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# Mating Systems, Copulatory Organ Size, and Scaling Relationship in Mollies (*Poecilia* spp.)

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Mating Systems, Copulatory Organ Size, and Scaling Relationship in mollies (*Poecilia spp.*)

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## **Abstract**

Copulatory organs rapidly evolve and are subject to complex selective pressures affecting mating success. One feature of copulatory organs that is subject to such selective pressures is size. Benefits of longer organs may include greater signal effectiveness in courtship and longer ‘reach’ when attempting copulations with evasive females. Costs of longer organs may include impaired locomotion, increased energetic cost or reduced mechanical compatibility with female genitalia. The optimal size for a copulatory organ may vary with mating behavior. The objective of this study is to examine among-species variability in copulatory organ size, body size and the relationship between copulatory organ size and body size, in a closely-related set of six species within the livebearing fishes (Poeciliidae). The copulatory organ in the Poeciliidae is called the gonopodium. Among these fishes there are pronounced differences in mating behavior. In some species courtship often precedes copulation, especially when the male is relatively large. In other species there is no courtship and males attempt to force copulate by inseminating evasive females. I hypothesize that species that court will have either a shorter or longer gonopodium than species that do not court. Because small males of species that court and force copulate have less “need” for a long gonopodium, I hypothesize that gonopodia in large males in species with courtship will be disproportionately short. Variation in functionality of the anal fin between the sexes also suggests variation in the scaling relationship between body size and anal fin size. The findings revealed hypoallometry in all male species examined as well as a longer absolute and relative gonopodium size in species that perform both courtship and forced copulations. Female anal fins of all species were found to scale isometrically to body size.

## Introduction

Male copulatory organs are subject to a variety of selective forces that influence reproductive success and fitness. Copulatory organs can be modified to enhance reproduction success by enhancing characters used in mate choice and male-male competition. Male organ characters exaggerated through mate choice can influence the receptiveness of a female {Brooks, 1995 #7; Rosenthal, 1998 #27}. However, increased copulatory organ size can reduce survival rate and increase energetic cost of the male {Langerhans, 2005 #23}. The copulatory organ of male fish in the family Poeciliidae is a highly specialized anal fin called a gonopodium. Gonopodium shape has been shown to influence fertilization success {Arnqvist, 1999 #43}. Previous studies have not addressed the influence of mating behavior on male copulatory organ length. This paper investigates how mating behaviors used by poeciliids influence the morphology of the gonopodium and how gonopodium morphology alters the reproduction success and fitness of the males.

Live-bearing fishes in the family Poeciliidae offer an opportunity to test for mating system effects on copulatory organ characteristics. This group of fish is very easy to sex and examine for genital morphological variation since the male copulatory organ is external. Poeciliids are ideal for this study because species can exhibit two different mating behaviors or a combination of both. Mating behaviors used by poeciliids include courtship, where a male attracts receptive females allowing for cooperative reproduction, and forced copulation, where a male attempts to sneak-copulate with an uncooperative female. Male poeciliids have been noted to perform courtship, gonopodial thrusts, or a combination of the two in attempt to copulate with a female {Farr, 1989 #41}. Some

poeciliid species only thrust, and some other species perform courtship preceding at least some of the copulations. Species that have been observed only thrusting include *P. orri*, which has a shortfin morphology {Ptacek, 1998 #48}. Three closely related species (*Poecilia latipinna*, *P. petenensis*, *P. velifera*) with a sailfin morphology have been observed to perform both courtship and gonopodial thrusts {Hankison, 2006 #46}. A combination of these mating systems has also been observed in poeciliids with shortfin morphology as well {Ptacek, 1998 #48}{Neimeitz, 2002 #55}. Farr et al {1989 #41} summarizes poeciliid species that perform one or a combination of mating behaviors. Species examined in this study include one thrusting only and five courting/thrusting species.

