.306	0.083	0.090		**0.480**	-0.354	-0.418	-0.437	-0.432	-0.453
LDH V_{max}	0.848	0.663	0.391	0.938	0.771	0.038		0.112	0.046	-0.001	0.042	-0.063
$\log_{10}$ sword	<0.001	0.908	0.002	0.016	0.047	0.137	0.647		**0.737**	**0.751**	**0.804**	**0.608**
$\log_{10}$ body mass	<0.001	0.268	<0.001	<0.001	<0.001	0.075	0.852	<0.001		**0.988**	**0.958**	**0.963**
$\log_{10}$ tail mass	<0.001	0.212	<0.001	<0.001	<0.001	0.061	0.996	<0.001	<0.001		**0.974**	**0.961**
$\log_{10}$ caudal fin	<0.001	0.288	<0.001	<0.001	0.001	0.065	0.865	<0.001	<0.001	<0.001		**0.902**
$\log_{10}$ BD	<0.001	0.059	<0.001	<0.001	<0.001	0.051	0.796	0.006	<0.001	<0.001	<0.001	

Table 3.4. Correlations of whole-organismal traits. Males, phylogenetic, two-tailed p. Pearson' r in upper diagonal, two-tailed p in lower diagonal. Correlations significant at $p < 0.05$ (unadjusted for multiple comparisons) are in **bold**.

	$\log_{10}$ SL	$\log_{10}$ U_{crit}	$\log_{10}$ VO_{2max}	$\log_{10}$ heart	$\log_{10}$ gill	CS V_{max}	LDH V_{max}	$\log_{10}$ sword	$\log_{10}$ body mass	$\log_{10}$ tail mass	$\log_{10}$ caudal fin	$\log_{10}$ BD
$\log_{10}$ SL		0.195	**0.870**	**0.832**	**0.869**	-0.404	-0.308	0.229	**0.979**	**0.966**	**0.955**	**0.950**
$\log_{10}$ U_{crit}	0.439		0.181	0.412	0.280	0.207	0.082	0.176	0.254	0.243	0.112	0.306
$\log_{10}$ VO_{2max}	<0.001	0.471		**0.703**	**0.713**	-0.243	-0.229	0.212	**0.876**	**0.891**	**0.831**	**0.841**
$\log_{10}$ heart	<0.001	0.089	0.001		**0.947**	-0.228	-0.226	-0.075	**0.877**	**0.835**	**0.757**	**0.896**
$\log_{10}$ gill	<0.001	0.261	0.001	<0.001		-0.446	-0.234	-0.019	**0.900**	**0.835**	**0.828**	**0.924**
CS V_{max}	0.096	0.409	0.330	0.363	0.064		0.396	-0.011	-0.318	-0.233	-0.456	-0.330
LDH V_{max}	0.214	0.748	0.361	0.368	0.349	0.103		0.107	-0.281	-0.288	-0.320	-0.304
$\log_{10}$ sword	0.361	0.486	0.399	0.767	0.940	0.965	0.672		0.221	0.250	0.302	0.178
$\log_{10}$ body mass	<0.001	0.308	<0.001	<0.001	<0.001	0.198	0.259	0.377		**0.985**	**0.938**	**0.988**
$\log_{10}$ tail mass	<0.001	0.331	<0.001	<0.001	<0.001	0.352	0.247	0.317	<0.001		**0.928**	**0.961**
$\log_{10}$ caudal fin	<0.001	0.658	<0.001	<0.001	<0.001	0.057	0.196	0.223	<0.001	<0.001		**0.919**
$\log_{10}$ BD	<0.001	0.217	<0.001	<0.001	<0.001	0.181	0.220	0.481	<0.001	<0.001	<0.001	

Table 3.5. Correlations of whole-organismal traits. Females, non-phylogenetic, two-tailed p. Pearson' r in upper diagonal, two-tailed p in lower diagonal. Correlations significant at $p < 0.05$ (unadjusted for multiple comparisons) are in **bold**.

	$\log_{10}$ SL	$\log_{10}$ U_{crit}	$\log_{10}$ VO_{2max}	$\log_{10}$ heart	$\log_{10}$ gill	CS V_{max}	LDH V_{max}	$\log_{10}$ sword	$\log_{10}$ body mass	$\log_{10}$ tail mass	$\log_{10}$ caudal fin	$\log_{10}$ BD
$\log_{10}$ SL		**0.504**	**0.798**	**0.719**	0.377	-0.193	0.312	-0.077	**0.972**	**0.967**	**0.918**	**0.930**
$\log_{10}$ U_{crit}	0.028		**0.492**	**0.524**	0.357	-0.180	0.300	0.150	**0.495**	**0.465**	0.426	**0.589**
$\log_{10}$ VO_{2max}	<0.001	0.032		**0.522**	0.232	0.086	0.372	0.064	**0.788**	**0.791**	**0.784**	**0.721**
$\log_{10}$ heart	0.001	0.021	0.022		**0.818**	-0.056	0.137	-0.136	**0.750**	**0.706**	**0.688**	**0.789**
$\log_{10}$ gill	0.111	0.134	0.339	<0.001		-0.013	0.116	-0.068	0.425	0.360	0.407	0.451
CS V_{max}	0.428	0.461	0.728	0.819	0.957		0.353	0.166	-0.181	-0.161	-0.128	-0.239
LDH V_{max}	0.193	0.212	0.117	0.577	0.637	0.138		0.137	0.303	0.226	0.120	0.231
$\log_{10}$ sword	0.753	0.540	0.795	0.578	0.782	0.497	0.576		-0.024	-0.007	0.080	-0.049
$\log_{10}$ body mass	<0.001	0.031	<0.001	<0.001	0.069	0.458	0.207	0.922		**0.982**	**0.918**	**0.972**
$\log_{10}$ tail mass	<0.001	0.045	<0.001	0.001	0.130	0.510	0.351	0.978	<0.001		**0.947**	**0.952**
$\log_{10}$ caudal fin	<0.001	0.069	<0.001	0.001	0.084	0.600	0.625	0.744	<0.001	<0.001		**0.857**
$\log_{10}$ BD	<0.001	0.008	<0.001	<0.001	0.053	0.324	0.342	0.841	<0.001	<0.001	<0.001	

Table 3.6. Correlations of whole-organismal traits. Females, phylogenetic, two-tailed p. Pearson' r in upper diagonal, two-tailed p in lower diagonal. Correlations significant at $p < 0.05$ (unadjusted for multiple comparisons) are in **bold**.

	$\log_{10}$ SL	$\log_{10}$ U_{crit}	$\log_{10}$ VO_{2max}	$\log_{10}$ heart	$\log_{10}$ gill	CS V_{max}	LDH V_{max}	$\log_{10}$ sword	$\log_{10}$ body mass	$\log_{10}$ tail mass	$\log_{10}$ caudal fin	$\log_{10}$ BD
$\log_{10}$ SL		-0.060	**0.640**	**0.512**	0.189	-0.040	0.317	-0.375	**0.927**	**0.923**	**0.837**	**0.841**
$\log_{10}$ U_{crit}	0.813		0.200	0.168	0.189	0.236	0.399	0.268	0.016	-0.070	0.065	0.093
$\log_{10}$ VO_{2max}	0.004	0.427		0.274	0.071	0.142	0.355	0.052	**0.675**	**0.698**	**0.680**	**0.606**
$\log_{10}$ heart	0.030	0.504	0.271		**0.753**	0.245	0.281	-0.290	**0.606**	**0.483**	0.457	**0.678**
$\log_{10}$ gill	0.452	0.454	0.779	<0.001		-0.100	0.021	-0.076	0.160	0.126	0.190	0.320
CS V_{max}	0.874	0.346	0.574	0.327	0.694		0.629	0.169	0.176	0.041	-0.037	0.144
LDH V_{max}	0.200	0.101	0.148	0.258	0.934	0.005		-0.002	0.381	0.205	0.073	0.295
$\log_{10}$ sword	0.125	0.283	0.837	0.243	0.764	0.503	0.993		-0.292	-0.278	-0.064	-0.343
$\log_{10}$ body mass	<0.001	0.949	0.002	0.008	0.525	0.486	0.118	0.239		**0.944**	**0.840**	**0.939**
$\log_{10}$ tail mass	<0.001	0.783	0.001	0.042	0.619	0.871	0.413	0.264	<0.001		**0.916**	**0.904**
$\log_{10}$ caudal fin	<0.001	0.797	0.002	0.056	0.451	0.884	0.774	0.801	<0.001	<0.001		**0.792**
$\log_{10}$ BD	<0.001	0.714	0.008	0.002	0.195	0.569	0.235	0.164	<0.001	<0.001	<0.001	

Table 3.7. Allometric equations with $\log_{10}$ standard length (SL) for males only from Regressionv2.m, three models of evolution (OLS, PGLS, RegOU). Chi-squares (2*difference in ln likelihoods) reported for RegOU vs. OLS, and RegOU vs. PGLS. * = significant difference in models (chi-square > 3.841). Used Ucrit_VO2max_size_enzymes_19_aggr_males.tip and sword_4.dsc

Comparison	OLS	PGLS	RegOU	Chi-Square RegOU vs. OLS	Chi-Square RegOU vs. PGLS
$\log_{10}$ U_{crit} on $\log_{10}$ SL :					
β	0.3080	0.2047	0.2318		
P	0.2539	0.4247	0.3857		
C.I. (lower, upper)	-0.179,0.805	-0.307, 0.637	-0.168, 0.794		
intercept	0.8760	1.1275	1.0365		
ln Likelihood	16.36	17.49	18.26	3.80	1.54
d			0.792		
r^2	0.0758	0.0379	0.0446		
$\log_{10}VO_{2max}$ on $\log_{10}$ SL :					
β	1.2354	1.4684	1.3921		
P	<0.001	<0.001	<0.001		
C.I. (lower, upper)	0.961,1.546	1.058, 1.807	1.044, 1.740		
intercept	-0.8502	-1.2138	-1.0845		
ln Likelihood	25.40	21.61	26.71	2.62	10.20*
d			0.150		
$\log_{10}VO_{2max}$ on $\log_{10}$ mass :					
β	0.4035	0.4746	0.4434		
P	<0.001	<0.001	<0.001		
C.I. (lower, upper)	0.313, 0.499	0.340, 0.563	0.334, 0.561		
intercept	1.0689	1.0606	1.0651		
ln Likelihood	25.14	22.00	25.95	1.62	7.90*
d			0.165		
[&]$\log_{10}$ heart mass on $\log_{10}$ SL :					
β	2.6817	2.8373	2.8089		
P	<0.001	<0.001	<0.001		
C.I. (lower, upper)	2.012, 3.394	1.974, 3.621	1.943, 3.708		

Comparison	OLS	PGLS	RegOU	Chi-Square RegOU vs. OLS	Chi-Square RegOU vs. PGLS
intercept	-7.4468	-7.7341	-7.6729		
ln Likelihood	9.10	5.95	9.35	0.50	6.80*
d			0.340		
&$\log_{10}$ gill mass on $\log_{10}$ SL :					
β	2.3143	3.0512	3.0350		
P	<0.001	<0.001	<0.001		
C.I. (lower, upper)	1.382, 3.152	2.289, 3.852	2.133, 3.825		
intercept	-5.6395	-6.8678	-6.8374		
ln Likelihood	4.73	7.59	7.97	6.48*	0.76
d			0.910		
CS V_{max} on $\log_{10}$ SL :					
β	-139.129	-155.295	-120.524		
P	0.0538	0.0856	0.1387		
C.I. (lower, upper)	-263.1, -192.0	-311.4, 136.0	-269.8, 394.9		
intercept	354.853	376.728	327.015		
ln Likelihood	-89.11	-93.27	-89.03	0.16	8.48*
d			0.214		
LDH V_{max} on $\log_{10}$ SL :					
β	13.2697	-90.1653	-30.2178		
P	0.8484	0.1994	0.6790		
C.I. (lower, upper)	-112.7, 143.2	-225.8, 11.64	-186.0, 100.4		
intercept	108.280	257.205	175.465		
ln Likelihood	-89.44	-88.87	-87.32	4.24*	3.10
d			0.524		
$\log_{10}$ sword length on $\log_{10}$ SL:					
β	4.1723	0.8342	1.1220		
P	<0.001	0.3478	0.2249		
C.I. (lower, upper)	2.562, 5.727	-0.107, 2.777	-0.734, 2.800		
intercept	-5.7665	-0.8520	-1.2830		
ln Likelihood	-6.00	-6.05	-5.27	1.46	1.56
d			0.809		
$\log_{10}$ mass on $\log_{10}$ SL:					
β	3.0057	3.0496	3.0599		
P	<0.001	<0.001	<0.001		

Comparison	OLS	PGLS	RegOU	Chi-Square RegOU vs. OLS	Chi-Square RegOU vs. PGLS
C.I. (lower, upper)	2.710, 3.310	2.775, 3.339	2.760, 3.382		
intercept	-4.6314	-4.7249	-4.7403		
ln Likelihood	26.08	26.88	28.33	4.50*	2.90
d			0.667		
log_{10} tail mass on log_{10} SL:					
β	3.3335	3.0389	3.1563		
P	<0.001	<0.001	<0.001		
C.I. (lower, upper)	3.019, 3.640	2.660, 3.397	2.810, 3.575		
intercept	-5.5478	-5.0877	-5.2853		
ln Likelihood	24.86	21.95	25.02	0.32	6.14*
d			0.364		
log_{10} caudal fin length on log_{10} SL:					
β	1.1846	1.0397	1.1294		
P	<0.001	<0.001	<0.001		
C.I. (lower, upper)	1.073, 1.301	0.896, 1.189	0.994, 1.261		
intercept	-0.8473	-0.6203	-0.7594		
ln Likelihood	44.16	39.64	44.76	1.20	10.24*
d			0.111		
log_{10} body depth on log_{10} SL:					
β	1.0415	1.1970	1.1941		
P	<0.001	<0.001	<0.001		
C.I. (lower, upper)	0.841, 1.237	1.050, 1.400	1.020, 1.396		
intercept	-0.6020	-0.8209	-0.8210		
ln Likelihood	33.41	35.85	36.11	5.40*	0.52
d			0.959		

[&] These equations do not account for the effect of preservation time.

Table 3.8. Allometric equations with $\log_{10}$ standard length (SL) for females only from Regressionv2.m, three models of evolution (OLS, PGLS, RegOU). Chi-squares (2*difference in ln likelihoods) reported for RegOU vs. OLS, and RegOU vs. PGLS. * = significant difference in models (chi-square > 3.841). Used Ucrit_VO2max_size_repro_enzymes_19_aggr_females.tip and sword_4.dsc

Comparison	OLS	PGLS	RegOU	Chi-Square RegOU vs. OLS	Chi-Square RegOU vs. PGLS
$\log_{10}$ U_{crit} on $\log_{10}$ SL :					
β	0.8820	-0.1186	0.1641		
P	0.0279	0.8060	0.7327		
C.I. (lower, upper)	0.192, 1.602	-0.675, 0.745	-0.792, 1.045		
intercept	-0.0481	1.6279	1.1174		
ln Maximum Likelihood	15.66	14.31	15.79	0.26	2.96
d			0.672		
r^2	0.2537	0.0036	0.0070		
$\log_{10}VO_{2max}$ on $\log_{10}$ SL :					
β	1.6905	1.5065	1.6710		
P	<0.001	0.003	<0.001		
C.I. (lower, upper)	1.101, 2.244	0.722, 2.350	0.928, 2.349		
intercept	-1.5706	-1.2586	-1.5369		
ln Maximum Likelihood	18.87	15.84	19.44	1.14	7.20*
d			0.245		
[&]$\log_{10}$ heart mass on $\log_{10}$ SL :					
β	2.5573	2.4040	2.4562		
P	<0.001	0.0248	0.002		
C.I. (lower, upper)	1.469, 3.643	1.036, 4.393	1.241, 3.809		
intercept	-7.3520	-7.0780	-7.1879		
ln Maximum Likelihood	6.35	0.63	6.49	0.28	11.72*
d			0.044		
[&]$\log_{10}$ gill mass on $\log_{10}$ SL :					
β	1.3695	1.0268	1.2082		
P	0.1115	0.4366	0.2081		

Comparison	OLS	PGLS	RegOU	Chi-Square RegOU vs. OLS	Chi-Square RegOU vs. PGLS
C.I. (lower, upper)	-0.095, 2.929	-1.465, 3.407	-0.623, 3.027		
intercept	-4.1975	-3.7186	-3.9376		
ln Maximum Likelihood	0.48	-4.64	0.51	0.06	10.30*
d			0.091		
CS V_{max} on log_{10} SL :					
β	-43.7033	-16.0249	-43.1584		
P	0.4276	0.8750	0.4344		
C.I. (lower, upper)	-145.32, 54.162	-238.57, 134.61	-142.25, 55.542		
intercept	181.343	134.298	180.460		
ln Maximum Likelihood	-79.10	-87.38	-79.10	0.00	16.56*
d			1.907e-9		
LDH V_{max} on log_{10} SL :					
β	104.281	166.164	150.456		
P	0.1932	0.1857	0.0965		
C.I. (lower, upper)	-37.534, 245.78	-89.044, 362.69	-18.449, 313.85		
intercept	-41.1686	-146.581	-116.311		
ln Maximum Likelihood	-85.91	-90.86	-85.08	1.66	11.56*
d			0.147		
log_{10} mass on log_{10} SL:					
β	3.0139	2.8974	3.0139		
P	<0.001	<0.001	<0.001		
C.I. (lower, upper)	2.678, 3.340	2.312, 3.397	2.635, 3.374		
intercept	-4.6119	-4.4426	-4.6119		
ln Maximum Likelihood	29.52	24.14	29.52	0.00	10.76*
d			2.602e-17		
log_{10} tail mass on log_{10} SL:					
β	3.6171	3.4101	3.5322		
P	<0.001	<0.001	<0.001		
C.I. (lower, upper)	3.178, 4.028	2.811, 4.072	3.004, 4.078		
intercept	-6.1043	-5.7943	-5.9742		
ln Maximum Likelihood	24.58	20.49	24.98	0.8	8.98*
d			0.181		
log_{10} tail length on log_{10} SL:					
β	1.2527	0.9252	0.9841		

Comparison	OLS	PGLS	RegOU	Chi-Square RegOU vs. OLS	Chi-Square RegOU vs. PGLS
P	<0.001	<0.001	<0.001		
C.I. (lower, upper)	1.003, 1.490	0.662, 1.199	0.709, 1.250		
intercept	-0.9875	-0.47843	-0.5756		
ln Maximum Likelihood	35.24	36.70	38.23	5.98*	3.06
d			0.701		
$\log_{10}$ body depth on $\log_{10}$ SL:					
β	1.1750	0.9932	1.0995		
P	<0.001	<0.001	<0.001		
C.I. (lower, upper)	0.938, 1.382	0.788, 1.288	0.832, 1.397		
intercept	-0.8095	-0.5082	-0.6937		
ln Maximum Likelihood	38.04	35.62	38.05	0.02	4.86*
d			0.424		
[&]$\log_{10}$ ovary mass on $\log_{10}$ SL:					
β	0.8190	-0.3877	0.9439		
P	0.568	0.868	0.848		
C.I. (lower, upper)	-1.780, 3.406	-4.929, 3.765	-2.118, 3.716		
intercept	-2.3750	-0.4121	-2.5740		
ln Maximum Likelihood	-9.85	-15.65	-9.83	0.04	11.64*
d			0.032		
$\log_{10}$ # regressors on $\log_{10}$ SL:					
β	0.9243	0.4711	1.0947		
P	0.315	0.750	0.278		
C.I. (lower, upper)	-0.842, 2.484	-2.401, 3.161	-0.881, 3.048		
intercept	-0.2696	0.3792	-0.5446		
ln Maximum Likelihood	-1.23	-6.94	-1.07	0.32	11.74*
d			0.049		
[&]$\log_{10}$ reg. dry mass on $\log_{10}$ SL:					
β	0.3839	-0.9372	0.4803		
P	0.762	0.654	0.723		
C.I. (lower, upper)	-2.159, 2.622	-0.283, 3.638	-2.231, 3.061		
intercept	-2.1814	-0.0882	-2.3359		
ln Maximum Likelihood	-7.60	-13.50	-7.60	0.00	11.80*
d			0.010		

[&] These equations do not account for the effect of preservation time.

Table 3.9. Correlations of residual trait values obtained from non-phylogenetic regressions on log standard length, for males. Pearson's r in upper diagonal ($p<0.05$ in **bold**), two-tailed p-values in lower diagonal (not adjusted for multiple comparisons).

	U_{crit}	VO_{2max}	heart	gill	CS V_{max}	LDH V_{max}	sword	body mass	tail mass	caudal fin length	body depth
U_{crit}		-0.3667	0.2262	0.1651	-0.1469	-0.1248	-0.2966	-0.0077	0.1571	-0.0621	**0.4991**
VO_{2max}	0.1225		-0.0726	0.0933	0.3491	0.3540	-0.0327	0.1623	-0.0252	-0.1272	-0.1539
heart	0.3517	0.7678		**0.8803**	0.0716	0.0401	**-0.6314**	**0.5275**	0.2467	-0.4519	**0.5359**
gill	0.4995	0.7039	<0.0001		0.0213	0.1642	**-0.5902**	**0.5889**	0.1884	**-0.5980**	**0.5206**
CS V_{max}	0.5485	0.1429	0.7709	0.9310		**0.5611**	-0.0173	0.1100	0.0129	0.0393	-0.1143
LDH V_{max}	0.6107	0.1370	0.8707	0.5019	0.0124		0.1188	-0.0001	-0.2337	-0.0208	-0.2755
sword	0.2176	0.8943	0.0037	0.0078	0.9441	0.6282		-0.0939	0.0004	0.4059	-0.3991
body mass	0.9750	0.5068	0.0203	0.0080	0.6538	0.9996	0.7022		**0.7226**	0.0206	**0.7615**
tail mass	0.5206	0.9185	0.3087	0.4398	0.9581	0.3355	0.9988	0.0005		0.3744	**0.7479**
caudal fin	0.8006	0.6038	0.0521	0.0068	0.8730	0.9325	0.0847	0.9332	0.1143		0.0037
body depth	0.0296	0.5292	0.0180	0.0223	0.6413	0.2536	0.0906	0.0002	0.0002	0.9880	

Table 3.10. Correlations of residual trait values obtained from phylogenetic regressions on log standard length, for males. Pearson's r in upper diagonal ($p<0.05$ in **bold**), two-tailed p-values in lower diagonal (not adjusted for multiple comparisons).

	U_{crit}	VO_{2max}	heart	gill	CS V_{max}	LDH V_{max}	sword	body mass	tail mass	caudal fin length	body depth
U_{crit}		0.0245	0.4333	0.1720	0.3188	0.1517	0.1373	0.3211	0.2155	-0.2555	0.3943
VO_{2max}	0.9230		-0.0628	-0.1752	0.2403	0.0838	0.0261	0.2377	0.3959	0.0001	0.0915
heart	0.0725	0.8045		**0.7852**	0.3409	0.1799	**-0.5981**	0.4662	0.2406	-0.4242	**0.5015**
gill	0.4948	0.4869	0.0001		-0.0743	0.2708	**-0.6462**	0.3730	-0.0321	-0.2902	**0.5106**
CS V_{max}	0.1973	0.3368	0.1662	0.7695		0.3124	0.0914	0.4188	**0.6627**	-0.2600	0.1879
LDH V_{max}	0.5478	0.7409	0.4751	0.2771	0.2069		0.1918	0.1082	0.0405	-0.0898	-0.0375
sword	0.5870	0.9182	0.0088	0.0038	0.7183	0.4459		-0.0133	0.1158	0.2911	-0.1304
body mass	0.1939	0.3423	0.0512	0.1274	0.0836	0.6692	0.9582		**0.7478**	0.0479	**0.9044**
tail mass	0.3905	0.1039	0.3363	0.8995	0.0027	0.8731	0.6474	0.0004		0.0726	**0.5350**
caudal fin	0.3062	0.9997	0.0793	0.2427	0.2974	0.7231	0.2411	0.8503	0.7748		0.1187
body depth	0.1054	0.7180	0.0340	0.0304	0.4554	0.8826	0.6060	<0.0001	0.0221	0.6391	

Table 3.11. Female correlations of residual trait values, non-phylogenetic. Residuals obtained from regressions with standard length as the predictor. Pearson's r in upper diagonal ($p<0.05$ in **bold**), two-tailed p-values in lower diagonal.

	U_{crit}	VO_{2max}	heart	gill	CS V_{max}	LDH V_{max}	sword	body mass	tail mass	caudal fin length	body depth
U_{crit}		0.1740	0.1843	0.1011	-0.0975	0.1739	0.2195	0.0280	-0.1041	-0.1080	0.3802
VO_{2max}	0.4761		0.0160	0.0445	0.4053	0.2139	0.2090	0.0888	0.1248	0.2142	-0.0922
heart	0.4501	0.9482		**0.8094**	0.3149	-0.0353	-0.2112	0.1532	-0.1054	0.0169	0.2487
gill	0.6804	0.8563	<0.0001		0.2976	0.1364	-0.1492	0.0753	-0.2306	0.0796	0.0028
CS V_{max}	0.6913	0.0851	0.1892	0.2159		0.4439	0.1543	0.0293	0.1051	0.1268	-0.1645
LDH V_{max}	0.4761	0.3792	0.8859	0.5776	0.0569		0.1702	0.1702	-0.3149	-0.4433	-0.1701
sword	0.3665	0.3905	0.3854	0.5420	0.5281	0.4860		0.2178	0.2696	0.3835	0.0617
body mass	0.9093	0.7176	0.5312	0.7594	0.9052	0.9992	0.3704		**0.6955**	0.2706	**0.6955**
tail mass	0.6715	0.6108	0.6677	0.3422	0.6684	0.1892	0.2644	0.0009		**0.5801**	**0.5801**
caudal fin	0.6598	0.3786	0.9452	0.7461	0.6051	0.0573	0.1050	0.2625	0.0092		0.0243
body depth	0.1083	0.7073	0.3045	0.9909	0.5009	0.4863	0.8018	0.0001	0.0124	0.9214	

Table 3.12. Female correlations of residual trait values, phylogenetic. Residuals obtained from regressions with standard length as the predictor through the origin. Pearson's r in upper diagonal ($p<0.05$ in **bold**), two-tailed p-values in lower diagonal.

	U_{crit}	VO_{2max}	heart	gill	CS V_{max}	LDH V_{max}	sword	body mass	tail mass	caudal fin length	body depth
U_{crit}		0.3104	0.2204	0.1933	0.2342	0.4411	0.2650	0.1924	-0.0379	0.2118	0.2654
VO_{2max}	0.2099		-0.0474	-0.0173	0.2184	0.2091	0.4100	0.2827	0.3641	0.3427	0.1627
heart	0.3796	0.8519		**0.7365**	0.3877	0.2298	-0.1447	0.3593	-0.0709	-0.0324	0.4148
gill	0.4423	0.9457	0.0005		-0.0292	0.0589	-0.0242	-0.1678	-0.3069	-0.0770	0.1180
CS V_{max}	0.3495	0.3840	0.1120	0.9085		**0.6774**	0.1662	**0.5699**	0.2046	-0.0064	0.3284
LDH V_{max}	0.0669	0.4051	0.3589	0.8163	0.0020		0.1329	0.2456	-0.2406	-0.3721	0.0546
sword	0.2880	0.0910	0.5666	0.9241	0.5098	0.5991		0.1603	0.1930	**0.4940**	-0.0545
body mass	0.4443	0.2557	0.1430	0.5058	0.0136	0.3259	0.5251		**0.6102**	0.3103	**0.7877**
tail mass	0.8813	0.1375	0.7798	0.2155	0.4153	0.3362	0.4428	0.0072		**0.6789**	**0.6119**
caudal fin	0.3989	0.1639	0.8984	0.7615	0.9798	0.1283	0.0372	0.2102	0.0019		0.2969
body depth	0.2872	0.5189	0.0870	0.6409	0.1833	0.8296	0.8300	0.0001	0.0070	0.2316	

Table 3.13. Non-phylogenetic multiple regressions for males, for the effect of candidate predictors on residual $\log_{10} U_{crit}$. All predictors are residual $\log_{10}$ values from regressions with $\log_{10}$ standard length. Several candidate models examined, including a model with potential physiological predictors, a model with potential morphological predictors, and a full model with all predictors included. Additionally, a backwards stepwise regression was used to determine any potential significant combinations of physiological and morphological predictors. Only significant regression models with several significant predictors from backwards stepwise regression shown.