Mating strategy can influence male copulatory organ morphology {Arnqvist, 1998 #37}. In poeciliids that use a combination of forced copulations and courtship {Farr, 1989 #41; Ptacek, 1996 #26}, relative gonopodium length is related to precopulatory behavior, where species with males possessing either long or short gonopodia utilize different sexual activities based on their ability to maximize insemination {Rosen, 1961 #35}. Courting males have been observed using their gonopodia as a mating display {Brooks, 1995 #10; Rosen, 1961 #35}, of which males with longer gonopodia are considered more attractive to females {Langerhans, 2005 #23}. We will specifically examine length morphology of the male copulatory organ in response to specific mating types in members of *Poecilia*.

Copulatory organ size can be influenced by male-male competition. Some mating systems include females that are receptive year-round. Males in these systems tend to be highly sexually active and aggressive towards other males {Farr, 1989 #41} and males

that cannot compete well need an alternative solution for successful copulation. Males compete with other males by either physically chasing each other {Itzkowitz, 1971 #30} or by ‘guarding’ receptive females {Bisazza, 1996 #6}. Males can also indirectly compete through sperm competition. Modified copulatory organs in several insect taxa are used for sperm competition, where previously deposited sperm is removed or displaced in the female’s reproductive organ {Eberhard, 1985 #14; Waage, 1979 #44} or through sperm displacement {Hosken, 2000 #51}. This may not be the case in poeciliids since the sperm storage area in female genitalia is physically separated from the region where the gonopodium penetrates the female {Rosen, 1953 #52}. Small-bodied males cannot compete well with larger males since larger males are more suited to monopolize females and chase away smaller males. Small-bodied males need an alternative strategy to compete with large-bodied males. One strategy is sneak-copulation. Smaller-bodied males already have an advantage since it is easier for them to sneak up on females without being seen {Pilastro, 1997 #25}. Additional advantage could come from having an elongated gonopodium to extend their ‘reach’ as they attempt to make contact with a female.

Female mate choice may select for larger copulatory organs {Langerhans, 2005 #23}, especially if the organ is mostly external, however it may be costly to develop and maintain {Moller, 1993 #47}. Maximum speed and endurance are reduced in one species of spider due to their exterior pedipalps {Ramos, 2004 #50}. Poeciliids with long gonopodia have a reduced burst-swimming speed as well as elongated gonopodia in predator-free environments {Langerhans, 2005 #23}. The tradeoff between selection for elongated gonopodia due to female choice and selection against elongated gonopodia

from restricted locomotion is evident in interpopulation differences, wherein gonopodium length varies inversely with predation risk.

To examine how the gonopodium's function as a copulatory organ has affected its size and scaling relationship in poeciliids, we also analyze the scaling of the homologous female anal fin for comparison. The anal fin is used for propulsion {Arreola, 1996 #56} and is suggested to be used for balance in fish {Standen, 2005 #58}. The gonopodium is used by males in copulation {Collier, 1936 #57}, which could alter the developmental pattern of the gonopodium from the female anal fin.

The objective of the present study is to test a set of hypotheses for how gonopodium features should covary with mating behavior in mollies. First, absolute and relative gonopodium length of males that only thrust and males that perform a combination of courtship and thrusting are analyzed. It is hypothesized that courting/thrusting (CT) males will have longer gonopodia than thrusting only (TO) males since they are signaling and attracting more females for copulation with it. Alternatively, thrusting only males may have longer gonopodia since they are thrusting at females all the time and could increase their success rate by increasing their 'reach'. Second, differences in the scaling of gonopodium length between males in TO and CT species are compared. It is hypothesized that scaling will be especially hypoallometric in CT species since smaller individuals are expected to mate primarily by thrusting, thus requiring a longer gonopodium to increase their rate of success. In contrast, the relative size of gonopodia in TO species and the female anal fin will be the same regardless of body size (isometric scaling). Finally, scaling of the males and females of each mating type are

compared, with the hypothesis that male gonopodia will scale differently from female anal fins since there is a difference in the functionality of each organ.