	Unstandardized Coefficients		Standardized Coefficients	F	Sig.	Collinearity Statistics	
	B	Std. Error	Beta			Tolerance	VIF
Physiological Predictors							
Model (d.f. = 6, 12)				0.615	0.715		
(Constant)	-6.770E-17	0.026		0.000	1.000		
sword length	-0.100	0.105	-0.323	0.900	0.361	0.549	1.821
VO_{2max}	-0.622	0.478	-0.387	1.697	0.217	0.723	1.382
heart mass	-0.009	0.488	-0.012	0.000	0.986	0.162	6.184
gill mass	0.005	0.397	0.008	0.000	0.990	0.164	6.108
CS V_{max}	-2.530E-04	0.001	-0.065	0.037	0.850	0.558	1.792
LDH V_{max}	3.289E-04	0.001	0.086	0.063	0.806	0.539	1.857
Morphological Predictors							
Model (d.f. = 4, 14)				2.113	0.133		
(Constant)	2.141E-16	0.022		0.000	1.000		
sword length	0.011	0.084	0.037	0.019	0.893	0.603	1.659
body depth	**2.459**	**1.011**	**1.003**	**5.921**	**0.029**	**0.262**	**3.812**
tail mass	-1.023	0.635	-0.654	2.597	0.129	0.270	3.700
caudal fin	0.708	1.131	0.164	0.392	0.541	0.649	1.541
Full Model							
Model (d.f. = 9, 9)				1.044	0.475		
(Constant)	-8.749E-17	0.024		.000	1.000		
sword length	-0.055	0.111	-0.178	0.244	0.633	0.418	2.395
VO_{2max}	-0.428	0.466	-0.266	0.844	0.382	0.647	1.546
heart mass	0.116	0.466	0.149	0.062	0.810	0.151	6.626
gill mass	-0.470	0.483	-0.740	0.949	0.355	0.094	10.604
CS V_{max}	-0.001	0.001	-0.136	0.172	0.688	0.508	1.970
LDH V_{max}	0.001	0.001	0.383	0.963	0.352	0.356	2.810
body depth	2.506	1.257	1.022	3.976	0.077	0.207	4.829
tail mass	-0.498	0.805	-0.319	0.383	0.551	0.205	4.875

	Unstandardized Coefficients		Standardized Coefficients	F	Sig.	Collinearity Statistics	
	B	Std. Error	Beta			Tolerance	VIF
caudal fin	-1.165	1.835	-0.270	0.403	0.541	0.301	3.323
Backwards Stepwise Regression							
Model (d.f. = 1, 17)				5.639	0.030		
(Constant)	-2.127E-16	0.021		0.000	1.000		
body depth	**1.224**	**0.515**	**0.499**	**5.639**	**0.030**	**1.000**	**1.000**
Model From Phylogenetic Backwards Stepwise Regression							
Model (d.f. = 2, 16)				0.796	0.468		
(Constant)	3.225E-17	0.024		0.000	1.000		
sword length	-0.079	0.095	-0.256	0.691	0.418	0.601	1.663
heart mass	0.050	0.239	0.065	0.045	0.836	0.601	1.663

Table 3.14. Phylogenetic multiple regressions for males, for the effect of candidate predictors on residual $\log_{10} U_{crit}$. All predictors are residual $\log_{10}$ values from regressions with $\log_{10}$ standard length. All regressions analyzed on independent contrasts through the origin. Several candidate models examined, including a model with potential physiological predictors, a model with potential morphological predictors, and a full model with all predictors included. Additionally, a backwards stepwise regression was used to determine any potential significant combinations of physiological and morphological predictors. Only significant regression models with several significant predictors from backwards stepwise regression shown.

	Unstandardized Coefficients		Standardized Coefficients	F	Sig.	Collinearity Statistics	
	B	Std. Error	Beta			Tolerance	VIF
Physiological Predictors							
Model (d.f. = 6, 12)				1.681	0.209		
sword length	0.190	0.097	0.656	3.847	0.073	0.405	2.468
VO_{2max}	0.087	0.282	0.070	0.094	0.764	0.878	1.139
heart mass	0.586	0.291	0.994	4.054	0.067	0.186	5.380
gill mass	-0.109	0.375	-0.153	0.084	0.776	0.163	6.149
CS V_{max}	-2.333E-04	0.001	-0.079	0.059	0.813	0.423	2.362
LDH V_{max}	-3.424E-04	0.001	-0.092	0.102	0.755	0.541	1.848
Morphological Predictors							
Model (d.f. = 4, 14)				1.820	0.181		
sword length	0.097	0.069	0.334	1.996	0.180	0.841	1.189
body depth	1.385	0.703	0.527	3.876	0.069	0.656	1.524
tail mass	-0.095	0.334	-0.075	0.082	0.779	0.676	1.479
caudal fin	-1.315	0.739	-0.410	3.168	0.097	0.887	1.128
Full Model							
Model (d.f. = 9, 9)				1.165	0.421		
sword length	0.118	0.141	0.407	0.697	0.425	0.216	4.623
VO_{2max}	0.045	0.354	0.036	0.016	0.902	0.632	1.583
heart mass	0.546	0.314	0.926	3.026	0.116	0.181	5.520
gill mass	-0.486	0.583	-0.683	0.693	0.427	0.076	13.131
CS V_{max}	-2.738E-04	0.001	-0.093	0.037	0.853	0.217	4.613
LDH V_{max}	4.826E-04	0.002	0.130	0.088	0.774	0.266	3.763
body depth	1.429	1.298	0.544	1.211	0.300	0.210	4.754
tail mass	-0.390	0.541	-0.308	0.520	0.489	0.281	3.557
caudal fin	-0.751	1.001	-0.234	0.564	0.472	0.527	1.896

	Unstandardized Coefficients		Standardized Coefficients	F	Sig.	Collinearity Statistics	
	B	Std. Error	Beta			Tolerance	VIF
Backwards Stepwise Regression							
Model (d.f. = 2, 16)				6.093	0.011		
sword length	**0.179**	**0.068**	**0.617**	**6.895**	**0.018**	**0.642**	**1.557**
heart mass	**0.474**	**0.139**	**0.802**	**11.655**	**0.004**	**0.642**	**1.557**
Model from Non-phylogenetic Backwards Stepwise Regression							
Model (d.f. = 1, 17)				3.130	0.095		
body depth	1.037	0.586	0.394	3.130	0.095	1.000	1.00

Table 3.15. Non-phylogenetic multiple regressions for females, for the effect of candidate predictors on residual $\log_{10} U_{crit}$. All predictors are residual $\log_{10}$ values from regressions with $\log_{10}$ standard length. Several candidate models examined, including a model with potential physiological predictors, a model with potential morphological predictors, and a full model with all predictors included. Additionally, a backwards stepwise regression was used to determine any potential significant combinations of physiological and morphological predictors. Only significant regression models with several significant predictors from backwards stepwise regression shown. Residual $\log_{10}$ sword length and residual $\log_{10}$ regressor dry mass included in all models.

	Unstandardized Coefficients		Standardized Coefficients	F	Sig.	Collinearity Statistics	
	B	Std. Error	Beta			Tolerance	VIF
Physiological Predictors							
Model (d.f. = 7, 11)				0.934	0.518		
(Constant)	3.083E-17	0.025		0.000	1.000		
sword length	0.761	0.601	0.327	1.603	0.232	0.855	1.170
regressor dry mass	-0.087	0.084	-0.281	1.071	0.323	0.773	1.294
VO_{2max}	0.305	0.315	0.258	0.940	0.353	0.807	1.240
heart mass	0.404	0.305	0.593	1.757	0.212	0.285	3.505
gill mass	-0.063	0.240	-0.119	0.068	0.798	0.274	3.648
CS V_{max}	-0.004	0.002	-0.613	3.594	0.085	0.546	1.833
LDH V_{max}	0.002	0.001	0.366	1.657	0.224	0.706	1.416
Morphological Predictors							
Model (d.f. = 5, 13)				4.083	0.019		
(Constant)	-2.665E-15	0.018		.000	1.000		
sword length	0.903	0.444	0.388	4.140	0.063	0.822	1.217
regressor dry mass	**-0.171**	**0.066**	**-0.551**	**6.812**	**0.022**	**0.671**	**1.490**
body depth	**3.160**	**0.807**	**0.973**	**15.345**	**0.002**	**0.485**	**2.061**
tail mass	**-1.918**	**0.550**	**-1.199**	**12.168**	**0.004**	**0.253**	**3.951**
caudal fin	1.001	0.720	0.357	1.934	0.188	0.453	2.206
Full Model							
Model (d.f. = 10, 8)				1.772	0.215		
(Constant)	-2.771E-15	0.021		0.000	1.000		
sword length	0.879	0.619	0.378	2.013	0.194	0.549	1.822
regressor dry mass	-0.189	0.083	-0.607	5.190	0.052	0.547	1.827
VO_{2max}	0.350	0.266	0.296	1.730	0.225	0.769	1.301
heart mass	-0.130	0.330	-0.191	0.156	0.703	0.167	5.999
gill mass	0.117	0.236	0.223	0.246	0.633	0.193	5.169
CS V_{max}	-0.001	0.002	-0.102	0.098	0.762	0.366	2.730

	Unstandardized Coefficients		Standardized Coefficients	F	Sig.	Collinearity Statistics	
	B	Std. Error	Beta			Tolerance	VIF
LDH V_{max}	2.073E-04	0.002	-0.044	0.015	0.907	0.300	3.338
body depth	**3.346**	**1.249**	**1.030**	**7.180**	**0.028**	**0.263**	**3.801**
tail mass	**-1.946**	**0.760**	**-1.217**	**6.552**	**0.034**	**0.172**	**5.811**
caudal fin	0.785	1.094	0.280	0.514	0.494	0.255	3.921
Backwards Stepwise Regression							
Model (d.f. = 4, 14)				4.332	0.017		
(Constant)	-2.423E-15	0.019		.000	1.000		
sword length	**1.029**	**0.449**	**0.442**	**5.251**	**0.038**	**0.857**	**1.167**
regressor dry mass	**-0.145**	**0.065**	**-0.468**	**5.007**	**0.042**	**0.729**	**1.371**
body depth	**2.611**	**0.727**	**0.804**	**12.910**	**0.003**	**0.637**	**1.569**
tail mass	**-1.401**	**0.419**	**-0.876**	**11.208**	**0.005**	**0.466**	**2.146**

Table 3.16. Phylogenetic multiple regressions for females, for the effect of candidate predictors on residual $\log_{10} U_{crit}$. All predictors are residual $\log_{10}$ values from regressions with $\log_{10}$ standard length. All regressions analyzed on independent contrasts through the origin. Several candidate models examined, including a model with potential physiological predictors, a model with potential morphological predictors, and a full model with all predictors included. Additionally, a backwards stepwise regression was used to determine any potential significant combinations of physiological and morphological predictors. Only significant regression models with several significant predictors from backwards stepwise regression shown. Residual $\log_{10}$ sword length and residual $\log_{10}$ regressor dry mass included in all models.

	Unstandardized Coefficients		Standardized Coefficients	F	Sig.	Collinearity Statistics	
	B	Std. Error	Beta			Tolerance	VIF
Physiological Predictors							
Model (d.f. = 7, 11)				0.872	0.557		
sword length	0.514	0.510	0.330	1.016	0.335	0.547	1.829
regressor dry mass	-0.046	0.067	-0.198	0.480	0.503	0.718	1.393
VO_{2max}	0.170	0.301	0.157	0.320	0.583	0.758	1.319
heart mass	0.220	0.271	0.420	0.656	0.435	0.218	4.589
gill mass	-0.057	0.196	-0.132	0.084	0.778	0.281	3.564
CS V_{max}	-0.002	0.002	-0.370	0.712	0.417	0.304	3.286
LDH V_{max}	0.002	0.001	0.542	2.510	0.141	0.499	2.005
Morphological Predictors							
Model (d.f. = 5, 13)				2.434	0.091		
sword length	0.852	0.450	0.546	3.589	0.081	0.479	2.089
regressor dry mass	-0.123	0.064	-0.525	3.649	0.078	0.527	1.897
body depth	**2.453**	**0.857**	**0.800**	**8.197**	**0.013**	**0.509**	**1.965**
tail mass	**-1.511**	**0.533**	**-1.092**	**8.049**	**0.014**	**0.268**	**3.731**
caudal fin	1.259	1.028	0.388	1.501	0.242	0.397	2.522
Full Model							
Model (d.f. = 10, 8)				1.488	0.293		
sword length	0.482	0.705	0.309	0.467	0.514	0.214	4.673
regressor dry mass	-0.109	0.094	-0.466	1.363	0.277	0.274	3.653
VO_{2max}	0.254	0.293	0.235	0.752	0.411	0.597	1.676
heart mass	0.095	0.272	0.182	0.123	0.735	0.162	6.175
gill mass	-0.145	0.210	-0.338	0.477	0.509	0.183	5.471
CS V_{max}	-0.001	0.002	-0.147	0.127	0.730	0.258	3.875
LDH V_{max}	0.001	0.002	0.373	0.615	0.456	0.193	5.190
body depth	2.317	1.218	0.756	3.616	0.094	0.277	3.613
tail mass	-1.716	0.877	-1.240	3.826	0.086	0.109	9.204

	Unstandardized Coefficients		Standardized Coefficients	F	Sig.	Collinearity Statistics	
	B	Std. Error	Beta			Tolerance	VIF
caudal fin	2.151	1.314	0.663	2.681	0.140	0.267	3.747
Backwards Stepwise Regression							
Model (d.f. = 4, 14)[&]				3.958	0.024		
LDH V_{max}	**0.002**	**0.001**	**0.530**	**6.890**	**0.020**	**0.821**	**1.218**
body depth	1.320	0.743	0.430	3.158	0.097	0.571	1.750
tail mass	**-0.936**	**0.428**	**-0.677**	**4.776**	**0.046**	**0.350**	**2.859**
caudal fin	**2.404**	**0.855**	**0.741**	**7.912**	**0.014**	**0.483**	**2.069**
Backwards Stepwise Regression no B.D.							
Model (d.f. = 3, 15)				3.694	0.036		
LDH V_{max}	**0.002**	**0.001**	**0.608**	**8.314**	**0.011**	**0.861**	**1.161**
tail mass	-0.485	0.369	-0.351	1.728	0.208	0.539	1.856
caudal fin	**2.195**	**0.905**	**0.676**	**5.877**	**0.028**	**0.493**	**2.029**
Backwards Stepwise Regression no B.D. and tail mass							
Model (d.f. = 2, 16)				4.473	0.029		
LDH V_{max}	**0.002**	**0.001**	**0.603**	**7.827**	**0.013**	**0.862**	**1.161**
caudal fin	1.416	0.700	0.436	4.092	0.060	0.862	1.161

Table 3.17. Non-phylogenetic multiple regressions for males, for the effect of candidate predictors on residual $\log_{10} VO_{2max}$. All predictors are residual $\log_{10}$ values from regressions with $\log_{10}$ standard length. Several candidate models examined, including a model with potential physiological predictors, a model with potential morphological predictors, and a full model with all predictors included. Additionally, a backwards stepwise regression was used to determine any potential significant combinations of physiological and morphological predictors. Only significant regression models with several significant predictors from backwards stepwise regression shown.

	Unstandardized Coefficients		Standardized Coefficients	F	Sig.	Collinearity Statistics	
	B	Std. Error	Beta			Tolerance	VIF
Physiological Predictors							
Model (d.f. = 5, 13)				0.994	0.458		
(Constant)	-4.099E-16	0.015		.000	1.000		
sword length	-0.025	0.061	-0.132	0.174	0.683	0.556	1.797
heart mass	-0.378	0.263	-0.782	20.059	0.175	0.187	5.338
gill mass	0.269	0.218	0.680	1.518	0.240	0.183	5.469
CS V_{max}	0.001	0.001	0.330	1.190	0.295	0.609	1.642
LDH V_{max}	2.476E-04	0.001	0.104	0.106	0.749	0.543	1.842
Morphological Predictors							
Model (d.f. = 4, 14)				0.485	0.747		
(Constant)	-4.814E-16	0.016		.000	1.000		
sword length	-0.035	0.062	-0.181	0.316	0.583	0.603	1.659
body depth	-0.961	0.745	-0.630	1.661	0.218	0.262	3.812
tail mass	0.526	0.468	0.541	1.262	0.280	0.270	3.700
caudal fin	-0.682	0.835	-0.254	0.667	0.428	0.649	1.541
Full Model							
Model (d.f. = 8, 10)				0.682	0.700		
(Constant)	-6.078E-16	0.016		0.000	1.000		
sword length	-0.058	0.073	-0.301	0.621	0.449	0.443	2.255
heart mass	-0.368	0.294	-0.762	1.566	0.239	0.175	5.728
gill mass	0.233	0.319	0.590	0.535	0.481	0.099	10.066
CS V_{max}	0.001	0.001	0.229	0.429	0.527	0.530	1.889
LDH V_{max}	4.247E-04	0.001	0.179	0.180	0.681	0.362	2.760
body depth	-0.765	0.817	-0.502	0.877	0.371	0.225	4.439
tail mass	0.527	0.520	0.542	1.026	0.335	0.226	4.421
caudal fin	-0.544	10.232	-0.203	0.195	0.668	0.307	3.259

Note: no significant models with backwards stepwise regression; therefore, results not shown.

Table 3.18. Phylogenetic multiple regressions for males, for the effect of candidate predictors on residual $\log_{10}$ VO_{2max}. All predictors are residual $\log_{10}$ values from regressions with $\log_{10}$ standard length. All regressions analyzed on independent contrasts through the origin. Several candidate models examined, including a model with potential physiological predictors, a model with potential morphological predictors, and a full model with all predictors included. Additionally, a backwards stepwise regression was used to determine any potential significant combinations of physiological and morphological predictors. Only significant regression models with several significant predictors from backwards stepwise regression shown.

	Unstandardized Coefficients		Standardized Coefficients	F	Sig.	Collinearity Statistics	
	B	Std. Error	Beta			Tolerance	VIF
Physiological Predictors							
Model (d.f. = 5, 13)				0.361	0.866		
sword length	-0.068	0.093	-0.289	0.522	0.483	0.421	2.373
heart mass	-0.039	0.286	-0.083	0.019	0.893	0.186	5.372
gill mass	-0.188	0.365	-0.329	0.265	0.615	0.166	6.026
CS V_{max}	0.001	0.001	0.216	0.298	0.594	0.433	2.309
LDH V_{max}	0.001	0.001	0.176	0.253	0.624	0.552	1.812
Morphological Predictors							
Model (d.f. = 4, 14)				0.768	0.564		
sword length	-0.013	0.062	-0.056	0.046	0.834	0.841	1.189
body depth	-0.389	0.632	-0.184	0.379	0.548	0.656	1.524
tail mass	0.510	0.300	0.501	2.894	0.111	0.676	1.479
caudal fin	0.005	0.663	0.002	0.000	0.994	0.887	1.128
Full Model							
Model (d.f. = 8, 10)				0.729	0.666		
sword length	-0.170	0.114	-0.729	2.223	0.167	0.264	3.782
heart mass	-0.035	0.280	-0.075	0.016	0.902	0.181	5.511
gill mass	-0.633	0.480	-1.108	1.736	0.217	0.089	11.188
CS V_{max}	-0.001	0.001	-0.451	0.752	0.406	0.233	4.290
LDH V_{max}	0.002	0.001	0.649	2.154	0.173	0.323	3.096
body depth	1.003	1.114	0.474	0.809	0.389	0.227	4.398
tail mass	0.512	0.455	0.503	1.270	0.286	0.317	3.156
caudal fin	-0.756	0.861	-0.293	0.771	0.401	0.568	1.760

Note: no significant models with backwards stepwise regression; therefore, results not shown.

Table 3.19. Non-phylogenetic multiple regressions for females, for the effect of candidate predictors on residual $\log_{10}$ VO_{2max}. All predictors are residual $\log_{10}$ values from regressions with $\log_{10}$ standard length. Several candidate models examined, including a model with potential physiological predictors, a model with potential morphological predictors, and a full model with all predictors included. Additionally, a backwards stepwise regression was used to determine any potential significant combinations of physiological and morphological predictors. Only significant regression models with several significant predictors from backwards stepwise regression shown.

	Unstandardized Coefficients		Standardized Coefficients	F	Sig.	Collinearity Statistics	
	B	Std. Error	Beta			Tolerance	VIF
Physiological Predictors							
Model (d.f. = 6, 12)				0.479	0.812		
(Constant)	-3.046E-16	0.023		0.000	1.000		
sword length	0.241	0.547	0.123	0.195	0.667	0.868	1.151
regressor dry mass	0.005	0.077	0.021	0.005	0.945	0.773	1.293
heart mass	-0.062	0.279	-0.108	0.050	0.827	0.286	3.491
gill mass	0.009	0.220	0.019	0.002	0.970	0.274	3.647
CS V_{max}	0.002	0.002	0.417	1.602	0.230	0.618	1.617
LDH V_{max}	7.338E-06	0.001	0.002	0.000	0.995	0.706	1.416
Morphological Predictors							
Model (d.f. = 5, 13)				0.236	0.940		
(Constant)	1.313E-16	0.024		0.000	1.000		
sword length	0.303	0.576	0.154	0.277	0.608	0.822	1.217
regressor dry mass	-0.003	0.085	-0.012	0.001	0.971	0.671	1.490
body depth	-0.487	1.046	-0.177	0.216	0.649	0.485	2.061
tail mass	0.175	0.713	0.129	0.060	0.810	0.253	3.951
caudal fin	0.197	0.934	0.083	0.044	0.837	0.453	2.206
Full Model							
Model (d.f. = 9, 9)				0.301	0.956		
(Constant)	1.778E-16	0.026		0.000	1.000		
sword length	-0.054	0.775	-0.028	0.005	0.946	0.549	1.821
regressor dry mass	0.026	0.103	0.097	0.061	0.811	0.551	1.815
heart mass	0.035	0.412	0.061	0.007	0.934	0.167	5.994
gill mass	-0.084	0.294	-0.188	0.081	0.782	0.195	5.123
CS V_{max}	0.002	0.003	0.306	0.420	0.533	0.383	2.608
LDH V_{max}	0.001	0.002	0.254	0.232	0.642	0.307	3.254
body depth	-0.045	1.563	-0.017	0.001	0.977	0.263	3.801

	Unstandardized Coefficients		Standardized Coefficients	F	Sig.	Collinearity Statistics	
	B	Std. Error	Beta			Tolerance	VIF
tail mass	0.014	0.952	.010	.000	.989	.172	5.810
caudal fin	0.751	1.347	.317	.311	.591	.264	3.790

Note: no significant models with backwards stepwise regression; therefore, results not shown.

Table 3.20. Phylogenetic multiple regressions for females, for the effect of candidate predictors on residual $\log_{10}$ VO_{2max}. All predictors are residual $\log_{10}$ values from regressions with $\log_{10}$ standard length. All regressions analyzed on independent contrasts through the origin. Several candidate models examined, including a model with potential physiological predictors, a model with potential morphological predictors, and a full model with all predictors included. Additionally, a backwards stepwise regression was used to determine any potential significant combinations of physiological and morphological predictors. Only significant regression models with several significant predictors from backwards stepwise regression shown.

	Unstandardized Coefficients		Standardized Coefficients	F	Sig.	Collinearity Statistics	
	B	Std. Error	Beta			Tolerance	VIF
Physiological Predictors							
Model (d.f. = 6, 12)	0.669	0.450	0.464	2.210	0.163	0.647	1.545
sword length	-0.049	0.063	-0.225	0.604	0.452	0.754	1.326
regressor dry mass	-0.026	0.260	-0.053	0.010	0.923	0.218	4.585
heart mass	0.019	0.188	0.049	0.011	0.920	0.281	3.561
gill mass	0.001	0.002	0.123	0.073	0.792	0.306	3.266
CS V_{max}	3.347E-04	0.001	0.092	0.067	0.800	0.501	1.994
LDH V_{max}	0.669	0.450	0.464	2.210	0.163	0.647	1.545
Morphological Predictors							
Model (d.f. = 5, 13)				0.917	0.500		
sword length	0.639	0.496	0.444	1.658	0.220	0.479	2.089
regressor dry mass	-0.022	0.071	-0.103	0.098	0.759	0.527	1.897
body depth	0.092	0.946	0.032	0.009	0.924	0.509	1.965
tail mass	0.357	0.588	0.279	0.368	0.555	0.268	3.731
caudal fin	-0.262	1.134	-0.087	0.053	0.821	0.397	2.522
Full Model							
Model (d.f. = 9, 9)				0.676	0.715		
sword length	-0.194	0.799	-0.135	0.059	0.813	0.215	4.643
regressor dry mass	0.074	0.104	0.343	0.515	0.491	0.289	3.455
heart mass	-0.197	0.302	-0.407	0.424	0.531	0.170	5.897
gill mass	0.210	0.228	0.530	0.849	0.381	0.200	4.999
CS V_{max}	-0.001	0.002	-0.162	0.103	0.755	0.261	3.831
LDH V_{max}	0.002	0.002	0.680	1.579	0.240	0.227	4.415
body depth	-0.836	1.356	-0.295	0.380	0.553	0.288	3.466
tail mass	1.129	0.923	0.884	1.495	0.253	0.127	7.893
caudal fin	0.642	1.477	0.214	0.189	0.674	0.272	3.670

Note: no significant models with backwards stepwise regression; therefore, results not shown.

Figure 3.1. Hypothesized phylogenetic relationships among species of *Xiphophorus* and *Priapella*, with example pictures of males and females among species with varying sword length. Grids on pictures are 1 cm for scale. Branch lengths represent substitutions per site. Species Xv through Xu are platyfish, Xe through Xb are also referred to as Northern swordtails, and Xo through Xa as Southern swordtails.

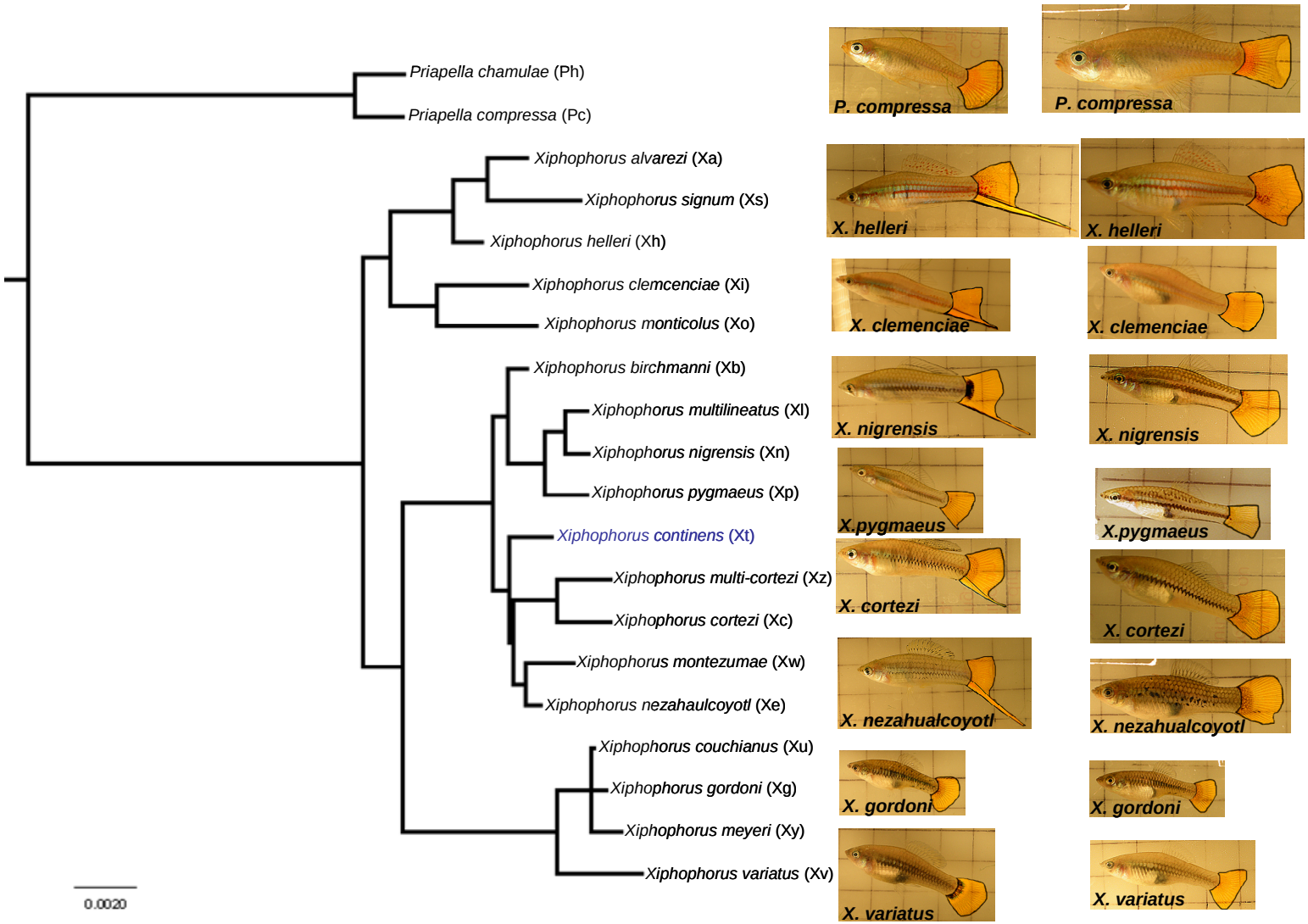

Priapella chamulae (Ph)
Priapella compressa (Pc)
Xiphophorus alvarezi (Xa)
Xiphophorus signum (Xs)
Xiphophorus helleri (Xh)
Xiphophorus clemcenciae (Xi)
Xiphophorus monticolus (Xo)
Xiphophorus birchmanni (Xb)
Xiphophorus multilineatus (Xl)
Xiphophorus nigrensis (Xn)
Xiphophorus pygmaeus (Xp)
Xiphophorus continens (Xt)
Xiphophorus multi-cortezi (Xz)
Xiphophorus cortezi (Xc)
Xiphophorus montezumae (Xw)
Xiphophorus nezahaulcoyotl (Xe)
Xiphophorus couchianus (Xu)
Xiphophorus gordoni (Xg)
Xiphophorus meyeri (Xy)
Xiphophorus variatus (Xv)
0.0020
P. compressa
P. compressa
X. helleri
X. helleri
X. clemenciae
X. clemenciae
X. nigrensis
X. nigrensis
X.pygmaeus
X.pygmaeus
X. cortezi
X. cortezi
X. nezahualcoyotl
X. nezahualcoyotl
X. gordoni
X. gordoni
X. variatus
X. variatus
Males
Females

Figure 3.1

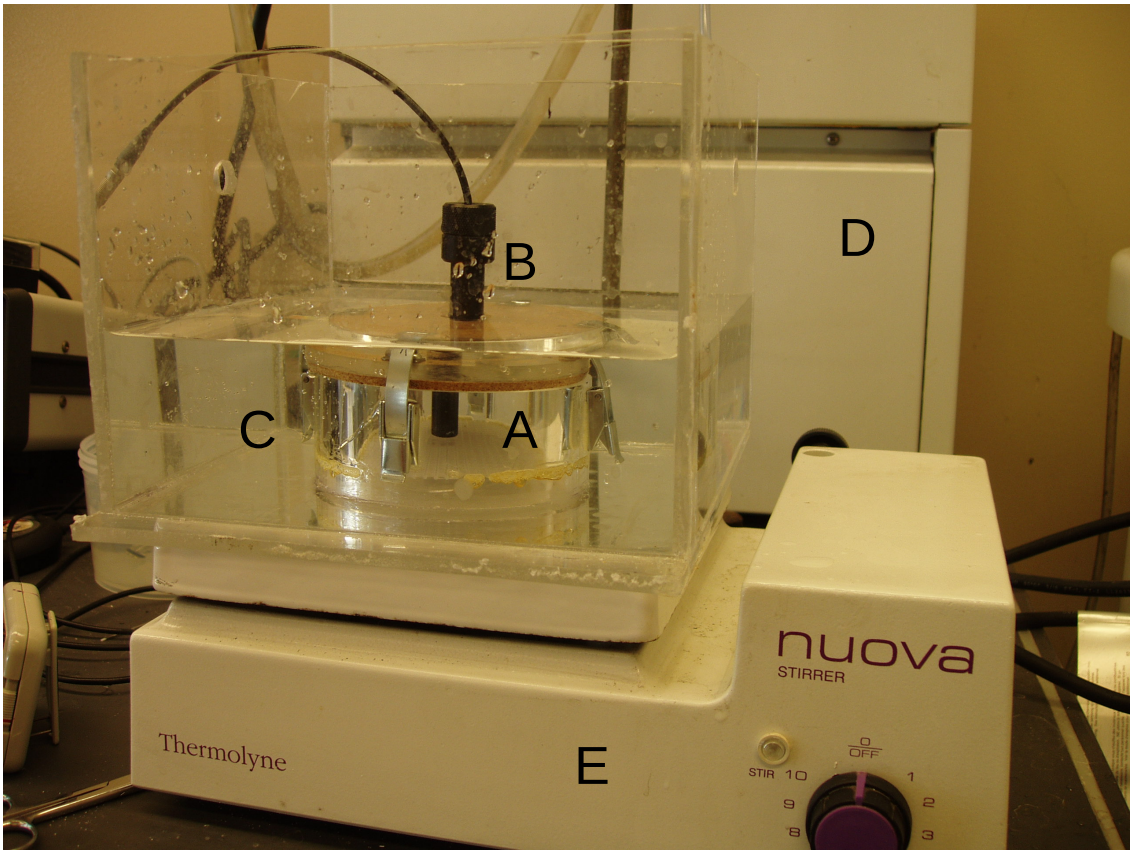


Figure 3.2. Maximum oxygen consumption experimental setup. We used closed system respirometry through forced locomotion to obtain the VO_{2max} of the fish. The respirometers (A) were saturated with O_2 and sealed by clamping the lid. The microelectrode (B) recorded the percent oxygen in the water. The entire respirometer was in a water bath (C) connected to a circulating water bath (D) that maintained temperature at 23°C. Forced locomotion was achieved by ramping the velocity of a stir bar in the respirometer (A) with the magnetic stirrer (E). The fish was considered fatigue when it could no longer maintain position in the flow.

Figure 3.3. Scatterplots for raw, non-phylogenetic trait values versus standard length (A-F, G, I, K, M, O, P) and for mass measurements against body mass (H, J, L, N) among males. Points represent species means ± standard errors.

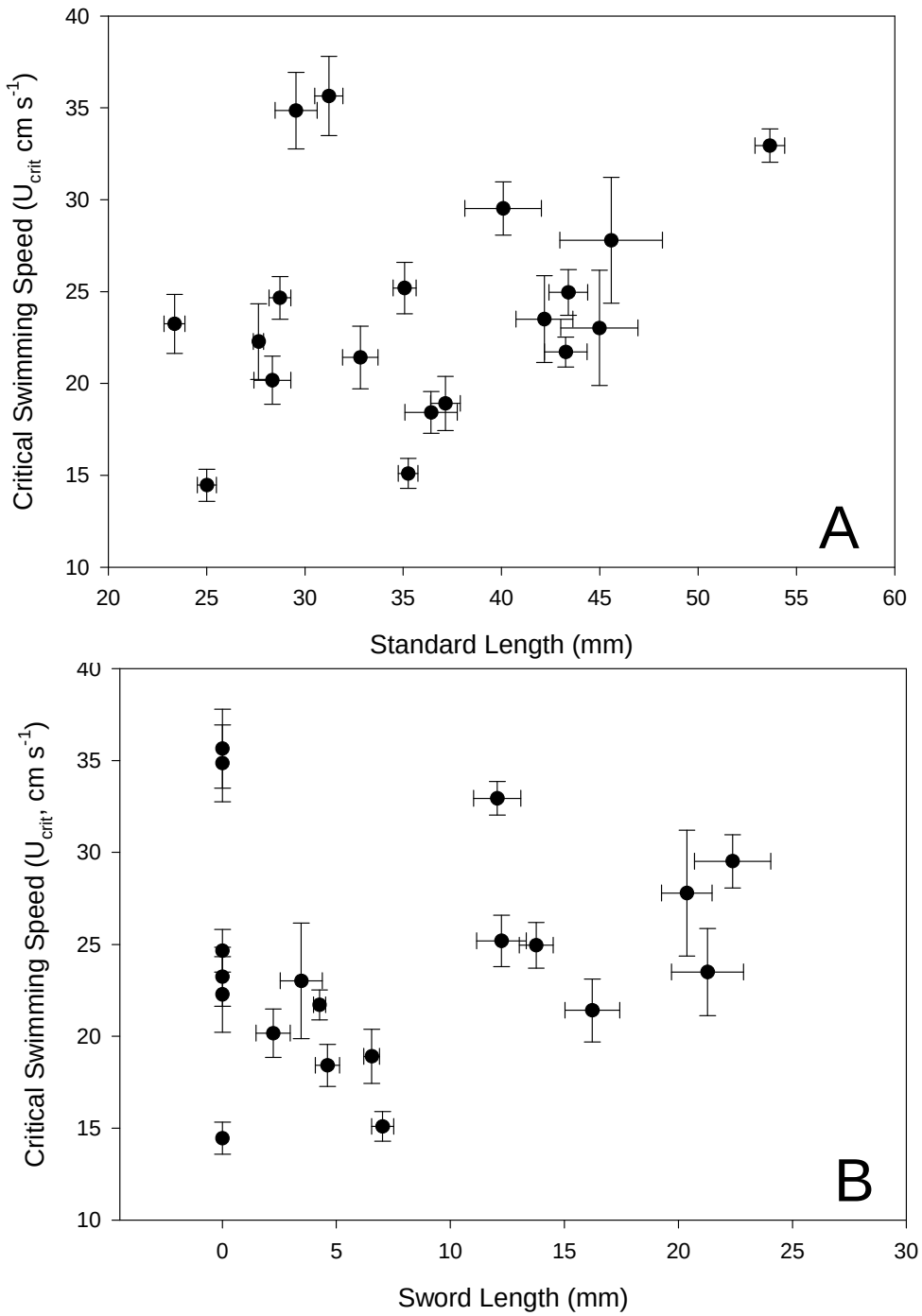


Figure 3.3 (Males)

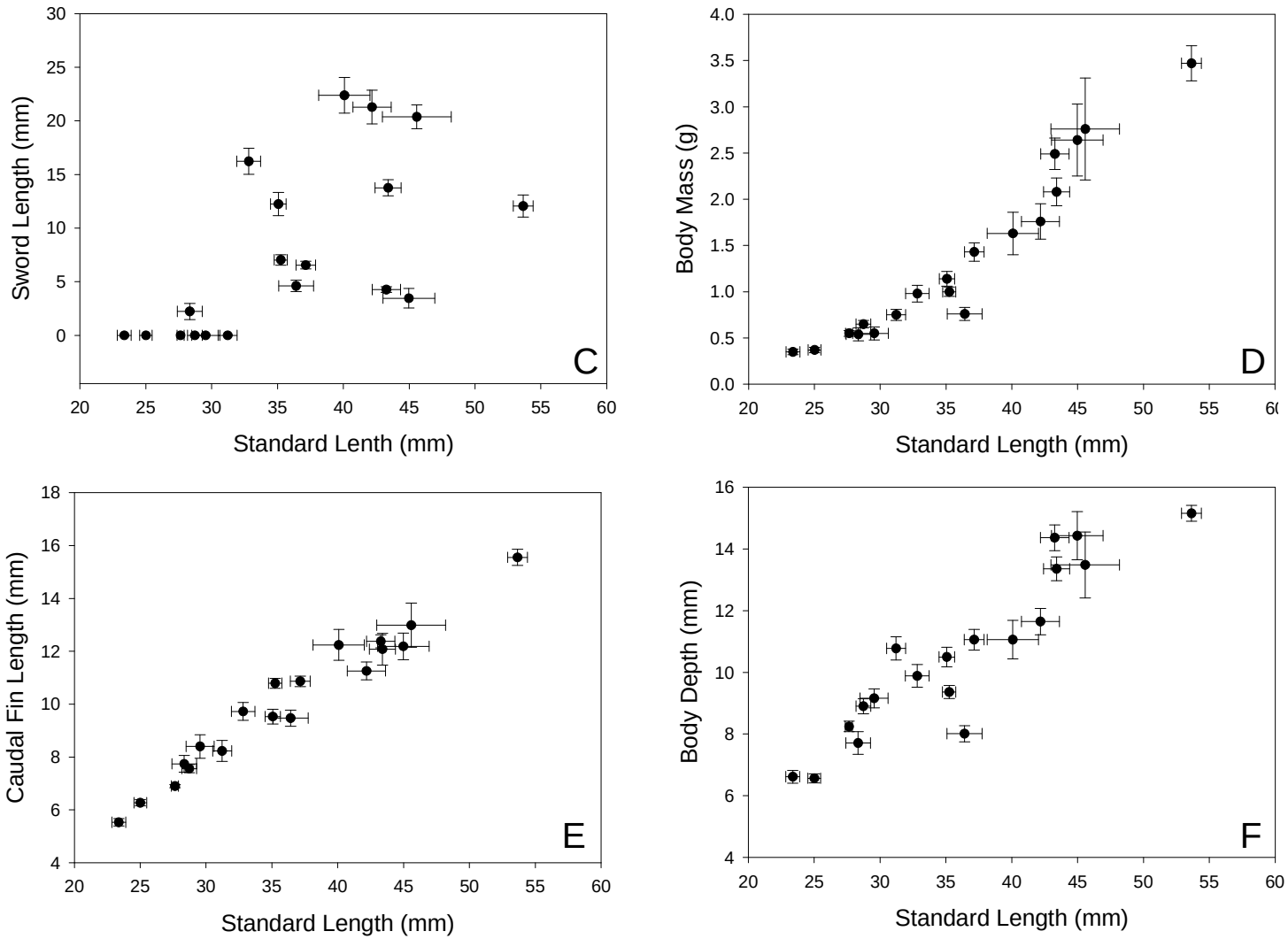


Figure 3.3, continued (Males)

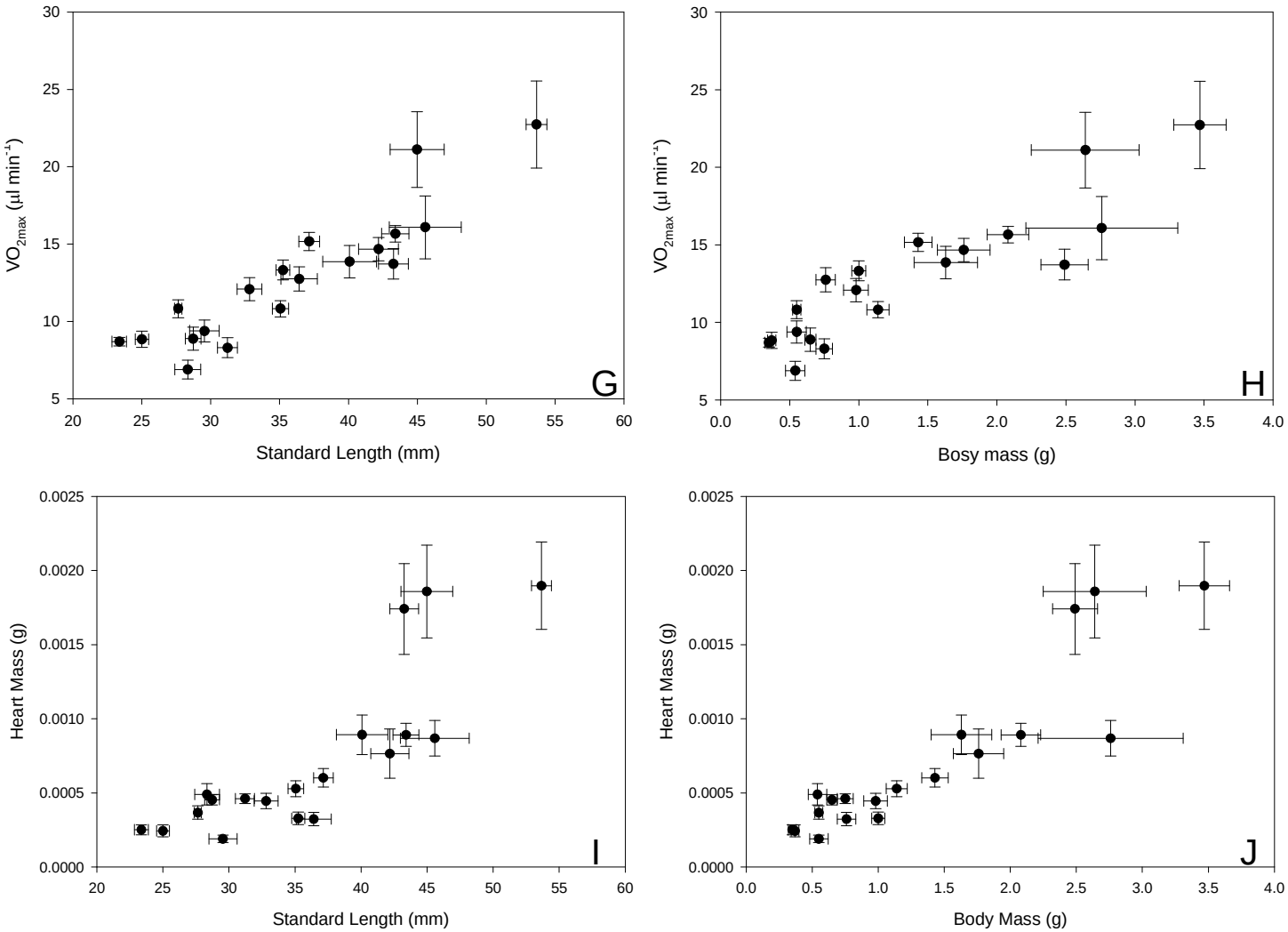


Figure 3.3, continued (Males)

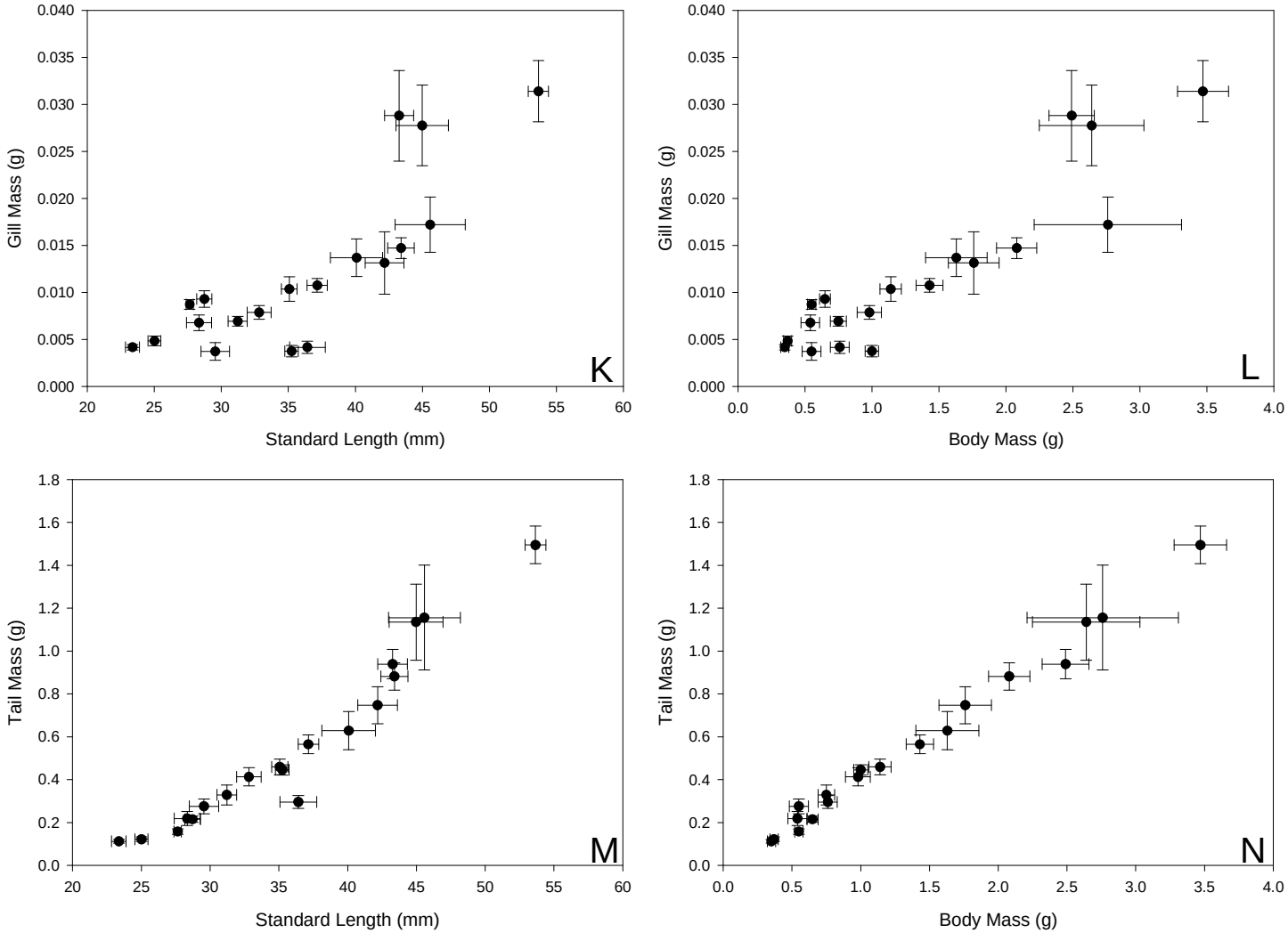


Figure 3.3, continued (Males)

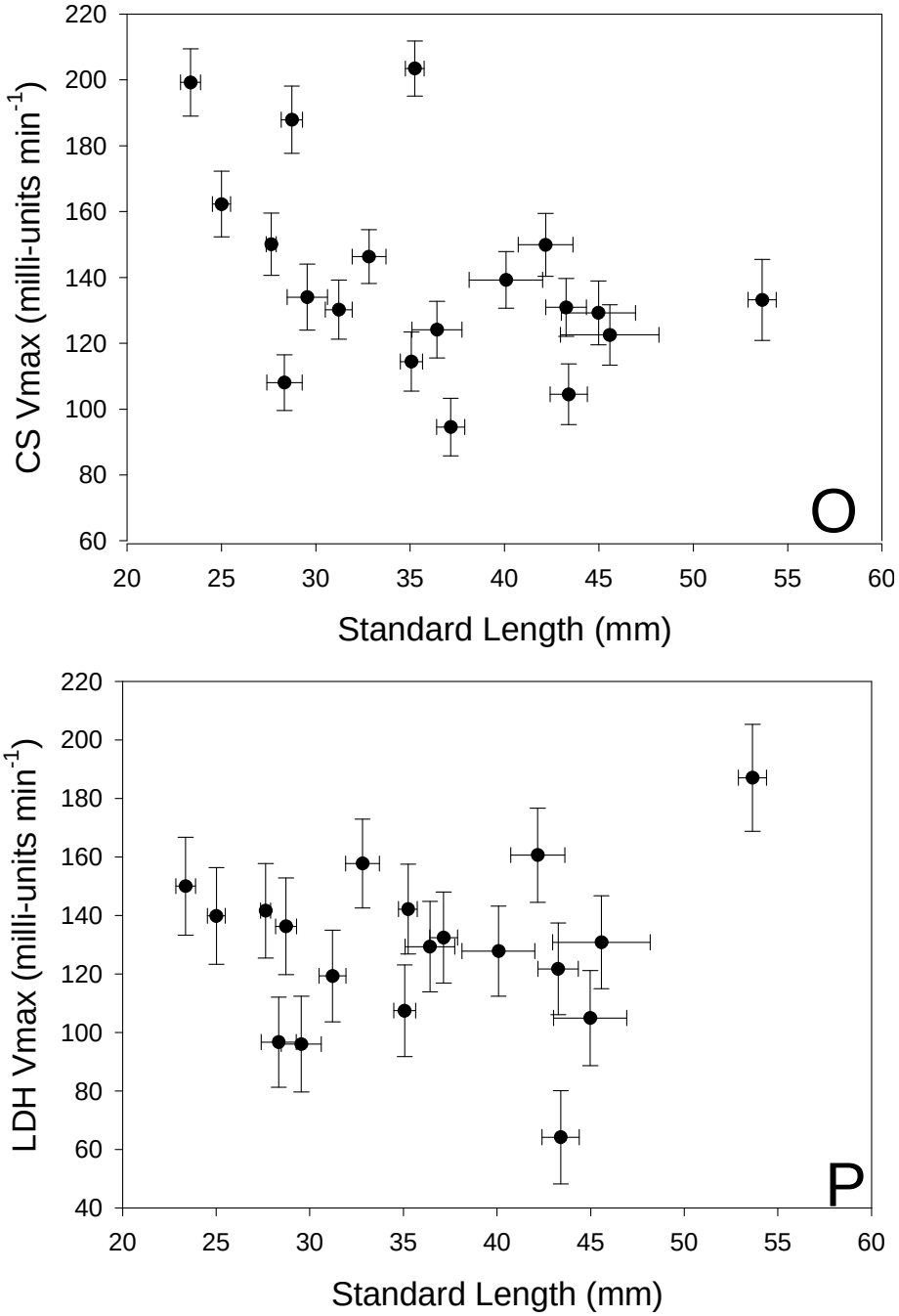


Figure 3, continued (Males)

Figure 3.4. Scatterplots for $\log_{10}$ transformed, non-phylogenetic trait values versus standard length (A-F, G, I, K, M) and for mass measurements against body mass (H, J, L, N) among males. Points represent species means ± standard errors.

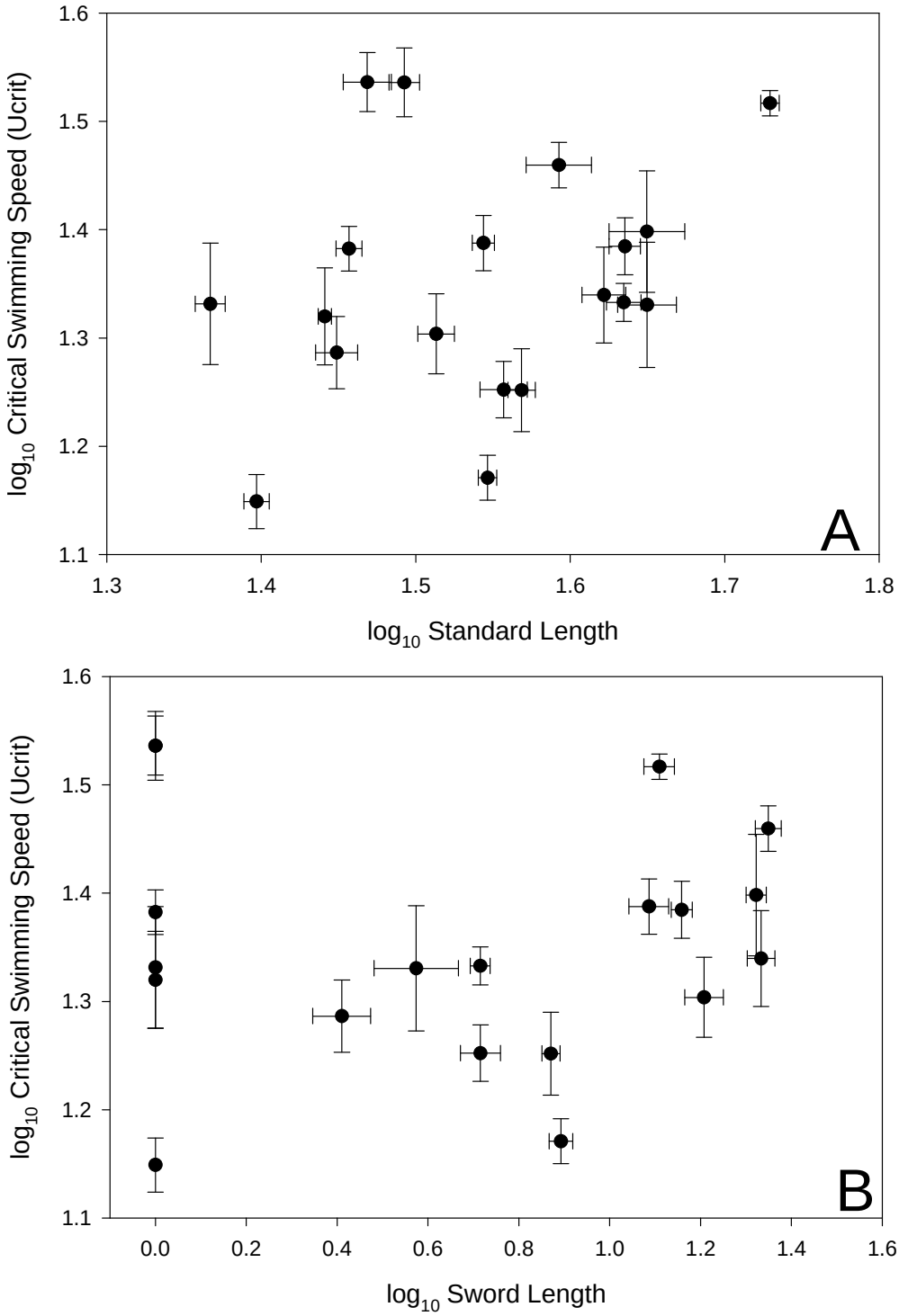


Figure 3.4 (Males)

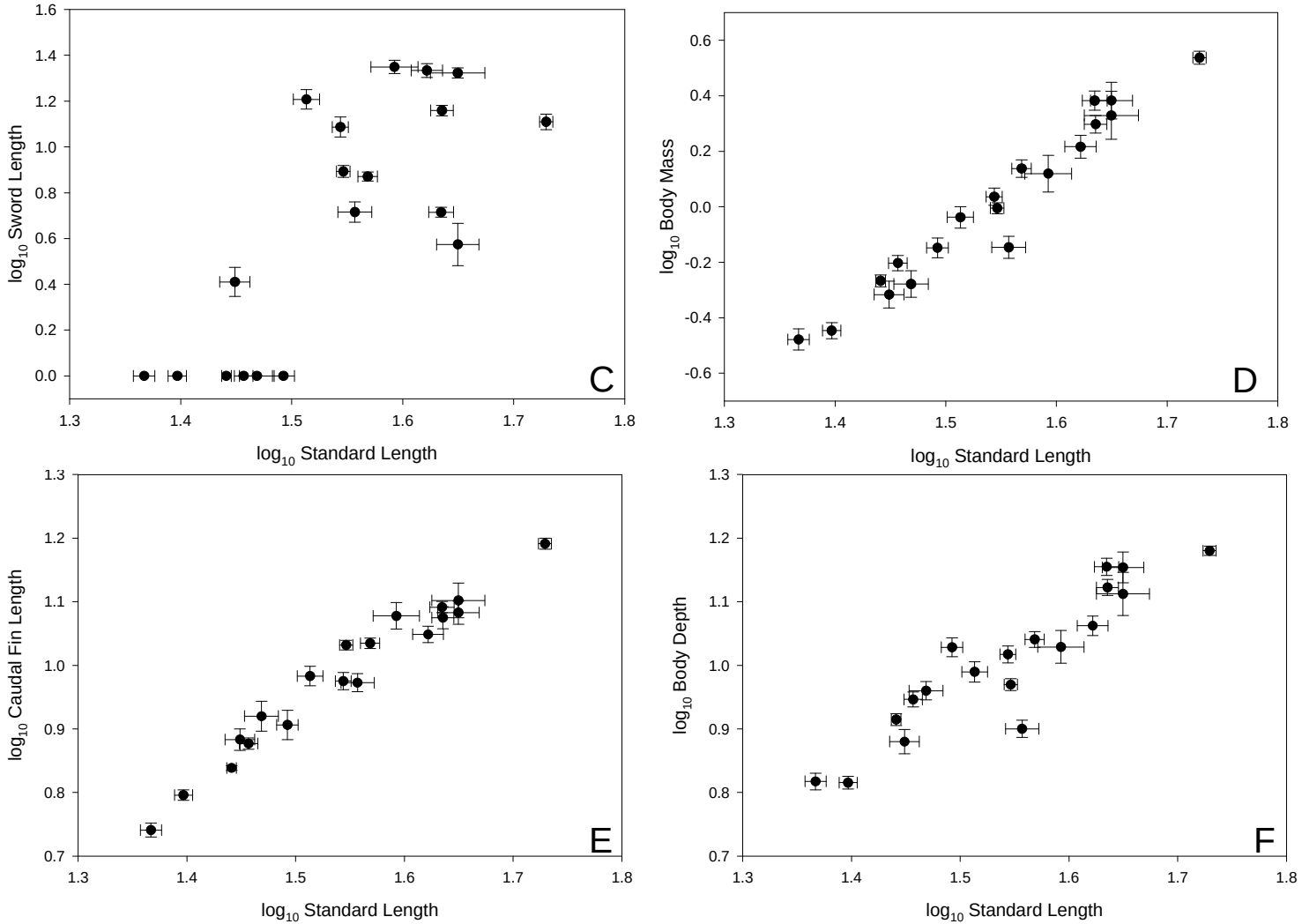


Figure 3.4, continued (Males)

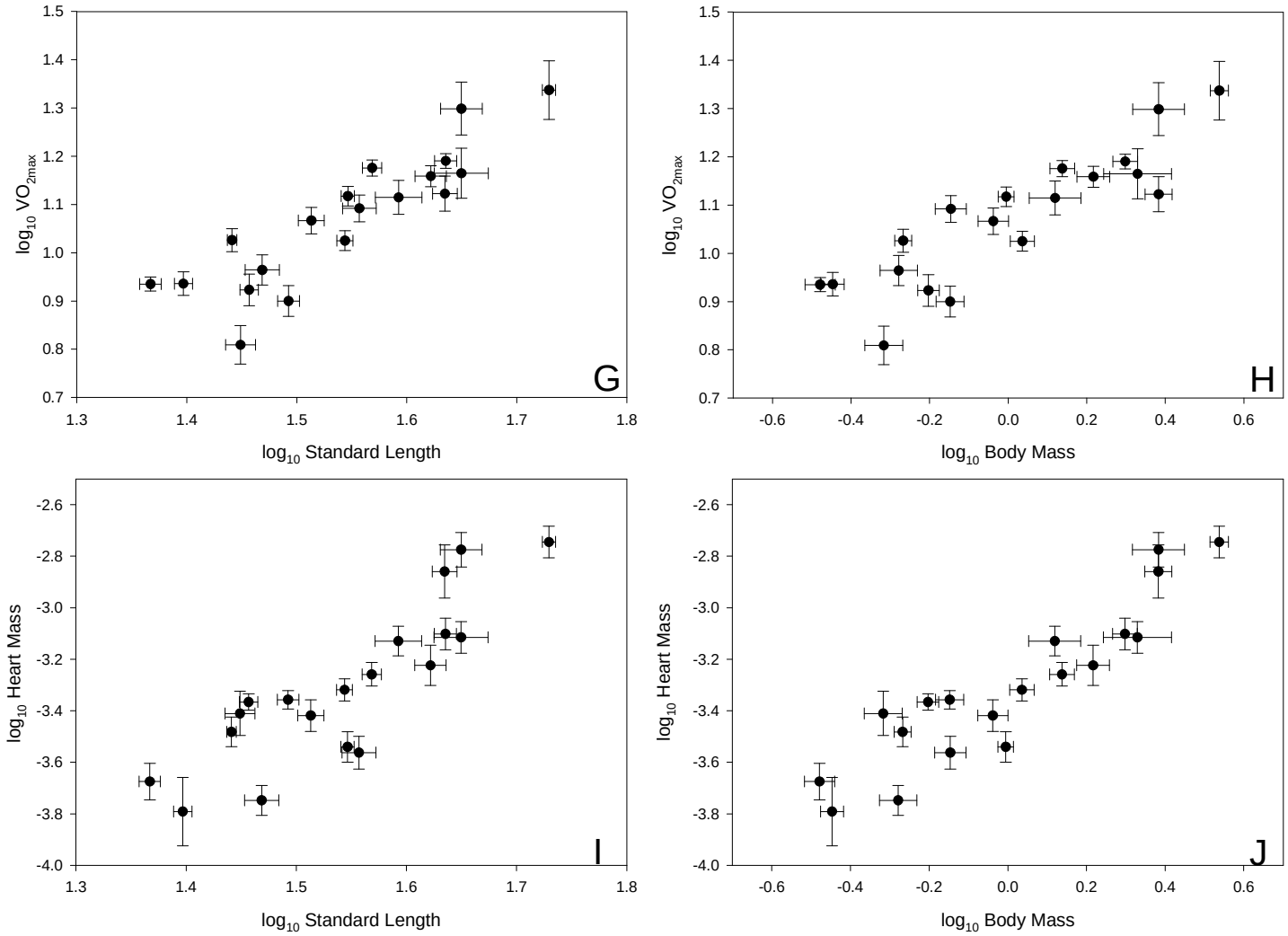


Figure 3.4, continued (Males)

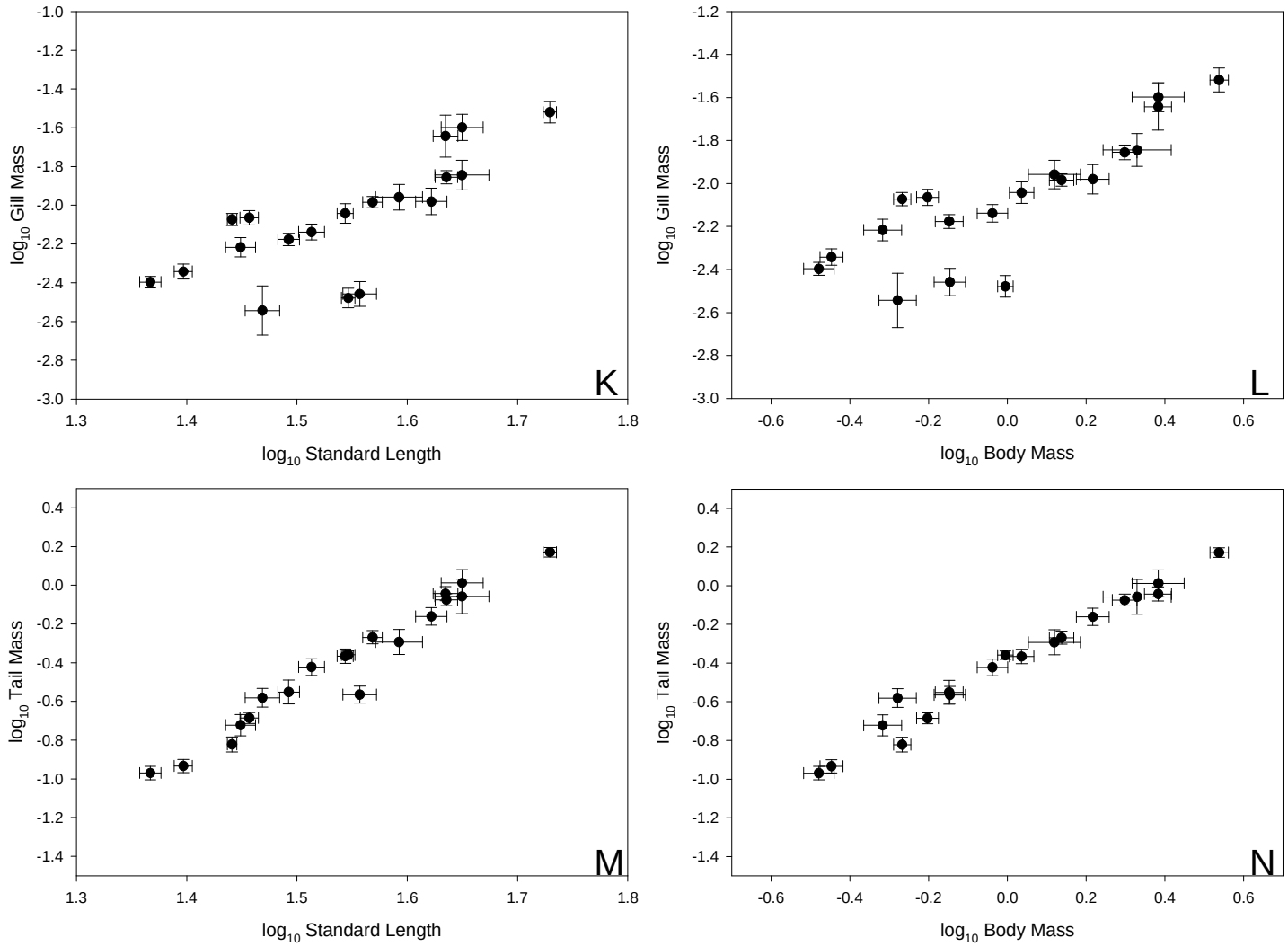


Figure 3.4, continued (Males)

Figure 3.5. Scatterplots for raw, non-phylogenetic trait values versus standard length (A-F, G, I, K, M, O, P) and for mass measurements against body mass (H, J, L, N) among females. Points represent species means ± standard errors.

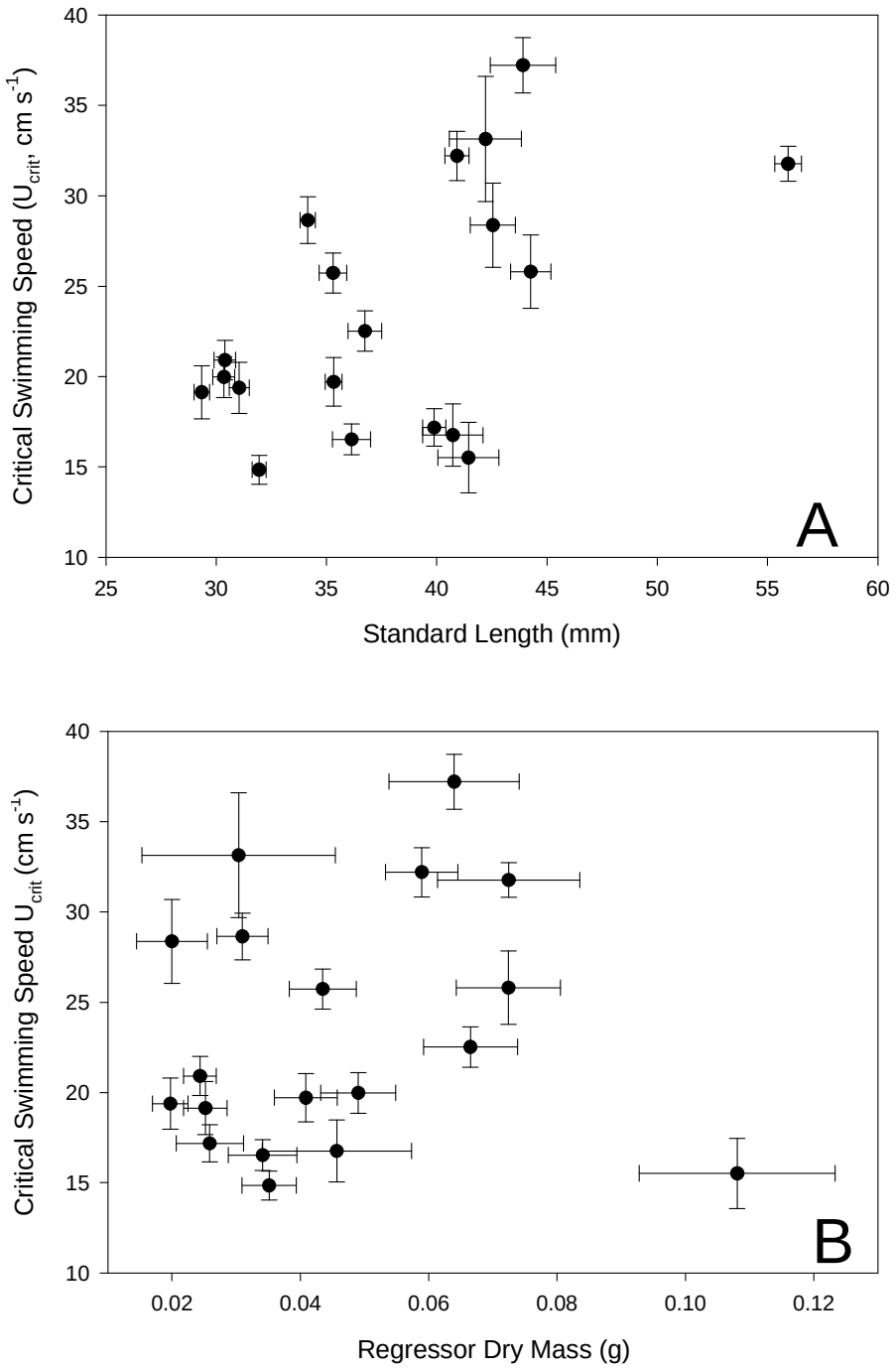


Figure 3.5 (Females)

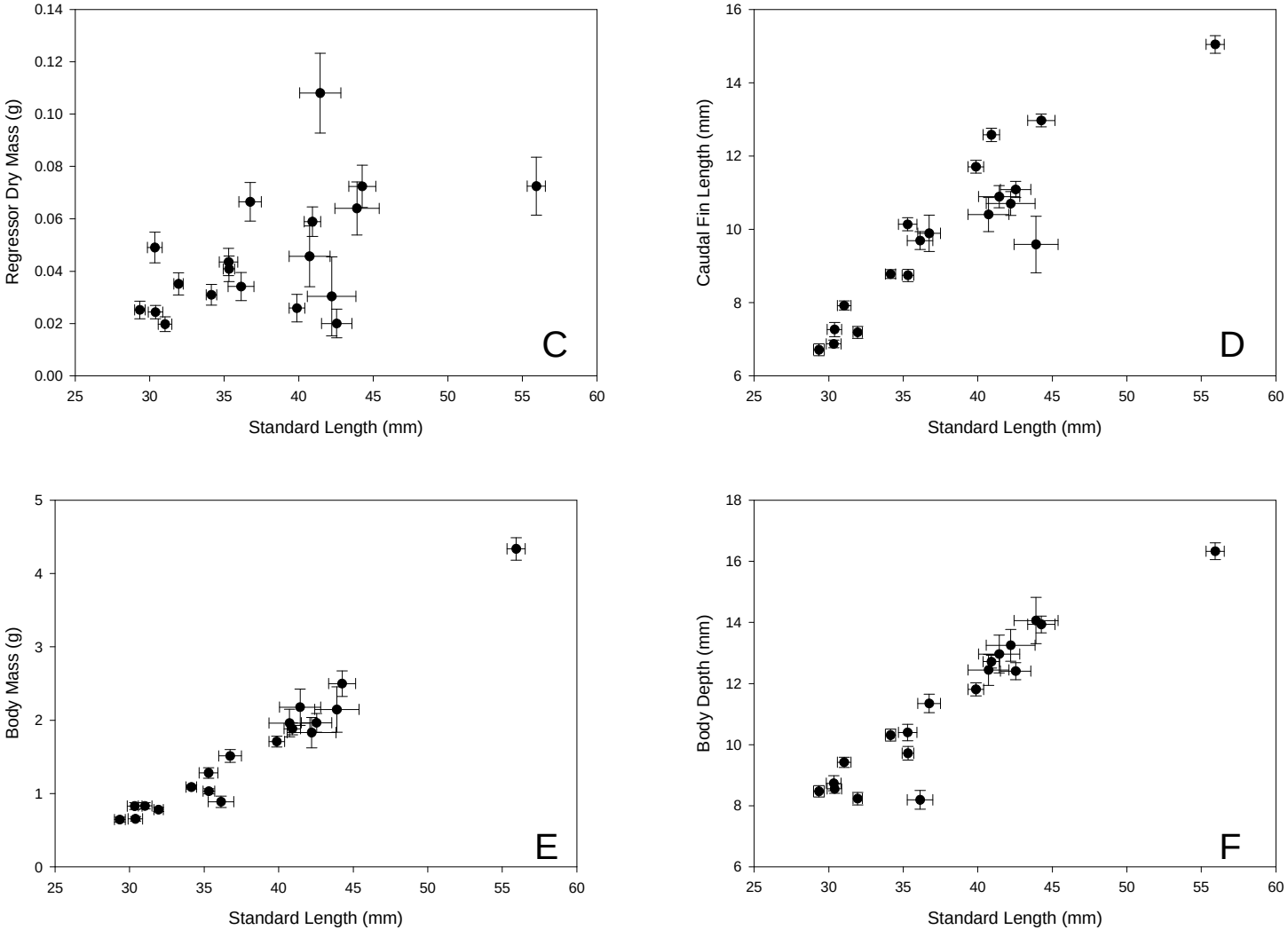


Figure 3.5, continued (Females)

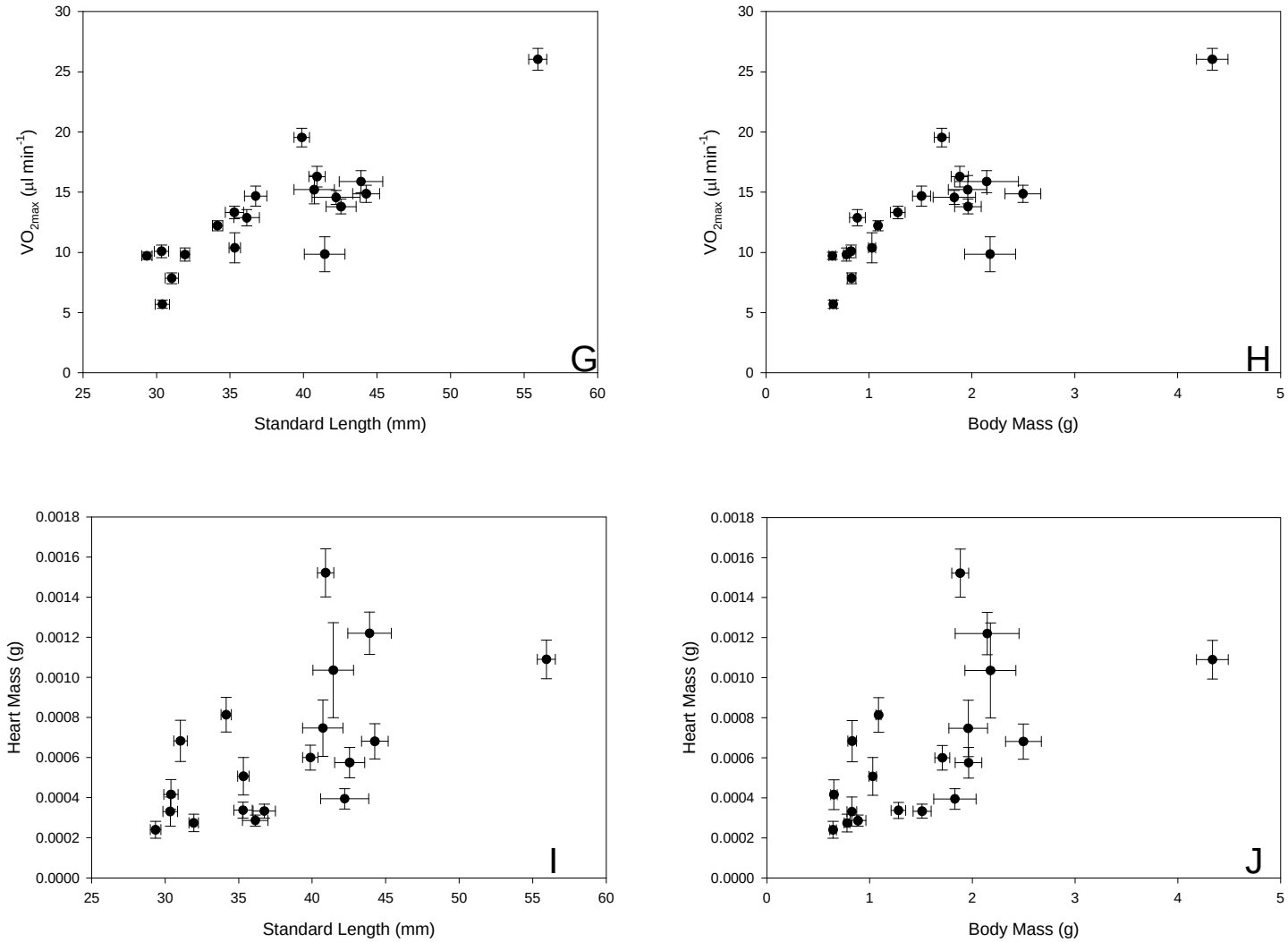


Figure 3.5, continued (Females)

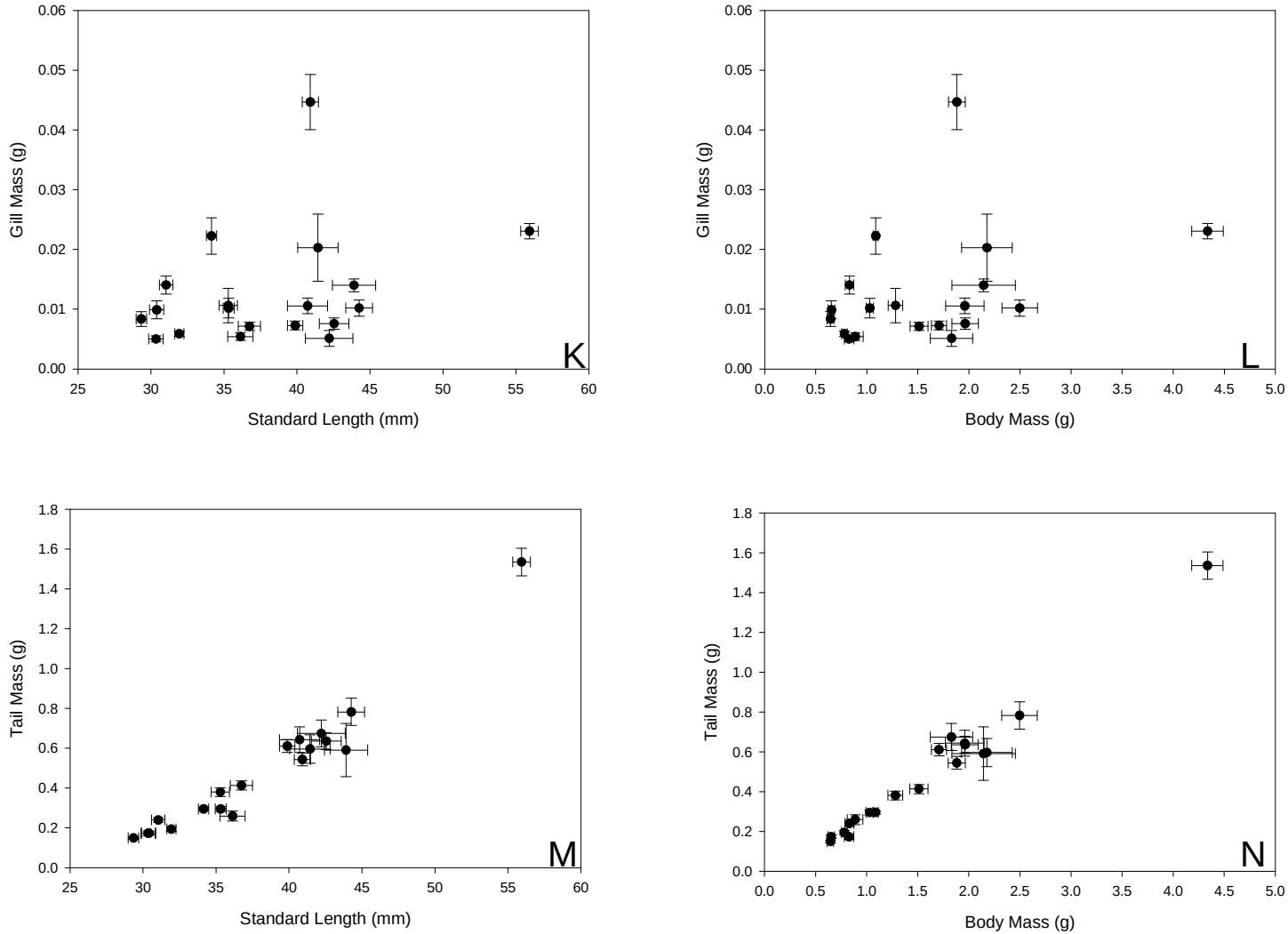
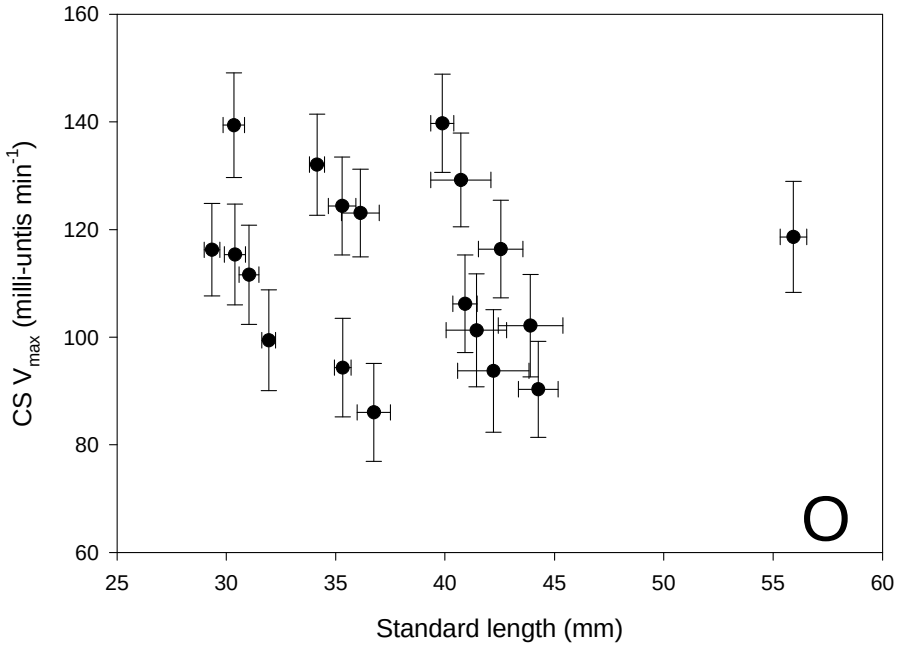


Figure 3.5, continued (Females)



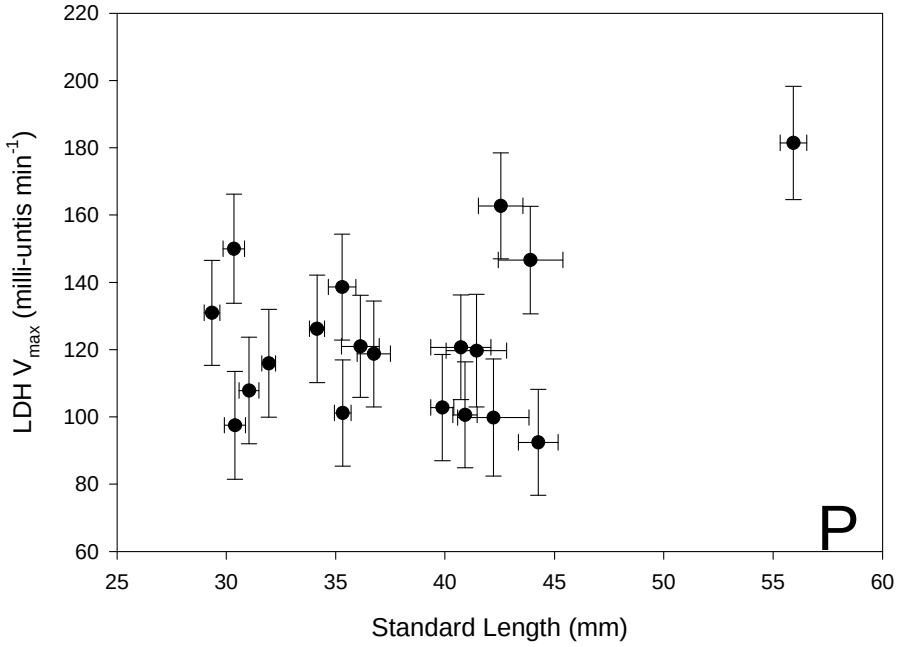
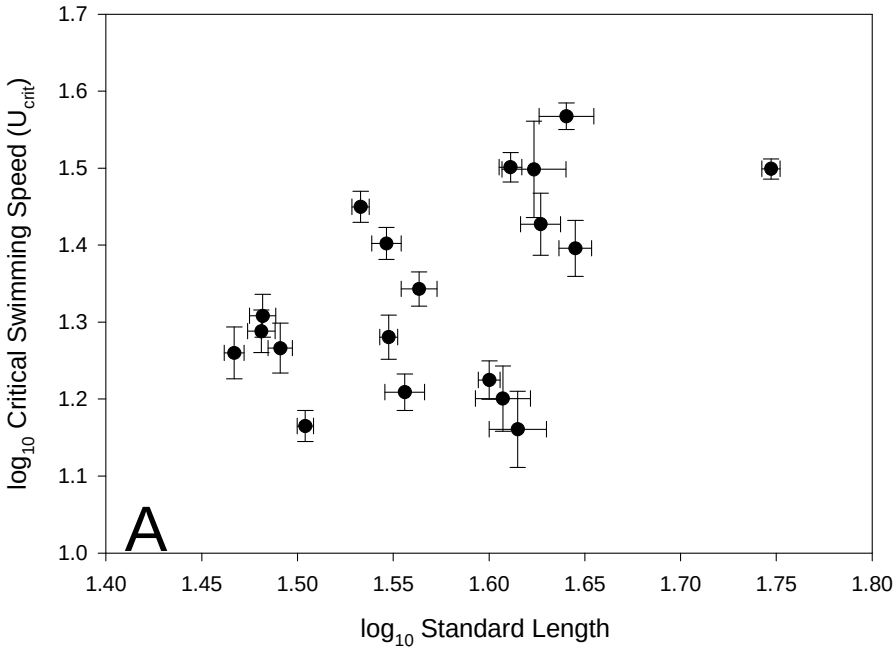


Figure 3.5, continued (Females)

Figure 3.6. Scatterplots for $\log_{10}$ transformed, non-phylogenetic trait values versus standard length A-F, G, I, K, M) and for mass measurements against body mass (H, J, L, N) among females. Points represent species means ± standard errors. Note the large standard errors in (B & C) are due to a small sample size (N = 3) for reproductive data in *Priapella chamulae* (see Appendices 3.7 & 3.8).



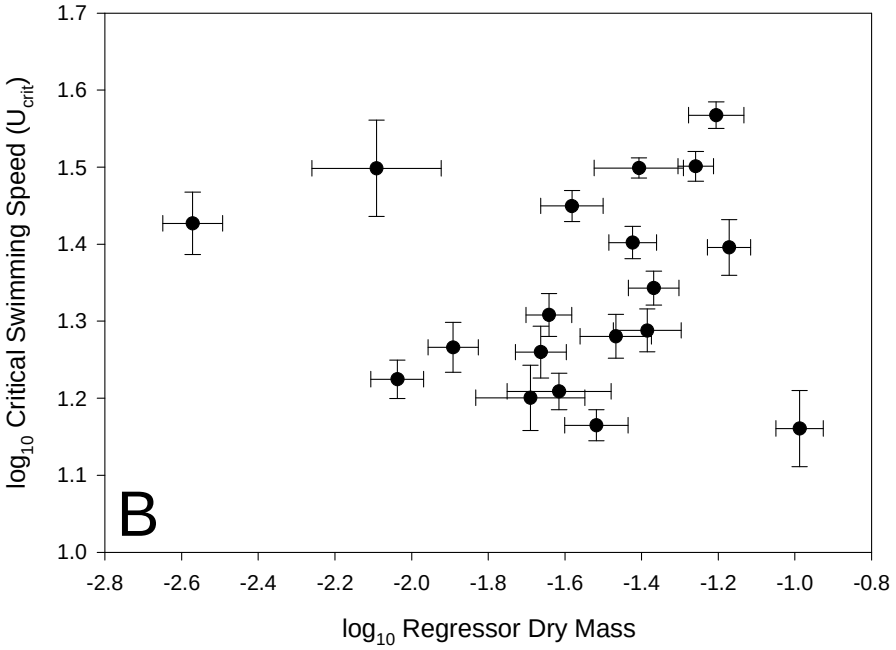


Figure 3.6 (Females)

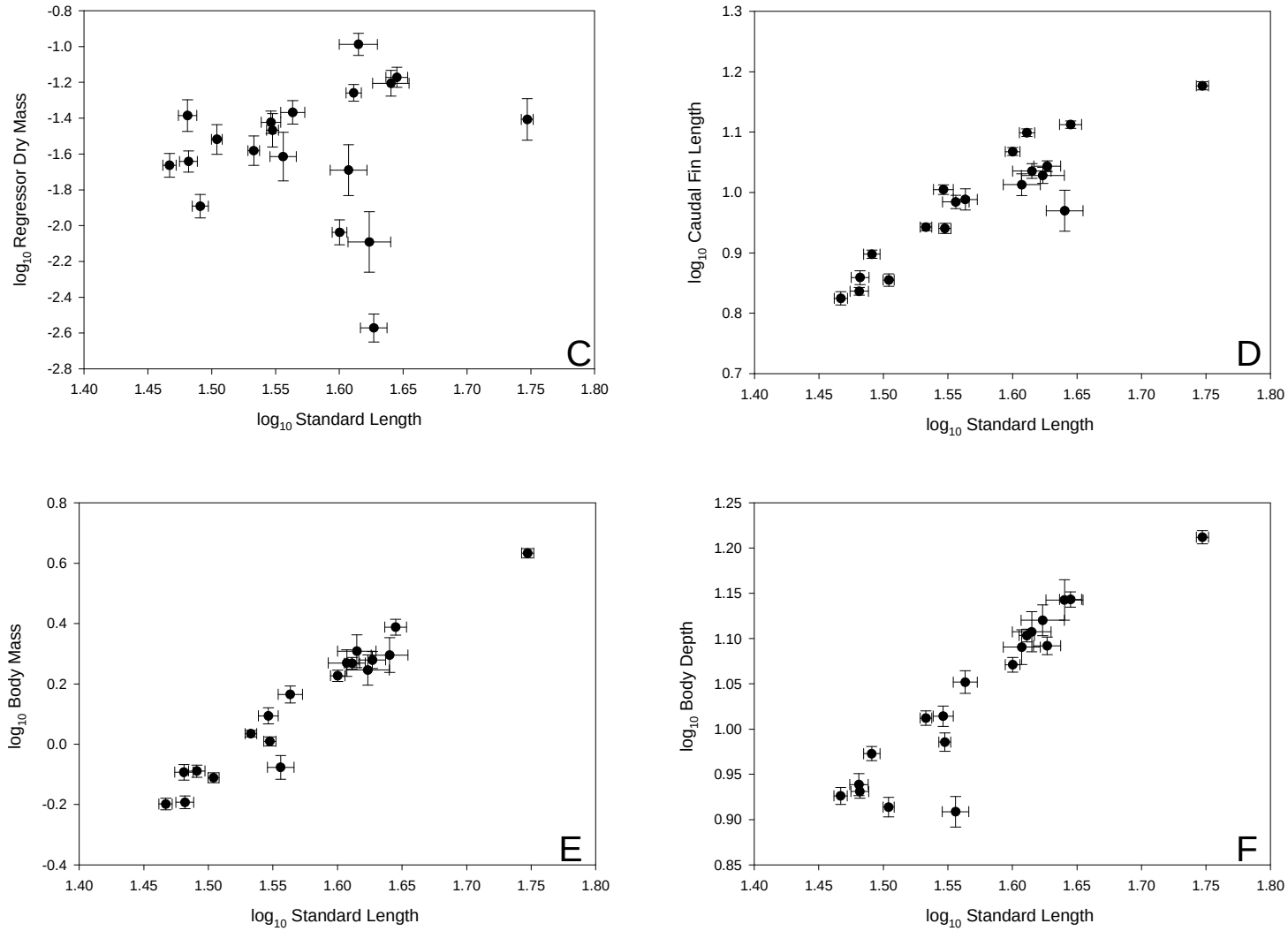


Figure 3.6, continued (Females)

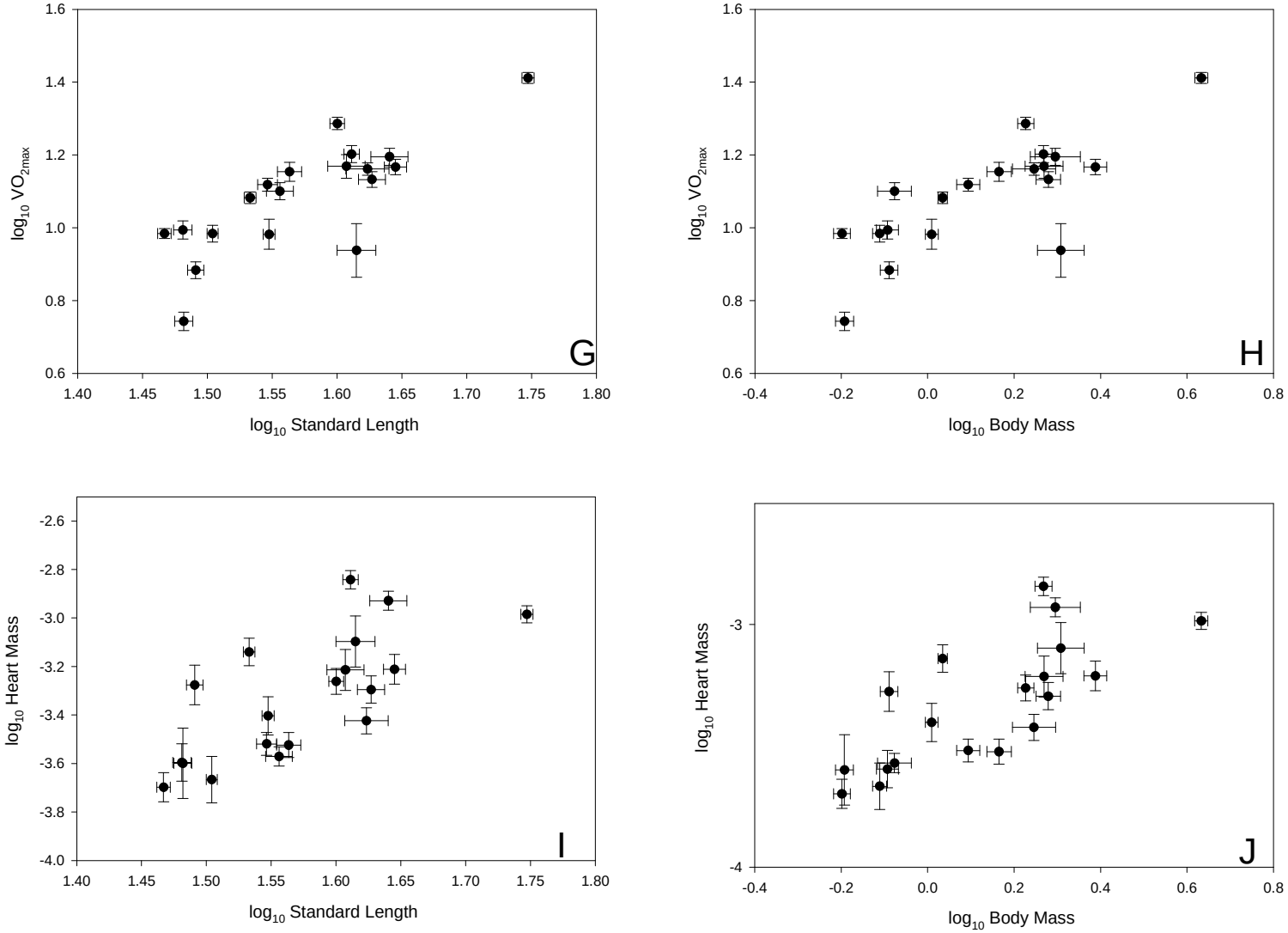


Figure 3.6, continued (Females)

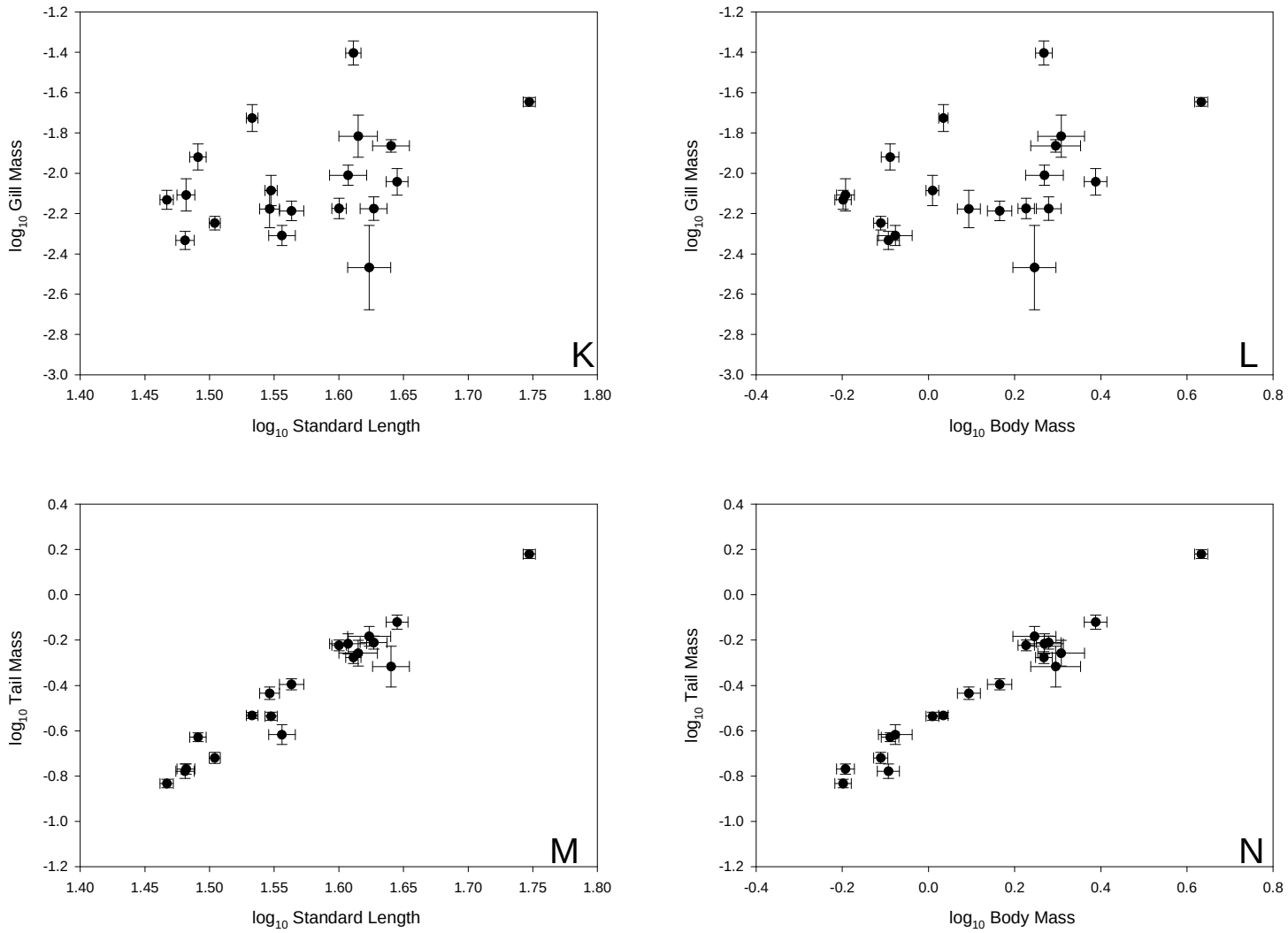


Figure 3.6, continued (Females)

Figure 3.7. Scatterplots of residual $\log_{10}$ critical swimming speed (U_{crit}) versus candidate residual $\log_{10}$ predictors used in multiple regression analyses (see *Statistical Analyses*) for males. Points represent residual $\log_{10}$ values based on non-phylogenetic species means. Residuals obtained from regressions with standard length, and standard length and preservation time for heart and gill mass.

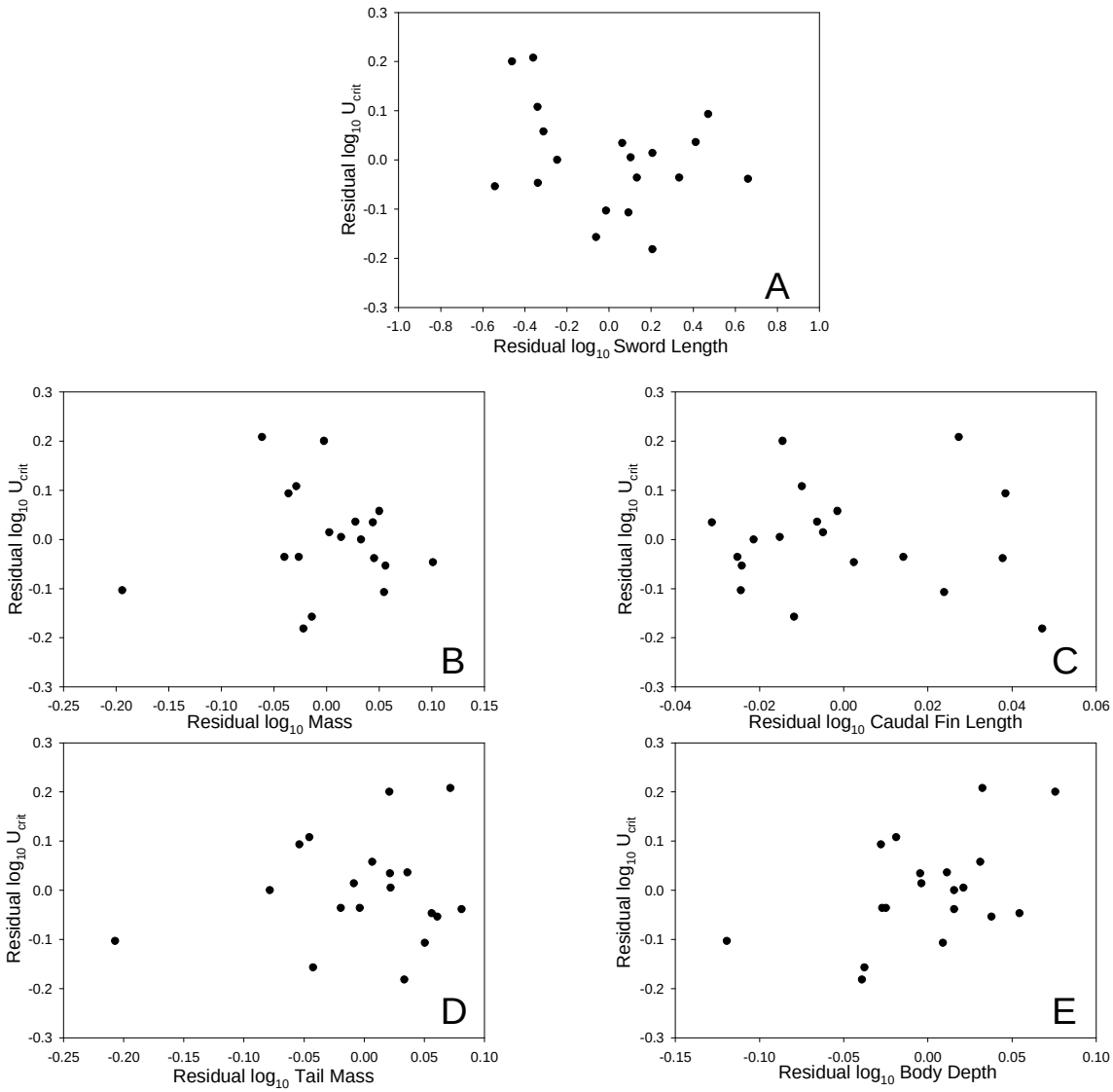


Figure 3.7 (Males)

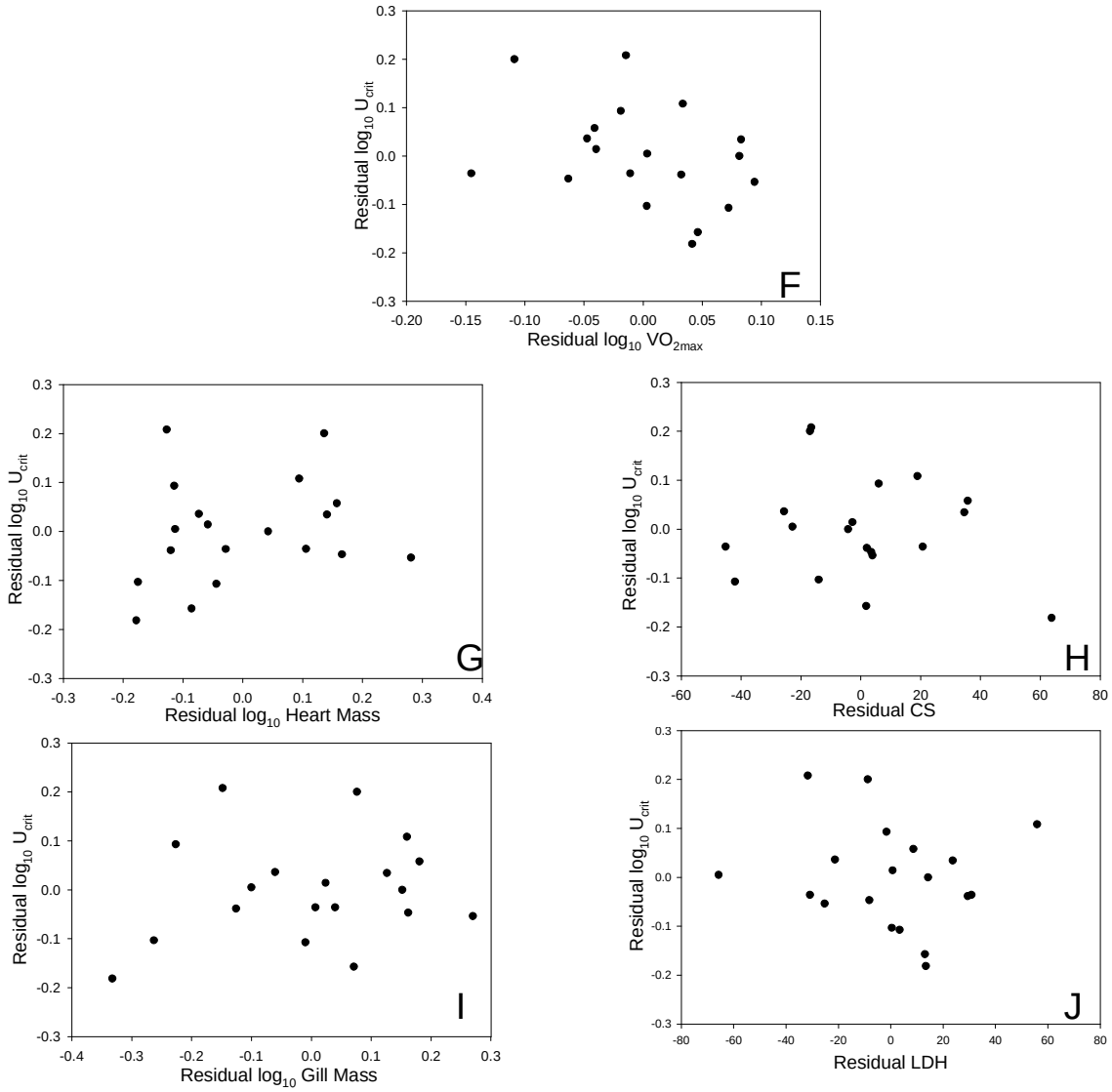


Figure 3.7, continued (Males)

Figure 3.8. Scatterplots of residual $\log_{10}$ critical swimming speed (U_{crit}) versus candidate residual $\log_{10}$ predictors used in multiple regression analyses (see *Statistical Analyses*) for females. Points represent residual $\log_{10}$ values based on non-phylogenetic species means. Residuals obtained from regressions with standard length, and standard length and preservation time for heart and gill mass.

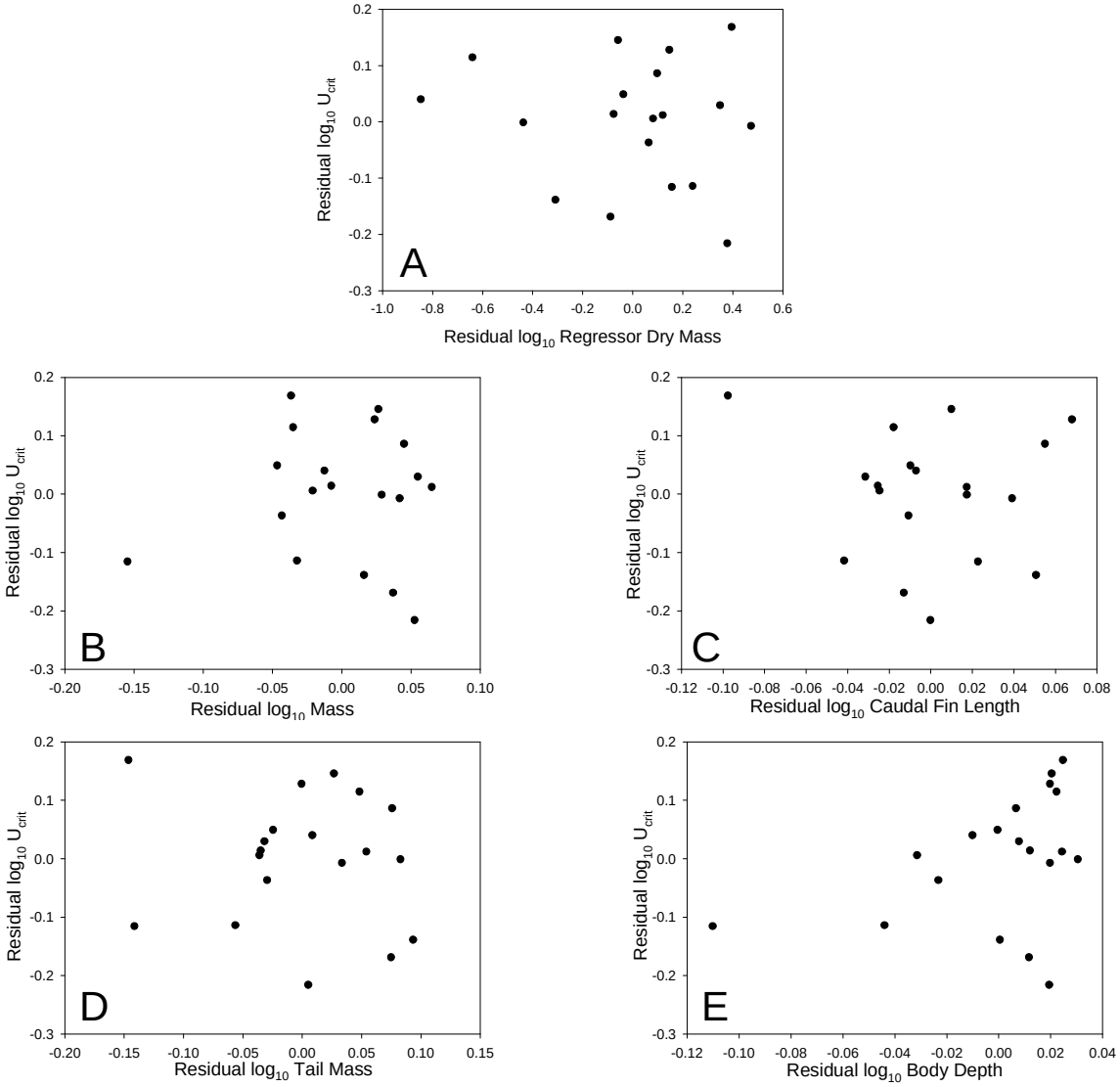


Figure 3.8 (Females)

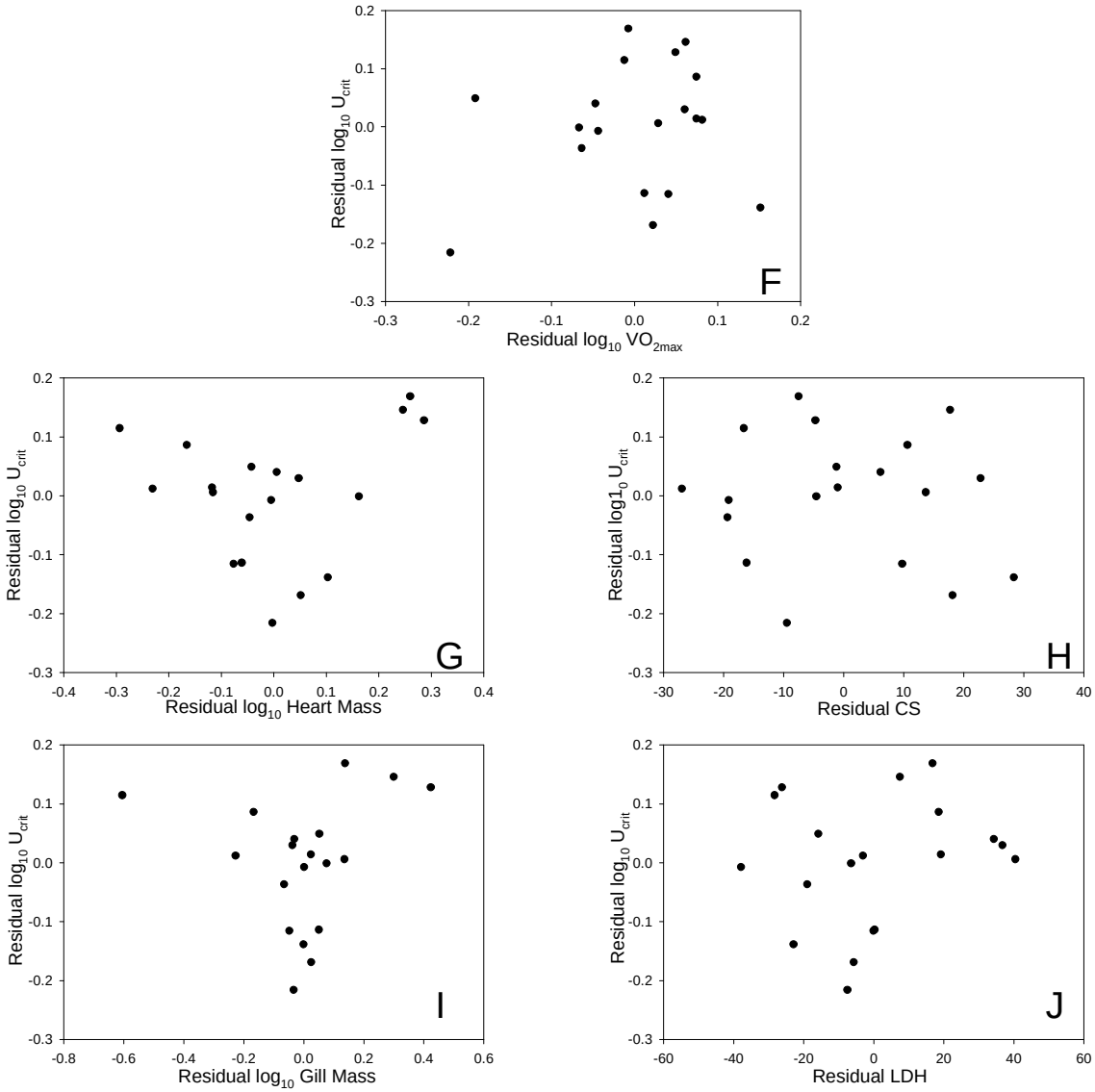


Figure 3.8, continued (Females)

3.7 Appendices

Appendix 3.1. Species list, sample sizes and sources for the species used in the study. Order matches the order of the phylogenetic hypothesis depicted in figure 3.1. *X. continens* and *X. cortezi* (Pop. 2) not used in final analyses.

Species (tip names)	Sex	N	Source
P. chamulae (Ph)	Males	8	Manfred Schartl's Lab, University of Wuerzburg
	Females	6	
P. compressa (Pc)	Males	16	Manfred Schartl's Lab, University of Wuerzburg
	Females	9	
X. alvarezi (Xa)	Males	14	Hobby Industry
	Females	12	
X. signum (Xs)	Males	6	The *Xiphophorus* Genetic Stock Center, Texas State University
	Females	15	
X. helleri (Xh)	Males	21	Hobby Industry
	Females	17	
X. clemenciae (Xi)	Males	16	Manfred Schartl's Lab, University of Wuerzburg
	Females	15	
X. monticolus (Xo)	Males	17	Manfred Schartl's Lab, University of Wuerzburg
	Females	15	
X. birchmanni (Xb)	Males	9	Mike Ryan's Lab University of Texas, Austin
	Females	11	
X. multilineatus (Xl)	Males	18	Mike Ryan's Lab University of Texas, Austin
	Females	16	
X. nigrensis (Xn)	Males	19	Mike Ryan's Lab University of Texas, Austin
	Females	18	
X. pygmaeus (Xp)	Males	17	Mike Ryan's Lab University of Texas, Austin
	Females	17	
X. continens (Xt)	Males	1	Mike Ryan's Lab University of Texas, Austin
	Females	1	

Species (tip names)	Sex	N	Source
X. multi-cortezi (Xz)	Males	12	Mike Ryan's Lab University of Texas, Austin
	Females	7	
X. cortezi (Xc)	Males	17	The *Xiphophorus* Genetic Stock Center, Texas State University
	Females	18	
X. cortezi (Pop. 2) (Xr)	Males	11	Manfred Schartl's Lab, University of Wuerzburg
	Females	14	
X. montezumae (Xw)	Males	15	Mike Ryan's Lab University of Texas, Austin
	Females	15	
X. nezahaulcoyotl (Xe)	Males	19	Molly Morris's Lab, Ohio University
	Females	19	
X. couchianus (Xu)	Males	16	The *Xiphophorus* Genetic Stock Center, Texas State University
	Females	16	
X. gordoni (Xg)	Males	14	The *Xiphophorus* Genetic Stock Center, Texas State University
	Females	18	
X. meyeri (Xy)	Males	16	The *Xiphophorus* Genetic Stock Center, Texas State University
	Females	14	
X. variatus (Xv)	Males	20	Molly Morris's Lab, Ohio University
	Females	17	

Appendix 3.2A. PDI file corresponding to the phylogenetic tree in Fig. 3.1, and used in analyses. *X. continens* not included in analyses or in PDI file. File is c:\xipho\MSWsluc.PDI. First trait is $\log_{10}$ standard length, second is $\log_{10}$ U_{crit}.

```
19

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Ph  1A   1.6485600000E-03
Pc  1A   1.5849510000E-03
1B  rt   1.0660050000E-02
1C  1B   8.8847000000E-04
1D  1C   1.9817920000E-03
1E  1D   1.1227640000E-03
Xa  1E   1.3101210000E-03
Xs  1E   3.0148140000E-03
Xh  1D   1.0059870000E-03
1F  1C   1.4603370000E-03
Xi  1F   2.9610930000E-03
Xo  1F   3.2596180000E-03
1G  1B   1.2852130000E-03
1H  1G   2.8702610000E-03
1I  1H   4.8041800000E-04
Xb  1I   6.8629500000E-04
1J  1I   1.1818130000E-03
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Xl  1K   7.7693000000E-04
Xn  1K   7.8437400000E-04
Xp  1J   1.4286280000E-03
1M  1H   6.7010500000E-04
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Xc  z1   1.7521730000E-03
1O  1M   3.8855000000E-04
Xw  1O   1.5659510000E-03
Xe  1O   5.2185800000E-04
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Xg  1R   5.2076100000E-04
Xy  1S   1.0350490000E-03
Xv  1Q   2.7864990000E-03

Ph   1.4686056070E+00   1.5364252670E+00
Pc   1.4925997640E+00   1.5360538800E+00
Xa   1.6496650870E+00   1.3983275220E+00
Xs   1.7293325550E+00   1.5168875060E+00
```

Xh	1.5926750430E+00	1.4598465250E+00
Xi	1.5465327130E+00	1.1709152330E+00
Xo	1.5569464130E+00	1.2524045210E+00
Xb	1.6497456500E+00	1.3307041860E+00
Xl	1.6354098910E+00	1.3846457550E+00
Xn	1.5438006710E+00	1.3877170480E+00
Xp	1.4488815820E+00	1.2865674460E+00
Xz	1.6346569730E+00	1.3329015920E+00
Xc	1.5685521720E+00	1.2519486800E+00
Xw	1.6218130450E+00	1.3397216920E+00
Xe	1.5132883650E+00	1.3038128060E+00
Xu	1.3670295590E+00	1.3315646440E+00
Xg	1.4411980370E+00	1.3199734200E+00
Xy	1.3969683920E+00	1.1491015220E+00
Xv	1.4567490010E+00	1.3825417000E+00

Appendix 3.2B. DSC file corresponding to the phylogenetic tree in Fig. 3.1, and used in analyses. *X. continens* not included in analyses or in PDI file. File is c:\xipho\sword_4.DSC

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0.01066
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0.01195 0.01195 0.01195 0.01195 0.01195 0.01195 0.01686 0.01686 0.01686
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Appendix 3.3. Means ± standard errors for critical swimming speed (U_{crit}), maximum oxygen consumption (VO_{2max}), citrate synthase activity (CS V_{max}) and lactate dehydrogenase activity (LDH V_{max}) for each sex among the 21 species/populations. Only one male and one female tested for *X. continens*. In final phylogenetic analyses *X. continens* and *X. cortezi* (Pop. 2) were not included. Order of species matches phylogenetic hypothesis depicted in Figure 3.1 and Appendix 3.2.

Species (tip names)	Sex	U_{crit} (cm s^{-1})	VO_{2max} (µl min^{-1})	CS V_{max} (milli-untis min^{-1})[&]	LDH V_{max} (milli-untis min^{-1})[&]
P. chamulae (Ph)	M	34.8500 ±2.0897	9.3773 ±0.7138	133.98 ±10.0187	96.05 ±16.3711
	F	33.1421 ±3.4620	14.5673 ±0.5757	93.73 ±11.4008	99.80 ±17.3914
P. compressa (Pc)	M	35.6424 ±2.1511	8.3011 ±0.6401	130.20 ±8.9437	119.34 ±15.6335
	F	37.2145 ±1.5202	15.8786 ±0.9136	102.12 ±9.5035	146.65 ±16.0217
X. alvarezi (Xa)	M	27.7856 ±3.4185	16.0782 ±2.0285	122.52 ±9.2015	130.86 ±15.8791
	F	25.8028 ±2.0274	14.8713 ±0.7185	90.30 ±8.9372	92.42 ±15.7200
X. signum (Xs)	M	32.9378 ±0.9096	22.7299 ±2.8180	133.16 ±12.2778	187.08 ±18.2665
	F	31.7676 ±0.9610	26.0355 ±0.9073	118.62 ±10.2947	181.46 ±16.8473
X. helleri (Xh)	M	29.5167 ±1.4521	13.8599 ±1.0501	139.19 ±8.5899	127.79 ±15.4134
	F	32.2005 ±1.3682	16.2973 ±0.8592	106.19 ±9.0487	100.60 ±15.7322
X. clemenciae (Xi)	M	15.0970 ±0.8105	13.3156 ±0.6367	203.43 ±8.3805	142.17 ±15.3422
	F	17.1810 ±1.0318	19.5388 ±0.7786	139.72 ±9.1404	102.76 ±15.8038
X. monticolus (Xo)	M	18.4162 ±1.1433	12.7504 ±0.7829	124.10 ±8.6438	129.35 ±15.4567
	F	16.5283 ±0.8583	12.8745 ±0.6790	123.06 ±8.1662	120.97 ±15.1872
X. birchmanni (Xb)	M	23.0171 ±3.1352	21.1132 ±2.4400	129.20 ±9.7151	104.94 ±16.2541

Species (tip names)	Sex	U_{crit} (cm s^{-1})	VO_{2max} (µl min^{-1})	CS V_{max} (milli-untis min^{-1})[&]	LDH V_{max} (milli-untis min^{-1})[&]
	F	16.7581 ±1.7228	15.2060 ±1.1772	129.20 ±8.7054	120.68 ±15.5281
X. multilineatus (Xl)	M	24.9455 ±1.2350	15.6580 ±0.5366	104.47 ±9.2209	64.19 ±15.8990
	F	19.7098 ±1.3362	10.3796 ±1.2551	94.32 ±9.1731	101.17 ±15.8146
X. nigrensis (Xn)	M	25.1933 ±1.3909	10.8213 ±0.5248	114.39 ±8.9943	107.47 ±15.6713
	F	19.3811 ±1.4149	7.8516 ±0.4583	111.59 ±9.2018	107.83 ±15.8537
X. pygmaeus (Xp)	M	20.1699 ±1.3100	6.8769 ±0.6145	108.03 ±8.4570	96.68 ±15.3867
	F	20.9099 ±1.0828	5.6867 ±0.3438	115.35 ±9.3735	97.50 ±16.0441
X. continens[¥] (Xt)	M	12.3280 ----	7.4577 ----	---- ----	---- ----
	F	21.4800 ----	7.7656 ----	---- ----	---- ----
X. multi-cortezi (Xz)	M	21.7088 ±0.8177	13.7221 ±0.9931	130.90 ±8.8134	121.77 ±15.6812
	F	15.5120 ±1.9452	9.8508 ±1.4439	101.27 ±10.4827	119.70 ±16.6899
X. cortezi (Xc)	M	18.9116 ±1.4685	15.1597 ±0.5858	94.53 ±8.7139	132.44 ±15.5218
	F	22.5192 ±1.1204	14.6768 ±0.8330	86.03 ±9.0870	118.75 ±15.7434
X. cortezi (Pop. 2)[¥] (Xr)	M	18.0508 ±1.5158	16.4834 ±0.8563	---- ----	---- ----
	F	20.7893 ±1.8182	14.2323 ±0.5575	---- ----	---- ----
X. montezumae (Xw)	M	23.4955 ±2.3679	14.6685 ±0.7540	149.88 ±9.5666	160.62 ±16.0995
	F	28.3697 ±2.3252	13.8058 ±0.6223	116.37 ±9.0745	162.73 ±15.7440
X. nezahaulcoyotl (Xe)	M	21.4125 ±1.7097	12.0751 ±0.7520	146.30 ±8.1803	157.77 ±15.1993

Species (tip names)	Sex	U_{crit} (cm s^{-1})	VO_{2max} (µl min^{-1})	CS V_{max} (milli-untis min^{-1})[&]	LDH V_{max} (milli-untis min^{-1})[&]
	F	25.7347 ±1.1063	13.3214 ±0.5211	124.35 ±9.0789	138.60 ±15.7334
X. couchianus (Xu)	M	23.2416 ±1.5995	8.6862 ±0.2844	199.24 ±10.2222	150.04 ±16.7094
	F	19.9693 ±1.1293	10.0814 ±0.5325	139.40 ±9.7173	150.01 ±16.2588
X. gordoni (Xg)	M	22.2781 ±2.0572	10.8228 ±0.5758	150.04 ±9.4847	141.63 ±16.1518
	F	19.1299 ±1.4673	9.7118 ±0.3047	116.22 ±8.5860	130.94 ±15.5937
X. meyeri (Xy)	M	14.4602 ±0.8709	8.8413 ±0.5269	162.26 ±9.9957	139.85 ±16.5764
	F	14.8453 ±0.7973	9.8205 ±0.5503	99.42 ±9.3587	115.89 ±16.0232
X. variatus (Xv)	M	24.6471 ±1.1600	8.8765 ±0.7530	187.88 ±10.1834	136.26 ±16.5440
	F	28.6485 ±1.2924	12.2143 ±0.4149	132.03 ±9.3999	126.15 ±15.9904

[&] Means are least squares means and associated standard errors from SAS PROC MIXED with assay batch as a random effect, sex, species, and the sex*species interaction as fixed effects.

[¥] *X. continens* and *X. cortezi* (Pop.2) were not used in final analyses, and were not included in estimation of least squares means for CS and LDH activity; therefore, there is no value to report for them.

Appendix 3.4. Means ± standard errors for $\log_{10}$ critical swimming speed (U_{crit}) and $\log_{10}$ maximum oxygen consumption (VO_{2max}) for each sex among the 21 species/populations. Only one male and one female tested for *X. continens*. In final phylogenetic analyses *X. continens* and *X. cortezi* (Pop. 2) were not included. The order of species matches that found in Figure 3.1 and Appendix 3.2.

Species	Sex	$\log_{10}$ U_{crit} (cm s^{-1})	$\log_{10}$ VO_{2max} (µl min^{-1})
P. chamulae (Ph)	M	1.5364 ±0.0271	0.9643 ±0.0312
	F	1.4986 ±0.0626	1.1614 ±0.0170
P. compressa (Pc)	M	1.5361 ±0.0317	0.8999 ±0.0320
	F	1.5676 ±0.0173	1.1949 ±0.0235
X. alvarezi (Xa)	M	1.3983 ±0.0562	1.1645 ±0.0519
	F	1.3958 ±0.0363	1.1667 ±0.0212
X. signum (Xs)	M	1.5169 ±0.0118	1.3371 ±0.0608
	F	1.4990 ±0.0132	1.4116 ±0.0151
X. helleri (Xh)	M	1.4598 ±0.0210	1.1145 ±0.0353
	F	1.5012 ±0.0193	1.2023 ±0.0234
X. clemenciae (Xi)	M	1.1709 ±0.0207	1.1173 ±0.0201
	F	1.2247 ±0.0250	1.2862 ±0.0170
X. monticolus (Xo)	M	1.2524 ±0.0261	1.0919 ±0.0276
	F	1.2090 ±0.0237	1.1005 ±0.0235
X. birchmanni (Xb)	M	1.3307 ±0.0578	1.2985 ±0.0547
	F	1.2008 ±0.0424	1.1686 ±0.0325
X. multilineatus (Xl)	M	1.3846 ±0.0263	1.1901 ±0.0151
	F	1.2804 ±0.0285	0.9819 ±0.0414
X. nigrensis (Xn)	M	1.3877 ±0.0255	1.0250 ±0.0205
	F	1.2661 ±0.0323	0.8832 ±0.0232
X. pygmaeus (Xp)	M	1.2866 ±0.0333	0.8090 ±0.0399
	F	1.3082	0.7426

Species	Sex	$\log_{10} U_{crit}$ (cm s^{-1})	$\log_{10} VO_{2max}$ (µl min^{-1})
		±0.0277	±0.0257
X. continens (Xt)	M	1.0909 ----	0.8726 ----
	F	1.3320 ----	0.8902 ----
X. multi-cortezi (Xz)	M	1.3329 ±0.0176	1.1224 ±0.0362
	F	1.1606 ±0.0495	0.9376 ±0.0739
X. cortezi (Xc)	M	1.2519 ±0.0383	1.1756 ±0.0166
	F	1.3431 ±0.0222	1.1538 ±0.0262
X. cortezi (Pop. 2) (Xr)	M	1.2398 ±0.0365	1.2107 ±0.0226
	F	1.2984 ±0.0352	1.1492 ±0.0162
X. montezumae (Xw)	M	1.3397 ±0.0442	1.1586 ±0.0218
	F	1.4271 ±0.0405	1.1325 ±0.0217
X. nezahaulcoyotl (Xe)	M	1.3038 ±0.0369	1.0667 ±0.0275
	F	1.4022 ±0.0209	1.1182 ±0.0178
X. couchianus (Xu)	M	1.3316 ±0.0561	0.9352 ±0.0148
	F	1.2882 ±0.0277	0.9935 ±0.0248
X. gordoni (Xg)	M	1.3200 ±0.0448	1.0260 ±0.0240
	F	1.2599 ±0.0336	0.9837 ±0.0134
X. meyeri (Xy)	M	1.1491 ±0.0250	0.9358 ±0.0245
	F	1.1649 ±0.0202	0.9839 ±0.0232
X. variatus (Xv)	M	1.3825 ±0.0207	0.9230 ±0.0328
	F	1.4498 ±0.0202	1.0825 ±0.0158

Appendix 3.5. Means ± S.E. for five linear measurements and four mass measurements for both sexes among the 21 species used in the study. *X. cortezi* (Pop. 2) and *X. continens* were not used in analyses. Order matches that depicted in Figure 3.1 and Appendix 3.2. Data from c:\xipho\ch3\Ucrit_VO2max_size_aggr_males.sav, Ucrit_VO2max_size_aggr_females.sav, Ucrit_VO2max_size_aggr_males_se.sav, Ucrit_VO2max_size_aggr_females_se.sav

Species (tip names)	Sex	Total Length (mm)	Standard Length (mm)	Body Depth (mm)	Mass (g)	Tail Mass (g)	Caudal Fin (mm)	Sword Length (mm)	Gill Mass (g)	Heart Mass (g)
P. chamulae (Ph)	M	37.95	29.55	9.16	0.5503	0.2754	8.40	----	0.0037	0.00019
		±1.49	±1.07	±0.31	±0.0654	±0.0346	±0.44	----	±0.000 94	±0.00002
	F	52.91	42.21	13.25	1.8305	0.6747	10.70	----	0.0051	0.00039
		±1.95	±1.64	±0.52	±0.2073	±0.0674	±0.33	----	±0.001 32	±0.00005
P. compressa (Pc)	M	39.45	31.22	10.78	0.7514	0.3286	8.23	----	0.0069	0.00046
		±1.01	±0.72	±0.38	±0.0645	±0.0463	±0.40	----	±0.000 50	±0.00003
	F	53.49	43.91	14.06	2.1443	0.5909	9.59	----	0.0140	0.00122
		±2.21	±1.48	±0.76	±0.31	±0.1339	±0.77	----	±0.00105	±0.00011
X. alvarezi (Xa)	M	58.56	45.58	13.48	2.7558	1.1559	12.9814	20.37	0.0172	0.00087
		±3.43	±2.61	±1.07	±0.5474	±0.2445	±0.8361	±1.11	±0. 00293	±0.00012
	F	57.23	44.26	13.93	2.4973	0.7827	12.9675	----	0.0102	0.00068
		±1.02	±0.91	±0.27	±0.1738	±0.0687	±0.1755	----	±0.0 0136	±0.00009
X. signum (Xs)	M	69.20	53.65	15.16	3.4733	1.4955	15.55	12.05	0.0314	0.00190
		±0.97	±0.75	±0.26	±0.1926	±0.0870	±0.30	±1.03	±0.00326	±0.00030
	F	70.97	55.93	16.33	4.3363	1.5357	15.04	----	0.0231	0.00109
		±0.75	±0.61	±0.27	±0.1535	±0.0693	±0.24	----	±0.00127	±0.0001
X. helleri (Xh)	M	52.32	40.08	11.06	1.6322	0.6286	12.24	22.38	0.0137	0.00089
		±2.51	±1.95	±0.62	±0.2262	±0.0891	±0.58	±1.67	±0.00 199	±0.00013
	F	53.49	40.92	12.72	1.8828	0.5441	12.58	0.07	0.0447	0.00152
		±0.70	±0.55	±0.21	±0.0829	±0.0321	±0.18	±0.07	±0.00 46	±0.00012
X. clemenciae (Xi)	M	46.04	35.25	9.36	1.0046	0.4460	10.7869	7.02	0.0038	0.00033
		±0.63	±0.50	±0.21	±0.0506	±0.0238	±0.1845	±0.48	±0.00061	±0.00004
	F	51.58	39.88	11.81	1.7083	0.6108	11.7053	----	0.0073	0.00060
		±0.69	±0.53	±0.22	±0.0735	±0.0318	±0.1763	----	±0.00073	±0.00006
X. monticolus (Xo)	M	45.89	36.42	8.01	0.7627	0.2954	9.47	4.61	0.0042	0.00032
		±1.46	±1.33	±0.26	±0.0688	±0.0299	±0.30	±0.53	±0.00 066	±0.00005

Species (tip names)	Sex	Total Length (mm)	Standard Length (mm)	Body Depth (mm)	Mass (g)	Tail Mass (g)	Caudal Fin (mm)	Sword Length (mm)	Gill Mass (g)	Heart Mass (g)
	F	45.82 ±1.09	36.13 ±0.86	8.19 ±0.31	0.8876 ±0.0766	0.2593 ±0.0248	9.69 ±0.24	---- ----	0.0071 ±0.000 64	0.00031 ±0.00003
X. birchmanni (Xb)	M	57.17 ±2.44	44.98 ±1.96	14.43 ±0.79	2.6444 ±0.3947	1.1354 ±0.1774	12.1844 ±0.5007	3.46 ±0.92	0.0278 ±0. 00428	0.00186 ±0.00031
	F	51.13 ±1.78	40.73 ±1.37	12.44 ±0.50	1.9602 ±0.1883	0.6431 ±0.065	10.4045 ±0.4665	---- ----	0.0105 ±0.00 13	0.00075 ±0.00014
X. multilineatus (Xl)	M	55.48 ±1.01	43.40 ±0.99	13.36 ±0.38	2.0823 ±0.1528	0.8815 ±0.0636	12.08 ±0.60	13.76 ±0.75	0.0147 ±0.00 111	0.00089 ±0.00008
	F	44.07 ±0.46	35.32 ±0.38	9.72 ±0.22	1.0307 ±0.0356	0.2951 ±0.0126	8.74 ±0.17	---- ----	0.0102 ±0.001 65	0.00051 ±0.00009
X. nigrensis (Xn)	M	44.60 ±0.83	35.07 ±0.59	10.50 ±0.32	1.1401 ±0.0813	0.4595 ±0.0358	9.53 ±0.28	12.24 ±1.09	0.0104 ±0.00 131	0.00053 ±0.00005
	F	38.96 ±0.56	31.04 ±0.46	9.42 ±0.17	0.8313 ±0.0419	0.2399 ±0.0115	7.92 ±0.12	---- ----	0.0140 ±0.001 51	0.00068 ±0.0001
X. pygmaeus (Xp)	M	36.08 ±1.21	28.34 ±0.94	7.71 ±0.37	0.5368 ±0.0694	0.2188 ±0.0325	7.74 ±0.31	2.23 ±0.75	0.0068 ±0.00 084	0.00049 ±0.00007
	F	37.66 ±0.56	30.39 ±0.49	8.55 ±0.15	0.6539 ±0.0319	0.1738 ±0.0086	7.27 ±0.19	---- ----	0.0099 ±0.001 5	0.00042 ±0.00007
X. continens (Xt)	M	33.80 ----	26.11 ----	6.21 ----	0.3760 ----	0.1590 ----	7.6900 ----	5.21 ----	0.0021 ----	0.0001 ----
	F	39.03 ----	31.55 ----	8.86 ----	0.8750 ----	0.1800 ----	7.4800 ----	---- ----	0.0064 ----	.00005 ----
X. multi-cortezi (Xz)	M	55.65 ±1.30	43.27 ±1.08	14.36 ±0.42	2.4898 ±0.1689	0.9388 ±0.0686	12.37 ±0.24	4.26 ±0.26	0.0288 ±0.00 482	0.00174 ±0.00031
	F	52.33 ±1.66	41.44 ±1.38	12.96 ±0.62	2.1774 ±0.2475	0.5963 ±0.0711	10.89 ±0.29	---- ----	0.0203 ±0.005 63	0.00104 ±0.00024
X. cortezi (Xc)	M	48.01 ±0.94	37.15 ±0.75	11.06 ±0.33	1.4315 ±0.1044	0.5652 ±0.0437	10.8606 ±0.2021	6.55 ±0.35	0.0108 ±0. 00073	0.00060 ±0.00006
	F	46.63 ±0.84	36.74 ±0.76	11.35 ±0.31	1.5119 ±0.0885	0.4132 ±0.0228	9.8911 ±0.4932	---- ----	0.0071 ±0.0 0069	0.00033 ±0.00003
X. cortezi (Pop. 2) (Xr)	M	54.53 ±1.00	42.47 ±0.73	13.03 ±0.36	2.2088 ±0.1638	0.8744 ±0.0586	12.061 ±0.3377	4.36 ±0.61	0.0092 ±0.00173	0.00076 ±0.00024
	F	54.08	43.12	12.37	2.3506	0.7479	10.9621	----	0.0083	0.00050

Species (tip names)	Sex	Total Length (mm)	Standard Length (mm)	Body Depth (mm)	Mass (g)	Tail Mass (g)	Caudal Fin (mm)	Sword Length (mm)	Gill Mass (g)	Heart Mass (g)
		±0.82	±0.70	±0.38	±0.1466	±0.0468	±0.1589	----	±0.00082	±0.00005
X. montezumae (Xw)	M	53.44 ±1.68	42.18 ±1.45	11.64 ±0.43	1.7621 ±0.1908	0.7085 ±0.0868	11.25 ±0.34	21.28 ±1.58	0.0131 ±0.00 333	0.00076 ±0.00017
	F	53.63 ±1.18	42.54 ±1.02	12.41 ±0.28	1.9630 ±0.1297	0.6359 ±0.042	11.09 ±0.22	---- ----	0.0076 ±0.0009 4	0.00058 ±0.00008
X. nezahaulcoyotl (Xe)	M	42.55 ±1.20	32.82 ±0.90	9.89 ±0.36	0.9835 ±0.0901	0.4132 ±0.0426	9.72 ±0.33	16.23 ±1.21	0.0079 ±0.00 073	0.00044 ±0.00005
	F	45.43 ±0.77	35.29 ±0.62	10.40 ±0.27	1.2812 ±0.0712	0.3802 ±0.022	10.14 ±0.18	0.65 ±0.09	0.0106 ±0.002 89	0.00034 ±0.00004
X. couchianus (Xu)	M	28.90 ±0.65	23.37 ±0.53	6.61 ±0.21	0.3523 ±0.0314	0.1124 ±0.0086	5.5306 ±0.1372	---- ----	0.0042 ±0.0 0030	0.00025 ±0.00003
	F	37.21 ±0.52	30.34 ±0.50	8.73 ±0.25	0.8278 ±0.0467	0.1731 ±0.0122	6.8725 ±0.1024	---- ----	0.0050 ±0.0 0051	0.00033 ±0.00007
X. gordoni (Xg)	M	34.54 ±0.30	27.64 ±0.27	8.24 ±0.17	0.5490 ±0.0254	0.1580 ±0.0129	6.90 ±0.06	---- ----	0.0087 ±0.000 52	0.00037 ±0.00005
	F	36.05 ±0.46	29.34 ±0.36	8.47 ±0.19	0.6450 ±0.032	0.1492 ±0.0065	6.71 ±0.16	---- ----	0.0084 ±0.0012 4	0.00024 ±0.00004
X. meyeri (Xy)	M	31.28 ±0.58	25.01 ±0.48	6.56 ±0.15	0.3706 ±0.0274	0.1223 ±0.0104	6.27 ±0.12	---- ----	0.0049 ±0.000 51	0.00024 ±0.00004
	F	39.13 ±0.39	31.94 ±0.31	8.23 ±0.21	0.7813 ±0.0279	0.1944 ±0.0108	7.19 ±0.16	---- ----	0.0059 ±0.000 46	0.00027 ±0.00004
X. variatus (Xv)	M	36.29 ±0.70	28.73 ±0.56	8.90 ±0.24	0.6520 ±0.0443	0.2153 ±0.0150	7.56 ±0.16	---- ----	0.0093 ±0.000 88	0.00045 ±0.00003
	F	42.92 ±0.46	34.15 ±0.35	10.31 ±0.19	1.0884 ±0.0255	0.2954 ±0.0086	8.78 ±0.12	---- ----	0.0223 ±0.003 04	0.00081 ±0.00009

Appendix 3.6. Means ± S.E. for log_{10}-transformed values for five linear measurements and four mass measurements for both sexes among the 21 species used in the study. *X. cortezi* (Pop. 2) and *X. continens* were not used in analyses. Order of species matches the order depicted in Figure 3.1 and Appendix 3.2. Data from c:\xipho\ch3\Ucrit_VO2max_size_aggr_males.sav, Ucrit_VO2max_size_aggr_females.sav, Ucrit_VO2max_size_aggr_males_se.sav, Ucrit_VO2max_size_aggr_females_se.sav

Species (tip names)	Sex	log_{10} Total Length	log_{10} Standard Length	log_{10} Body Depth	log_{10} Mass	log_{10} Tail Mass	log_{10} Tail Length	log_{10} Sword Length	log_{10} Gill mass	log_{10} Heart Mass
P. chamulae (Ph)	M	1.58 ±0.017	1.47 ±0.016	0.96 ±0.015	-0.28 ±0.048	-0.58 ±0.049	0.92 ±0.024	---- ----	-3.75 0.05 8	-2.54 ±0.127
	F	1.72 ±0.016	1.62 ±0.017	1.12 ±0.017	0.25 ±0.050	-0.18 ±0.044	1.03 ±0.013	---- ----	-3.42 0.05 4	-2.47 ±0.209
P. compressa (Pc)	M	1.59 ±0.011	1.49 ±0.010	1.03 ±0.015	-0.15 ±0.036	-0.55 ±0.061	0.91 ±0.023	---- ----	-3.36 0.03 5	-2.18 ±0.032
	F	1.73 ±0.017	1.64 ±0.014	1.14 ±0.022	0.30 ±0.058	-0.32 ±0.09	0.97 ±0.034	---- ----	-2.93 0.039	-1.86 ±0.031
X. alvarezi (Xa)	M	1.76 ±0.025	1.65 ±0.025	1.11 ±0.034	0.33 ±0.087	-0.06 ±0.089	1.10 ±0.027	1.32 ±0.022	-3.12 0. 062	-1.84 ±0.076
	F	1.76 ±0.007	1.65 ±0.008	1.14 ±0.008	0.39 ±0.026	-0.12 ±0.031	1.11 ±0.006	---- ----	-3.21 0.06 1	-2.04 ±0.066
X. signum (Xs)	M	1.84 ±0.006	1.73 ±0.006	1.18 ±0.007	0.54 ±0.023	0.17 ±0.025	1.19 ±0.009	1.11 ±0.034	-2.74 0. 061	-1.52 ±0.055
	F	1.85 ±0.005	1.75 ±0.005	1.21 ±0.007	0.63 ±0.015	0.18 ±0.020	1.18 ±0.007	---- ----	-2.98 0.03 5	-1.65 ±0.022
X. helleri (Xh)	M	1.71 ±0.021	1.59 ±0.021	1.03 ±0.026	0.12 ±0.066	-0.29 ±0.065	1.08 ±0.021	1.35 ±0.029	-3.13 0. 057	-1.96 ±0.066
	F	1.73 ±0.006	1.61 ±0.006	1.1 ±0.007	0.27 ±0.020	-0.28 ±0.026	1.10 ±0.006	0.02 ±0.02	-2.84 0.0 38	-1.40 ±0.06
X. clemenciae (Xi)	M	1.66 ±0.006	1.55 ±0.006	0.97 ±0.009	-0.01 ±0.020	-0.36 ±0.022	1.03 ±0.008	0.89 ±0.026	-3.54 0. 060	-2.48 ±0.050
	F	1.71 ±0.006	1.6 ±0.006	1.07 ±0.008	0.23 ±0.019	-0.22 ±0.024	1.07 ±0.007	---- ----	-3.26 0.05 3	-2.17 ±0.051
X. monticolus (Xo)	M	1.66 ±0.014	1.56 ±0.015	0.9 ±0.014	-0.15 ±0.040	-0.56 ±0.044	0.97 ±0.014	0.72 ±0.044	-3.56 0. 064	-2.46 ±0.064

Species (tip names)	Sex	log_{10} Total Length	log_{10} Standard Length	log_{10} Body Depth	log_{10} Mass	log_{10} Tail Mass	log_{10} Tail Length	log_{10} Sword Length	log_{10} Gill mass	log_{10} Heart Mass
	F	1.66 ±0.01	1.56 ±0.01	0.91 ±0.017	-0.08 ±0.039	-0.62 ±0.044	0.98 ±0.011	---- ----	-3.57 0.04	-2.31 ± 0.050
X. birchmanni (Xb)	M	1.75 ±0.019	1.65 ±0.019	1.15 ±0.024	0.38 ±0.066	0.01 ±0.069	1.08 ±0.018	0.57 ±0.093	-2.77 ±0 .067	-1.60 ±0.067
	F	1.71 ±0.014	1.61 ±0.014	1.09 ±0.019	0.27 ±0.044	-0.22 ±0.044	1.01 ±0.018	---- ----	-3.21 ±0.0 85	-2.01 ±0.050
X. multilineatus (Xl)	M	1.74 ±0.008	1.64 ±0.010	1.12 ±0.013	0.3 ±0.032	-0.07 ±0.031	1.07 ±0.017	1.16 ±0.023	-3.10 ±0 .061	-1.86 ±0.034
	F	1.64 ±0.004	1.55 ±0.005	0.99 ±0.010	0.01 ±0.015	-0.54 ±0.018	0.94 ±0.008	---- ----	-3.40 ±0.0 79	-2.09 ±0.075
X. nigrensis (Xn)	M	1.65 ±0.008	1.54 ±0.007	1.02 ±0.013	0.04 ±0.031	-0.37 ±0.038	0.98 ±0.014	1.09 ±0.044	-3.32 ±0 .043	-2.04 ±0.051
	F	1.59 ±0.006	1.49 ±0.006	0.97 ±0.008	-0.09 ±0.020	-0.63 ±0.020	0.90 ±0.007	---- ----	-3.28 ±0.0 82	-1.92 ±0.064
X. pygmaeus (Xp)	M	1.55 ±0.014	1.45 ±0.014	0.88 ±0.019	-0.32 ±0.048	-0.72 ±0.055	0.88 ±0.017	0.41 ±0.064	-3.41 ±0 .086	-2.22 ±0.050
	F	1.58 ±0.006	1.48 ±0.007	0.93 ±0.007	-0.19 ±0.021	-0.77 ±0.022	0.86 ±0.011	---- ----	-3.60 ±0.1 45	-2.11 ±0.080
X. continens (Xt)	M	1.53 ----	1.42 ----	0.79 ----	-0.42 ----	-0.80 ----	0.89 ----	0.79 ----	-4.30 ----	-2.67 ----
	F	1.59 ----	1.5 ----	0.95 ----	-0.06 ----	-0.74 ----	0.87 ----	---- ----	-3.32 ----	-2.19 ----
X. multi-cortezi (Xz)	M	1.74 ±0.011	1.63 ±0.011	1.16 ±0.014	0.38 ±0.034	-0.04 ±0.037	1.09 ±0.009	0.72 ±0.022	-2.86 ±0 .103	-1.64 ±0.109
	F	1.72 ±0.014	1.62 ±0.015	1.11 ±0.022	0.31 ±0.054	-0.26 ±0.057	1.04 ±0.012	---- ----	-3.1 ±0.1 05	-1.82 ±0.105
X. cortezi (Xc)	M	1.68 ±0.009	1.57 ±0.009	1.04 ±0.013	0.14 ±0.031	-0.27 ±0.034	1.03 ±0.008	0.87 ±0.020	-3.26 ±0 .046	-1.98 ±0.029
	F	1.67 ±0.008	1.56 ±0.009	1.05 ±0.013	0.17 ±0.028	-0.4 ±0.024	0.99 ±0.018	---- ----	-3.52 ±0.0 51	-2.19 ±0.048
X. cortezi (Pop. 2) (Xr)	M	1.74 ±0.008	1.63 ±0.007	1.11 ±0.012	0.33 ±0.032	-0.07 ±0.030	1.08 ±0.013	0.68 ±0.076	-3.31 ±0 .116	-2.12 ±0.082
	F	1.73	1.63	1.09	0.36	-0.14	1.04	----	-3.34	-2.11

Species (tip names)	Sex	log_{10} Total Length	log_{10} Standard Length	log_{10} Body Depth	log_{10} Mass	log_{10} Tail Mass	log_{10} Tail Length	log_{10} Sword Length	log_{10} Gill mass	log_{10} Heart Mass
		±0.007	±0.007	±0.013	±0.027	±0.028	±0.006	----	±0.0 44	±0.046
X. montezumae (Xw)	M	1.73 ±0.013	1.62 ±0.014	1.06 ±0.015	0.22 ±0.042	-0.16 ±0.045	1.05 ±0.013	1.33 ±0.030	-3.22 ±0 .078	-1.98 ±0.068
	F	1.73 ±0.01	1.63 ±0.010	1.09 ±0.010	0.28 ±0.028	-0.21 ±0.029	1.04 ±0.009	---- ----	-3.3 ±0.05 6	-2.18 ±0.058
X. nezahaulcoyotl (Xe)	M	1.63 0±.012	1.51 ±0.012	0.99 ±0.016	-0.04 ±0.039	-0.42 ±0.043	0.98 ±0.015	1.21 ±0.043	-3.42 ±0 .062	-2.14 ±0.041
	F	1.66 ±0. 007	1.55 ±0.008	1.01 ±0.011	0.09 ±0.027	-0.43 ±0.028	1.00 ±0.008	0.21 ±0.02	-3.52 ±0 .047	-2.18 ±0.092
X. couchianus (Xu)	M	1.46 ±0.010	1.37 ±0.010	0.82 ±0.013	-0.48 ±0.038	-0.97 ±0.035	0.74 ±0.011	---- ----	-3.67 ±0.0 71	-2.40 ±0.030
	F	1.57 ±0.006	1.48 ±0.007	0.94 ±0.012	-0.09 ±0.026	-0.78 ±0.032	0.84 ±0.007	---- ----	-3.6 ±0.0 77	-2.33 ±0.045
X. gordoni (Xg)	M	1.54 ±0.004	1.44 ±0.004	0.91 ±0.009	-0.27 ±0.022	-0.82 ±0.038	0.84 ±0.004	---- ----	-3.48 ±0.0 57	-2.07 ±0.031
	F	1.56 ±0.005	1.47 ±0.005	0.93 ±0.010	-0.2 ±0.019	-0.83 ±0.018	0.82 ±0.011	---- ----	-3.7 ±0.0 60	-2.13 ±0.047
X. meyeri (Xy)	M	1.49 ±0.008	1.40 ±0.008	0.82 ±0.010	-0.45 ±0.029	-0.93 ±0.034	0.80 ±0.008	---- ----	-3.79 ±0.1 33	-2.34 ±0.039
	F	1.59 ±0.004	1.50 ±0.004	0.91 ±0.011	-0.11 ±0.016	-0.72 ±0.024	0.85 ±0.010	---- ----	-3.67 ±0.0 96	-2.25 ±0.034
X. variatus (Xv)	M	1.56 ±0.008	1.46 ±0.008	0.95 ±0.012	-0.2 ±0.028	-0.69 ±0.028	0.88 ±0.009	---- ----	-3.37 ±0.0 32	-2.06 ±0.037
	F	1.63 ±0.005	1.53 ±0.004	1.01 ±0.008	0.03 ±0.011	-0.53 ±0.013	0.94 ±0.006	---- ----	-3.14 ±0.0 56	-1.73 ±0.067

Appendix 3.7. Samples sizes (N) of females dissected for reproductive traits, and means ± standard errors for ovary mass, number of regressors (unfertilized eggs), and regressor dry mass among the 21 species/populations. Only one female tested for *X. continens*. In final phylogenetic analyses *X. continens* and *X. cortezi* (Pop. 2) were not included. Order matches order of species in Figure 3.1 and Appendix 3.2.

Species	N	Ovary Mass (g)	Regressor #	Regressor Dry Mass (g)
P. chamulae (Ph)	3	0.0913 ±0.0523	6.8000 ±3.9299	0.0304 ±0.0151
P. compressa (Pc)	3	0.4166 ±0.1229	29.3333 ±6.3333	0.0640 ±0.0101
X. alvarezi (Xa)	11	0.2651 ±0.0221	40.0833 ±3.2699	0.0724 ±0.0081
X. signum (Xs)	14	0.2447 ±0.0359	42.3333 ±5.7918	0.0725 ±0.0111
X. helleri (Xh)	14	0.2249 ±0.0196	28.1333 ±3.2342	0.0589 ±0.0056
X. clemenciae (Xi)	12	0.0875 ±0.0141	16.9333 ±3.8155	0.0259 ±0.0052
X. monticolus (Xo)	13	0.1235 ±0.0197	17.2308 ±3.3668	0.0341 ±0.0054
X. birchmanni (Xb)	10	0.1464 ±0.0324	20.0000 ±5.7057	0.0457 ±0.0116
X. multilineatus (Xl)	13	0.0996 ±0.0112	13.7143 ±1.5752	0.0409 ±0.0049
X. nigrensis (Xn)	12	0.0551 ±0.0059	5.3077 ±0.4106	0.0197 ±0.0028
X. pygmaeus (Xp)	10	0.0587 ±0.0081	10.6364 ±1.0520	0.0244 ±0.0025
X. continens (Xt)	1	0.1960 ----	45.0000 -----	0.0710 -----
X. multi-cortezi (Xz)	6	0.2887 ±0.0442	40.1667 ±6.9350	0.1081 ±0.0153
X. cortezi (Xc)	17	0.2225 ±0.0243	30.9444 ±2.5689	0.0665 ±0.0073

Species	N	Ovary Mass (g)	Regressor #	Regressor Dry Mass (g)
X. cortezi (Pop. 2) (Xr)	14	0.0145 ±0.0063	41.1429 ±4.3876	0.0724 ±0.0074
X. montezumae (Xw)	8	0.0961 ±0.0209	9.6667 ±2.1023	0.0200 ±0.0055
X. nezahaulcoyotl (Xe)	17	0.1120 ±0.0140	21.6667 ±2.4974	0.0435 ±0.0052
X. couchianus (Xu)	14	0.1407 ±0.0120	28.3362 ±2.8101	0.0490 ±0.0059
X. gordoni (Xg)	16	0.0684 ±0.0076	13.9412 ±1.5135	0.0252 ±0.0034
X. meyeri (Xy)	13	0.1097 ±0.0086	18.6154 ±2.2088	0.0351 ±0.0042
X. variatus (Xv)	15	0.0954 ±0.0064	14.2530 ±1.7517	0.0310 ±0.0040

Appendix 3.8. Samples sizes (N) of females dissected for reproductive traits, means ± standard errors for $\log_{10}$ ovary mass, number of regressors (unfertilized eggs), and regressor dry mass. among the 21 species/populations. Only one female tested for *X. continens*. In final phylogenetic analyses *X. continens* and *X. cortezi* (Pop. 2) were not included. Order matches order of species in Figure 3.1 and Appendix 3.2.

Species	N	$\log_{10}$ Ovary Mass (g)	$\log_{10}$ Regressor #	$\log_{10}$ Regressor Dry Mass (g)
P. chamulae (Ph)	3	-1.6783 ±0.4691	0.6292 ±0.1339	-2.0912 ±0.1688
P. compressa (Pc)	3	-0.4169 ±0.1249	1.4593 ±0.1034	-1.2049 ±0.0719
X. alvarezi (Xa)	11	-0.6156 ±0.0390	1.5020 ±0.0326	-1.1715 ±0.0561
X. signum (Xs)	14	-0.9081 ±0.1136	1.4711 ±0.1046	-1.4067 ±0.1167
X. helleri (Xh)	14	-0.6773 ±0.0457	1.3529 ±0.0617	-1.2586 ±0.0463
X. clemenciae (Xi)	12	-1.6000 ±0.0570	1.0353 ±0.0608	-2.0372 ±0.0691
X. monticolus (Xo)	13	-1.0480 ±0.1300	1.1362 ±0.1086	-1.6148 ±0.1356
X. birchmanni (Xb)	10	-1.1749 ±0.1048	1.1273 ±0.1122	-1.6898 ±0.1423
X. multilineatus (Xl)	13	-1.0642 ±0.0846	1.1278 ±0.0632	-1.4670 ±0.0930
X. nigrensis (Xn)	12	-1.5154 ±0.0495	0.7569 ±0.0277	-1.8914 ±0.0657
X. pygmaeus (Xp)	10	-1.2787 ±0.0779	1.0154 ±0.0459	-1.6414 ±0.0596
X. continens (Xt)	1	-0.7075 ----	1.6628 -----	-1.1479 -----
X. multi-cortezi (Xz)	6	-0.5628 ±0.0627	1.5790 ±0.0819	-0.9875 ±0.0615
X. cortezi (Xc)	17	-0.8672 ±0.0612	1.4141 ±0.0469	-1.3679 ±0.0658

Species	N	$\log_{10}$ Ovary Mass (g)	$\log_{10}$ Regressor #	$\log_{10}$ Regressor Dry Mass (g)
X. cortezi (Pop. 2) (Xr)	14	-0.6579 ±0.0370	1.5879 ±0.0520	-1.1821 ±0.0607
X. montezumae (Xw)	8	-2.1272 ±0.0792	0.7019 ±0.0710	-2.5711 ±0.0783
X. nezahaulcoyotl (Xe)	17	-1.0127 ±0.0616	1.3131 ±0.0492	-1.4231 ±0.0620
X. couchianus (Xu)	14	-0.8737 ±0.0413	1.3754 ±0.0407	-1.3851 ±0.0884
X. gordoni (Xg)	16	-1.2132 ±0.0578	1.1396 ±0.0475	-1.6626 ±0.0665
X. meyeri (Xy)	13	-0.9793 ±0.0405	1.2626 ±0.0462	-1.5179 ±0.0828
X. variatus (Xv)	15	-1.0337 ±0.0291	1.1047 ±0.0451	-1.5816 ±0.0816

Appendix 3.9. Means ± standard errors for sprint speed, sword length/standard length and sword length/body depth for each sex among the 21 species/populations. Only one female tested for *X. continens*. In final phylogenetic analyses *X. continens* and *X. cortezi* (Pop. 2) were not included. Order matches order of species in Figure 3.1 and Appendix 3.2.

Species	Sex	Sprint Speed (m s^{-1})	Sword length/standard length	Sword length/body depth
P. chamulae	M	0.5363 ±0.0764	---- ----	---- ----
	F	0.9029 ±0.0704	---- ----	---- ----
P. compressa	M	0.5646 ±0.0462	---- ----	---- ----
	F	0.7589 ±0.0545	---- ----	---- ----
X. alvarezi	M	0.7560 ±0.0713	0.4511 ±0.0150	1.5626 ±0.0694
	F	0.6674 ±0.0428	---- ----	---- ----
X. signum	M	0.9560 ±0.1436	0.2254 ±0.0211	0.7975 ±0.0733
	F	1.1792 ±0.0751	---- ----	---- ----
X. helleri	M	0.7030 ±0.0715	0.5517 ±0.0214	2.0333 ±0.0946
	F	0.7369 ±0.0583	0.0016 ±0.0016	0.0051 ±0.0051
X. clemenciae	M	0.8116 ±0.0519	0.1982 ±0.0122	0.7433 ±0.0404
	F	0.9633 ±0.0564	---- ----	---- ----
X. monticolus	M	0.5205 ±0.0379	0.1255 ±0.0135	0.5669 ±0.0596
	F	0.5512 ±0.0497	---- ----	---- ----
X. birchmanni	M	0.8913 ±0.0809	0.0765 ±0.0213	0.233 ±0.0629

Species	Sex	Sprint Speed (m s^{-1})	Sword length/standard length	Sword length/body depth
	F	0.7174 ±0.0318	---- ----	---- ----
X. multilineatus	M	1.1413 ±0.0844	0.3201 ±0.0188	1.0425 ±0.0626
	F	0.7839 ±0.0510	---- ----	---- ----
X. nigrensis	M	0.7213 ±0.0463	0.3448 ±0.0284	1.1488 ±0.0907
	F	0.7166 ±0.0377	---- ----	---- ----
X. pygmaeus	M	0.6498 ±0.0528	0.0707 ±0.0197	0.2514 ±0.0650
	F	0.6905 ±0.0335	---- ----	---- ----
X. continens	M	0.3888 ----	0.1995 ----	0.839 ----
	F	0.6537 ----	---- ----	---- ----
X. multi-cortezi	M	0.7474 ±0.0910	0.0989 ±0.0061	0.299 ±0.0208
	F	0.8490 ±0.0812	---- ----	---- ----
X. cortezi	M	0.7117 ±0.0727	0.1786 ±0.0112	0.6061 ±0.0416
	F	0.7993 ±0.0589	---- ----	---- ----
X. cortezi (Pop. 2)	M	0.7436 ±0.0616	0.1018 ±0.0143	0.3324 ±0.0471
	F	0.7557 ±0.0536	---- ----	---- ----
X. montezumae	M	0.7839 ±0.0445	0.5118 ±0.0411	1.8664 ±0.1553
	F	0.7865 ±0.0502	---- ----	---- ----
X. nezahaulcoyotl	M	0.7892 ±0.0332	0.4866 ±0.0307	1.6194 ±0.1009

Species	Sex	Sprint Speed (m s^{-1})	Sword length/standard length	Sword length/body depth
	F	0.7927 ±0.0512	0.0179 ±0.0023	0.0609 ±0.0076
X. couchianus	M	0.4733 ±0.0295	---- ----	---- ----
	F	0.5158 ±0.0251	---- ----	---- ----
X. gordoni	M	0.6349 ±0.0384	---- ----	---- ----
	F	0.6657 ±0.0333	---- ----	---- ----
X. meyeri	M	0.5575 ±0.0383	---- ----	---- ----
	F	0.6347 ±0.0462	---- ----	---- ----
X. variatus	M	0.3978 ±0.0590	---- ----	---- ----
	F	0.5750 ±0.0427	---- ----	---- ----

Appendix 3.10. Means ± standard errors for stage 1 and 2 distance, maximum acceleration and maximum velocity during a c-srtart escape response for each sex among the 21 species/populations. Only one female tested for *X. continens*. In final phylogenetic analyses *X. continens* and *X. cortezi* (Pop. 2) were not included. Order matches order of species in Figure 3.1 and Appendix 3.2.

Species	Sex	S1 Distance (cm)	S2 distance (cm)	S1 max. acceleration (cm s^{-2})	S1 max. velocity (cm s^{-1})	S2 max acceleration (cm s^{-2})	S2 max. velocity (cm s^{-1})
P. chamulae	M	1.6928 ±0.2161	1.1271 ±0.0620	13859.5 ±434.80	121.3578 ±7.6795	9186.73 ±645.21	83.064 ±6.3982
	F	1.6967 ±0.2711	1.3482 ±0.2141	14092.38 ±860.93	120.9939 ±10.1205	8922.49 ±890.14	84.3905 ±8.6681
P. compressa	M	1.7658 ±0.1001	1.1424 ±0.0593	12392.47 ±598.34	113.6913 ±4.9289	10368.87 ±779.12	84.3365 ±4.7363
	F	2.4912 ±0.1784	1.7071 ±0.1942	16242.74 ±812.44	143.9121 ±4.5646	12709.97 ±1017.49	103.3472 ±6.9681
X. alvarezi	M	2.6466 ±0.3405	1.5598 ±0.1325	16216.61 ±720.66	149.8635 ±8.7904	10016.70 ±703.28	85.0847 ±6.2752
	F	2.4638 ±0.3195	1.7037 ±0.1671	15916.26 ±601.23	146.065 ±10.4901	10519.74 ±961.84	90.7068 ±7.7097
X. signum	M	3.4908 ±0.6200	2.1793 ±0.2157	19934.78 ±722.32	179.3318 ±10.9053	11861.13 ±824.72	108.4191 ±9.5222
	F	3.1481 ±0.3099	2.0714 ±0.1098	19684.59 ±495.89	177.9421 ±7.3431	11625.07 ±723.67	103.8154 ±6.7545
X. helleri	M	2.5355 ±0.1822	1.6244 ±0.1188	16909.09 ±493.68	148.4835 ±5.7018	9934.30 ±404.91	87.7199 ±3.7006
	F	2.0914 ±0.1887	1.9038 ±0.0856	15750.34 ±472.63	138.0445 ±6.4079	9604.68 ±517.29	88.7105 ±4.5976
X. clemenciae	M	2.1217 ±0.2332	1.537 ±0.1275	15428.91 ±531.93	131.4919 ±7.8736	9371.01 ±616.56	80.9622 ±5.1298
	F	2.3164 ±0.2649	1.632 ±0.1172	16335.89 ±544.51	140.7941 ±8.4136	11045.46 ±873.42	96.1371 ±5.8556
X. monticolus	M	2.3523 ±0.2038	1.7032 ±0.1054	11164.46 ±770.60	117.8381 ±6.0346	9830.82 ±563.47	92.1894 ±4.6415
	F	2.3088 ±0.2661	1.4326 ±0.0893	10116.65 ±479.56	102.5724 ±6.9474	8393.44 ±517.80	82.2965 ±4.6042

Species	Sex	S1 Distance (cm)	S2 distance (cm)	S1 max. acceleration (cm s^{-2})	S1 max. velocity (cm s^{-1})	S2 max acceleration (cm s^{-2})	S2 max. velocity (cm s^{-1})
X. birchmanni	M	2.0128 ±0.2327	1.6351 ±0.1191	13791.04 ±808.02	123.566 ±7.8163	8356.24 ±594.73	76.8888 ±4.9402
	F	2.321 ±0.2744	1.4384 ±0.0789	13857.58 ±855.05	123.3058 ±6.5228	9395.44 ±563.82	85.0644 ±5.4549
X. multilineatus	M	2.5767 ±0.2014	1.6181 ±0.0857	18058.41 ±529.39	161.6871 ±6.4343	11376.95 ±593.55	103.6819 ±4.8498
	F	1.8614 ±0.1208	1.267 ±0.0571	13764.15 ±630.10	124.0504 ±6.0340	9959.86 ±683.78	84.5829 ±5.1615
X. nigrensis	M	1.5681 ±0.1448	1.3948 ±0.1006	15376.48 ±539.77	124.3657 ±6.4672	9330.17 ±406.80	79.948 ±3.7640
	F	1.4804 ±0.1305	1.3941 ±0.0672	14466.97 ±537.46	116.5956 ±6.6147	10873.45 ±709.38	91.1743 ±5.8847
X. pygmaeus	M	1.4436 ±0.1242	1.0606 ±0.0904	13626.52 ±602.83	113.1933 ±6.8651	8839.61 ±705.21	74.5663 ±6.4132
	F	1.4800 ±0.1383	1.1654 ±0.0744	13602.92 ±528.27	112.4457 ±7.2228	11024.15 ±703.3814	89.7297 ±5.9001
X. continens	M	1.4956 ----	1.339 ----	11348.3 ----	98.2502 ----	9475.45 ----	85.7889 ----
	F	1.3648 ----	1.112 ----	11065.8 ----	89.3771 ----	6269.39 ----	57.0182 ----
X. multi-cortezi	M	0.984 ±0.2119	0.8655 ±0.0947	9938.554 ±1280.24	76.3192 ±12.1261	7346.08 ±660.45	56.563 ±5.9667
	F	1.7893 ±0.2450	1.5874 ±0.1941	13158.51 ±908.57	117.5503 ±9.6093	9481.03 ±1052.39	84.2145 ±9.5090
X. cortezi	M	2.0244 ±0.1653	1.5211 ±0.1156	13497.21 ±760.13	128.4857 ±7.8311	8722.52 ±586.18	77.7922 ±5.8284
	F	1.7744 ±0.1601	1.3571 ±0.0756	13427.45 ±493.36	120.0009 ±6.1880	9351.03 ±527.00	82.2811 ±3.9353
X. cortezi (Pop. 2)	M	2.499 ±0.2326	1.534 ±0.1042	14228.29 ±545.13	137.2115 ±6.8344	9697.86 ±617.39	89.0896 ±6.5063
	F	2.6495 ±0.1653	1.7359 ±0.1253	14821.48 ±544.74	140.1324 ±4.8781	9731.65 ±510.15	86.7756 ±4.0076
X. montezumae	M	2.0357 ±0.2284	1.3539 ±0.1235	13684.63 ±914.52	125.4536 ±9.9883	8741.57 ±807.24	78.1768 ±7.3267

Species	Sex	S1 Distance (cm)	S2 distance (cm)	S1 max. acceleration (cm s^{-2})	S1 max. velocity (cm s^{-1})	S2 max acceleration (cm s^{-2})	S2 max. velocity (cm s^{-1})
	F	2.108 ±0.2303	1.4287 ±0.0585	13978.69 ±679.98	126.253 ±7.5971	9438.74 ±390.48	84.4442 ±4.3639
X. nezahaulcoyotl	M	1.7476 ±0.1665	1.3294 ±0.0693	14626.47 ±610.80	122.8265 ±6.2712	8261.13 ±458.01	72.5317 ±3.8923
	F	2.1531 ±0.1511	1.5042 ±0.0699	14189.84 ±371.55	130.3513 ±5.0864	9905.21 ±622.65	86.9844 ±5.3710
X. couchianus	M	1.151 ±0.1099	0.9205 ±0.0502	10809.45 ±344.40	90.239 ±5.4634	8633.39 ±379.71	77.4657 ±3.5280
	F	1.4056 ±0.1423	1.1286 ±0.0505	10761.29 ±481.35	94.1445 ±6.2312	8299.30 ±312.10	75.6778 ±2.7456
X. gordoni	M	1.8535 ±0.1503	1.3398 ±0.0555	12839.69 ±400.71	120.8385 ±5.7081	9068.17 ±554.13	83.5457 ±4.8977
	F	1.7969 ±0.1650	1.2446 ±0.0643	12358.61 ±301.34	115.136 ±6.0956	9043.56 ±407.55	81.3489 ±3.7113
X. meyeri	M	1.2203 ±0.1095	0.9436 ±0.0644	11385.81 ±361.78	93.5088 ±5.0257	8415.31 ±591.25	73.9673 ±5.5565
	F	1.2774 ±0.1319	1.1025 ±0.0486	11270.04 ±339.35	96.7959 ±4.8873	8169.96 ±484.05	71.5643 ±4.0981
X. variatus	M	1.4722 ±0.1079	1.1750 ±0.0521	12517.95 ±331.25	107.1874 ±4.2923	8917.04 ±473.85	77.9059 ±3.5414
	F	1.8426 ±0.1561	1.3191 ±0.0952	13578.73 ±425.51	119.7075 ±5.1197	10033.27 ±706.45	88.9931 ±6.0181

Appendix 3.11. Files used for Chapter 3 analyses:

- **Ucrit.sav/ Ucrit.xls:** raw Ucrit data
- **VO2max.sav/ VO2max.xls:** raw VO2max data
- **CSVmax.sav/CSVmax.xls:** raw citrate synthase data
- **LDHVmax.sav/LDHVmax.xls:** raw lactate dehydrogenase data
- **Fish_Sizes_organs.xls/ Fish_Sizes_organs.sav:** raw size data with heart and gill mass
- **Fish_Sizes_organs_reproduction.xls/ Fish_Sizes_organs_reproduction.sav:** raw size data with heart and gill mass with reproductive traits
- **Enzyme_LSMean_Creation_LDH.sas:** to obtain batch corrected LS Means for LDH
- **Enzyme_LSMean_Creation_CS.sas:** to obtain batch corrected LS Means for CS
- **Fish_size_CS_LDH_noXt_Xr.sys:** Enzyme file used in SAS code
- **Ch3 Syntax.SPS:** Syntax file to obtain raw data files, merge and aggregate them; also has syntax to obtain a standard error file, and to obtain data files for enzymes
- **Ch3 Syntax Multiple Regressions.SPS:** Syntax to perform correlation analyses and multiple regression analyses presented in chapter 3 on non-phylogenetic and phylogenetic data
- **Ch3 FIC syntax.SPS:** File to obtain and merge .fic files from PDTREE for each trait
- **Ch3 Syntax_with_reproductive_traits.SPS:** File to obtain aggregated files including reproductive traits of the females.
- **Sword_4.pdi**
- **Sword_4.dsc:** phylogeny of the 19 taxa, excludes X. continens and X. cortezi (Pop. 2)

- **Ucrit_VO2max_size_enzymes_19_aggr_males.xls:** excel data file for U_{crit}, $_{VO2max,}$ and size measures, including log transformed values, for males
- **Ucrit_VO2max_size_enzymes_19_aggr_females.xls**: excel data file for U_{crit}, VO_{2max}, and size measures, including log transformed values, for males
- **Ucrit_VO2max_size_enzymes_19_aggr_males_for_MAtlab.xls:** excel data file used to generate .tip file for matlab Regressionv2 analyses for males
- **Ucrit_VO2max_size_enzymes_19_aggr_females_for_matlab.xls:** excel data file used to generate .tip file for matlab Regressionv2 analyses for females
- **Ucrit_VO2max_size_enzymes_19_aggr_males.tip:** .tip for males for matlab analyses, 44 columns with 20 rows (19 taxa) to be used with sword_4.dsc
- **Ucrit_VO2max_size_enzymes_19_aggr_females.tip:** .tip for males for matlab analyses, 44 columns with 20 rows (19 taxa) to be used with sword_4.dsc
- **Ucrit_VO2max_size_repro_enzymes_19_aggr_females.xls:** female excel file including reproductive data
- **Ucrit_VO2max_size_enzymes_19_aggr_males_Residuals.sav:** data file for 19 species, males with residual trait values
- **Ucrit_VO2max_size_enzymes_19_aggr_females_Residuals.sav:** data file for 19 species, females with residual trait values
- **Ucrit_VO2max_size_repro_19_aggr_females.sav:** data file for 19 species of females including reproductive traits
- **Ucrit_VO2max_size_repro_19_aggr_females_resid.sav:** data file for 19 species, females with residual trait values including reproductive traits
- **FSWSIC.sav:** SPSS data file of aggregated standardized independent contrast files for females

- **FSWSIC_repro.sav:** SPSS data file of aggregated standardized independent contrast files for females with reproductive traits
- **FSWSIC_resid.sav:** SPSS data file of aggregated standardized independent contrast files for females with residual trait values
- **FSWSIC_repro_resid.sav:** SPSS data file of aggregated standardized independent contrast files for females with reproductive traits and residual trait values
- **MSWSIC.sav:** SPSS data file of aggregated standardized independent contrast files for males
- **FSWSIC_resid.sav:** SPSS data file of aggregated standardized independent contrast files for males with residuals
- **MSWsluc.pdi:** pdi file for males with $\log_{10}$ standard length and $\log_{10}$ Ucrit
- **MSWslvo.pdi:** pdi file for males with $\log_{10}$ standard length and $\log_{10}$ VO2max
- **MSWsltms.pdi:** pdi file for males with $\log_{10}$ standard length and $\log_{10}$ tail mass
- **MSWsltln.pdi:** pdi file for males with $\log_{10}$ standard length and $\log_{10}$ caudal fin length
- **MSWslsw.pdi:** pdi file for males with $\log_{10}$ standard length and $\log_{10}$ sword length
- **MSWslpst.pdi:** pdi file for males with $\log_{10}$ standard length and preservation time
- **MSWslms.pdi:** pdi file for males with $\log_{10}$ standard length and $\log_{10}$ body mass
- **MSWslldh.pdi:** pdi file for males with $\log_{10}$ standard length and LDH Vmax
- **MSWslhrt.pdi:** pdi file for males with $\log_{10}$ standard length and $\log_{10}$ heart mass
- **MSWslgil.pdi:** pdi file for males with $\log_{10}$ standard length and $\log_{10}$ gill mass
- **MSWslcs.pdi:** pdi file for males with standard length and CS Vmax
- **MSWslbd.pdi:** pdi file for males with standard $\log_{10}$ length and $\log_{10}$ body depth
- **MrawsIsw.pdi:** pdi file for males with raw standard length and raw sword length

- **Mrawswsl.pdi:** pdi file for males with raw standard length and raw sword length/standard length
- **FSWsluc.pdi:** pdi file for females with log_{10} standard length and log_{10} Ucrit
- **FSWslvo.pdi:** pdi file for females with log_{10} standard length and log_{10} VO2max
- **FSWsltms.pdi:** pdi file for females with log_{10} standard length and log_{10} tail mass
- **FSWsltln.pdi:** pdi file for females with log_{10} standard length and log_{10} caudal fin length
- **FSWslpst.pdi:** pdi file for females with log_{10} standard length and preservation time
- **FSWslms.pdi:** pdi file for females with log_{10} standard length and log_{10} body mass
- **FSWslldh.pdi:** pdi file for females with log_{10} standard length and LDH Vmax
- **FSWslhrt.pdi:** pdi file for females with log_{10} standard length and log_{10} heart mass
- **FSWslgil.pdi:** pdi file for females with log_{10} standard length and log_{10} gill mass
- **FSWslcs.pdi:** pdi file for females with standard length and CS Vmax
- **FSWslbd.pdi:** pdi file for females with standard log_{10} length and log_{10} body depth
- **FSWslrn.pdi:** pdi file for females with standard log_{10} length and log_{10} # of regressors
- **FSWslrd.pdi:** pdi file for females with standard log_{10} length and log_{10} rergessor dry mass
- **FSWslom.pdi:** pdi file for females with standard log_{10} length and log_{10} ovary mass
- **Mslscvo.pdi:** pdi file for males with log10 standard length and size corrected log10 VO2max
- **Mslscuc.pdi:** pdi file for males with log10 standard length and size corrected log10 UCrit
- **Mslsctms.pdi:** pdi file for males with log10 standard length and size corrected log10 tail mass

- **Mslsctln.pdi:** pdi file for males with log10 standard length and size corrected log10 caudal fin length
- **Mslscsw.pdi:** pdi file for males with log10 standard length and size corrected log10 sword length
- **Mslscms.pdi:** pdi file for males with log10 standard length and size corrected log10 body mass
- **Mslscldh.pdi:** pdi file for males with log10 standard length and size corrected LDH Vmax
- **Mslschrt.pdi:** pdi file for males with log10 standard length and size corrected log10 heart mass
- **Mslscgil.pdi:** pdi file for males with log10 standard length and size corrected log10 gill mass
- **Mslsccs.pdi:** pdi file for males with log10 standard length and size corrected CS Vmax
- **Mslscbd.pdi:** pdi file for males with log10 standard length and size corrected log10 body depth
- **Fslscvo.pdi:** pdi file for females with log10 standard length and size corrected log10 VO2max
- **Fslscuc.pdi:** pdi file for females with log10 standard length and size corrected log10 UCrit
- **Fslsctms.pdi:** pdi file for females with log10 standard length and size corrected log10 tail mass
- **Fslsctln.pdi:** pdi file for females with log10 standard length and size corrected log10 caudal fin length

- **Fslscms.pdi:** pdi file for females with log10 standard length and size corrected log10 body mass
- **Fslscldh.pdi:** pdi file for females with log10 standard length and size corrected LDH Vmax
- **Fslschrt.pdi:** pdi file for females with log10 standard length and size corrected log10 heart mass
- **Fslscgil.pdi:** pdi file for females with log10 standard length and size corrected log10 gill mass
- **Fslsccs.pdi:** pdi file for females with log10 standard length and size corrected CS Vmax
- **Fslscbd.pdi:** pdi file for females with log10 standard length and size corrected log10 body depth
- **Fslscrd.pdi:** pdi file for females with log10 standard length and size corrected log10 regressor dry mass
- **Fslscrn.pdi:** pdi file for females with log10 standard length and size corrected log10 # of regressors
- **Fslscom.pdi:** pdi file for females with log10 standard length and size corrected log10 ovary mass
- **MSWsluc.fic:** fic file for males with $\log_{10}$ standard length and $\log_{10}$ Ucrit
- **MSWslvo.fic:** fic file for males with $\log_{10}$ standard length and $\log_{10}$ VO2max
- **MSWsltms.fic:** fic file for males with $\log_{10}$ standard length and $\log_{10}$ tail mass
- **MSWsltln.fic:** fic file for males with $\log_{10}$ standard length and $\log_{10}$ caudal fin length
- **MSWslsw.fic:** fic file for males with $\log_{10}$ standard length and $\log_{10}$ sword length
- **MSWslpst.fic:** fic file for males with $\log_{10}$ standard length and preservation time

- **MSWslms.fic:** fic file for males with $\log_{10}$ standard length and $\log_{10}$ body mass
- **MSWslldh.fic:** fic file for males with $\log_{10}$ standard length and LDH Vmax
- **MSWslhrt.fic:** fic file for males with $\log_{10}$ standard length and $\log_{10}$ heart mass
- **MSWslgil.fic:** fic file for males with $\log_{10}$ standard length and $\log_{10}$ gill mass
- **MSWslcs.fic:** fic file for males with standard length and CS Vmax
- **MSWslbd.fic:** fic file for males with standard $\log_{10}$ length and $\log_{10}$ body depth
- **Mrawslsw.fic:** fic file for males with raw standard length and raw sword length
- **Mrawswsl.fic:** fic file for males with raw standard length and raw sword length/standard length
- **FSWsluc.fic:** fic file for females with $\log_{10}$ standard length and $\log_{10}$ Ucrit
- **FSWslvo.fic:** fic file for females with $\log_{10}$ standard length and $\log_{10}$ VO2max
- **FSWsltms.fic:** fic file for females with $\log_{10}$ standard length and $\log_{10}$ tail mass
- **FSWsltln.fic:** fic file for females with $\log_{10}$ standard length and $\log_{10}$ caudal fin length
- **FSWslpst.fic:** fic file for females with $\log_{10}$ standard length and preservation time
- **FSWslms.fic:** fic file for females with $\log_{10}$ standard length and $\log_{10}$ body mass
- **FSWslldh.fic:** fic file for females with $\log_{10}$ standard length and LDH Vmax
- **FSWslhrt.fic:** fic file for females with $\log_{10}$ standard length and $\log_{10}$ heart mass
- **FSWslgil.fic:** fic file for females with $\log_{10}$ standard length and $\log_{10}$ gill mass
- **FSWslcs.fic:** fic file for females with standard length and CS Vmax
- **FSWslbd.fic:** fic file for females with standard $\log_{10}$ length and $\log_{10}$ body depth
- **FSWslrn.fic:** fic file for females with standard $\log_{10}$ length and $\log_{10}$ # of regressors
- **FSWslrd.fic:** fic file for females with standard $\log_{10}$ length and $\log_{10}$ rergessor dry mass
- **FSWslom.fic:** fic file for females with standard $\log_{10}$ length and $\log_{10}$ ovary mass

- **MSTARPST.PDI:** pdi file of preservation time on a star phylogeny for males
- **FSTARPST.PDI:** pdi file of preservation time on a star phylogeny for females
- **MStarPST.FIC:** fic file for preservation time on a star phylogeny for males
- **FStarPST.FIC:** fic file for preservation time on a star phylogeny for females

Concluding Remarks

My dissertation has focused on the swimming performance of fish, theorizing and experimentally examining the potential impact of sexual selection, and examining the stability of locomotor performance over time. As depicted in Figure 12.1 of Garland (1994b), natural and sexual selection often act rather directly on behavior (what an animal does), but behavior is constrained by whole-animal performance abilities, which in turn are limited by an animals lower-level morphological, physiological, and biochemical characteristics. Thus, the swimming performance of fish has potential impacts on Darwinian fitness due to its importance in escaping from predators, finding food, finding mates, and dispersing (Oufiero & Garland 2007). A reduction in swimming performance caused by the elaboration of a sexually selected trait may, therefore, compromise aspects of Darwinian fitness. If a trait evolves to increase matings, it may come at a cost to locomotion, and potentially reduce survival.

The first chapter of my dissertation introduced an integrative approach to examine performance costs of sexually selected traits. Most theories of sexual selection assume there is a cost to sexually selected traits, such that natural selection ultimately limits their expression (Fisher 1930, Zahavi 1975, Andersson 1994). Although costs have been examined in a variety of taxa and in a variety of organisms, most studies ignore other traits that may evolve because they mitigate the adverse effects of the sexually selected trait, i.e., partially offset its costs. Using simulated data, I showed that ignoring such compensatory traits may mask the negative effect of the sexually selected trait on locomotor performance. Future studies should incorporate a more integrative approach to examine the cost of sexually selected traits (Wilson *et al.* 2010), and take into account compensatory traits (Kirkpatrick 1987, Balmford, Jones & Thomas 1994,

Møller 1996, Oufiero & Garland 2007). These approaches will provide insight into the effects of sexually selected traits, and elucidate their potential costs.

The second chapter of my dissertation experimentally examined the repeatability of several swimming performance measures. The repeatability of a trait is important for many reasons (e.g., see Dohm 2002, Adolph & Hardin 2007, Adolph & Pickering 2008, Garland 2010); for example, if a trait is not repeatable, then there may be no stable 'target' for selection. Therefore, before beginning ecological and evolutionary studies of locomotor performance, the repeatability of the trait should be examined to ensure variation in the measure is at least somewhat consistent among individuals. In the second chapter of my dissertation, I showed that several swimming performance measures are significantly repeatable, over varying time scales (critical swimming speed, maximum speed, and c-start burst speed). This study was one of the first to examine the long-term repeatability of these measures of swimming performance, and it showed that most traits decreased over the span of a year, consistent with theories of senescence (Reznick *et al.* 2004). However, although these measures are repeatable, it is not known whether they are ecologically relevant. Future studies should focus on determining the fitness advantages of various performance measures in natural populations (e.g., see Irschick *et al.* 2008). Future studies should also seek to explore the ecological and evolutionary impacts of variation in these measures of swimming performance (Plaut 2001, Claireaux *et al.* 2007, Handelsman, Claireaux & Nelson 2010, Oufiero *et al.* 2010).

In the third chapter of my dissertation, I examined how the evolution of a sexually selected trait affects the critical swimming speed of *Xiphophorus* and *Priapella*, and how varying amounts of reproductive allocation might affect critical swimming speed in

females. Incorporating several potential compensatory traits and phylogenetic information, I found that when size and phylogeny are accounted for, the length of the sword and the mass of the heart both have statistically significant positive effects on critical swimming speed. In females, I found that under non-phylogenetic models, regressor dry mass (an index of reproductive allocation) had a significant negative effect on critical swimming speed. In females, there were also several significant effects of potential compensatory traits, including positive effects of body depth and negative effects of tail mass, but only in nonphylogenetic analyses. The results from the males suggest that the sword does not impose a cost in terms of critical swimming speed.

The results from this dissertation raise many additional questions and approaches to examine the relationship between the sword of these fish and locomotor performance. The positive effect of sword length on critical swimming speed suggests that the sword may be an indicator of the male's quality (Husak *et al.* 2006, Husak & Fox 2008). However, intraspecific studies are needed, examining the relationship between sword length and a male's critical swimming speed; if males with longer swords are of better quality, then they should have increased critical swimming speeds, similar to the interspecific results.

Additional interspecific analyses may shed light on the effect of the sword on performance. For instance, incorporation of path analyses could determine the direct and indirect effects of traits on locomotion (as discussed in Chapter 1) and may better indicate the cost (or lack thereof) of the sword (Fig. 0.4). Incorporation of additional swimming performance measures may also allow for the examination of potential trade-offs between aspects of performance. Future plans include examination of the effect of sword length on sprint speed (see Chapter 3) and various aspects of kinematics during a

c-start escape response. These two swimming performances are potentially important to escape predation (Domenici & Blake 1997, Walker *et al.* 2005, Oufiero *et al.* 2010). Future plans also include examining the morphometric shape among species and determining if and how shape interacts with the sexually selected trait and locomotor performance. Does shape evolve as a compensatory trait to help offset the cost of the sword?

The locomotor performance of organisms is a complex trait incorporating many levels of biological organization (Garland 1994a, Garland 1994b, Garland & Kelly 2006). Many studies have examined locomotion from ecological and evolutionary perspectives (Garland & Losos 1994, Walker *et al.* 2005, Langerhans 2009, Oufiero *et al.* 2010), and even more are beginning to examine the effects of sexual selection on locomotor performance (Langerhans, Layman & DeWitt 2005, Irschick *et al.* 2007, Irschick *et al.* 2008). Although locomotor performance has been studied in many taxa, for many years, there remain many unanswered questions related to mechanism, function, behavior, sexual selection, ecology, and evolution (e.g., Feder *et al.* 2010).

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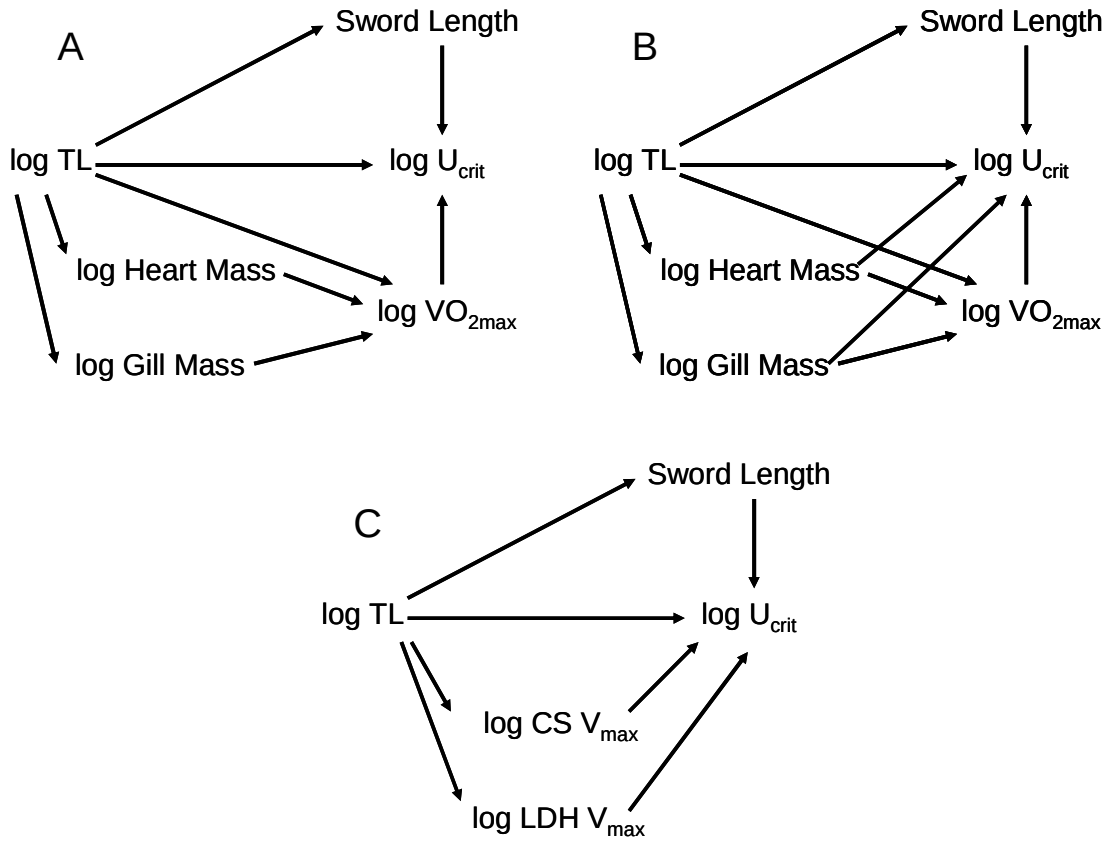

Figure 0.4. Potential path models that could be analyzed to determine the direct and indirect effects of potential predictors on critical swimming speed and to infer whether the sword causes a locomotor cost. Path models A and B incorporate maximum oxygen consumption (VO_{2max}), heart mass, and gill mass as potential compensatory traits, while model C incorporates citrate synthase activity and lactate dehydrogenase enzyme activities in tail muscle. More complex models can also be tested. log TL represents log total length. For further discussion, see Oufiero & Garland (2007).