## **Materials and Methods**

Species selected for analysis represented a diversity of mating behavior and ample specimen availability. The six species selected were *Poecilia orri*, *P. mexicana*, *P. latipinna*, *P. latipunctata*, *P. petenensis*, and *P. velifera*. Only the thrusting behavior has been observed in *P. orri* {Ptacek, 1998 #48}. In the remaining species, both courtship and thrusting behaviors have been observed {Farr, 1989 #41; Bisazza, 1993 #53; Hankison, 2006 #46; Ptacek, 1998 #48; Ptacek, 2005 #49; Ptacek, 1996 #26}.

Specimens were borrowed from the Field Museum of Natural History, the Florida Museum of Natural History, Tulane University, and the University of Michigan Museum of Zoology. We examined 618 males and 550 females of ‘courting and thrusting’ (CT) species, and 189 males and 149 females of ‘thrusting only’ (TO) *P. orri* (Table 1). Specimens were sorted based on sex and male maturity. Immature males were distinguished from females based on shape and position of the anal fin. Any uncertain specimens were not included in the selection. Mature males were distinguished from immature males based on complete differentiation of the fin rays into gonopodial structures.

We conducted morphological analysis on up to 60 mature males and 30 females in each lot. When the number available in a lot exceeded the desired sample size, we selected the specimens based on formal or informal size stratification. The lengths of all

mature male specimens were initially measured with calipers. The length distribution of mature males in the lot was divided into length classes from which equal numbers of specimens were randomly selected. We did not so divide the length distribution of females into classes, but selected the largest and smallest individuals in the lot and randomly selected females of intermediate size for a subsample with an approximately uniform size distribution. .

We recorded morphometrics from digital lateral views of each specimen in the selected subsample of mature males and females. Each specimen was carefully oriented on a dissecting tray below a digital camera (Canon EOS 20D) mounted to an easel. Insect pins were pushed into the tray to keep pelvic fins away from the body, providing a clear view of the entire anal fin. We used pieces of modeling clay under the caudal fin to level the specimen. A ruler was included in each image for calibration. We measured standard length and anal fin length on each specimen's image. Measurement of digital images was conducted in SigmaScan© Pro; the same investigator measured every specimen.

I conducted analyses on absolute gonopodium length, relative gonopodium length, and the scaling relationship between anal fin and standard length. SAS® was used for all data analyses. Absolute anal fin length was assessed by collecting raw anal fin measurements. Relative anal fin length was expressed as anal fin length divided by standard length. Tests of differences in absolute and relative anal fin lengths of CT and TO males were conducted via two-tailed t-tests. The scaling relationship was tested by comparing the exponent in the equation  $AL = aSL^b$ . Hypoallometry is indicated if  $b$  is significantly less than 1; if  $b$  is greater than 1, then hyperallometry is indicated. The exponent was estimated as the slope in reduced major axis regression of log-transformed

AL and SL. Tests of difference in  $b$  between CT and TO males were conducted via two-tailed  $t$ -tests.  $T$ -tests were also used to test for deviations from isometry ( $b=1$ ) and for differences between male and female values for  $b$ .

## Results

Males in the TO species have shorter gonopodia than males in CT species. There was a significant difference between the two groups in mean gonopodium length ( $\bar{x}_{TO} = 9.18 \pm 1.33$ ;  $\bar{x}_{CT} = 9.64 \pm 2.11$ ;  $t$ -test of between-group difference with unequal variances,  $t = 3.5$ ,  $df = 497$ ,  $P = 0.0005$ ; Fig 1) and relative gonopodium length (Figs 2 and 3;  $\bar{x}_{TO} = 0.242 \pm 0.028$ ;  $\bar{x}_{CT} = 0.261 \pm 0.031$ ;  $t$ -test of unequal variances,  $t = 7.85$ ,  $df = 340$   $P < .0001$ ).

The scaling relationship between gonopodium length and body length was strongly hypoallometric; small males in each population had relatively long gonopodia. The mean scaling exponents of both mating types were less than 1 (Fig. 4;  $\bar{x}_{TO} = 0.6 \pm 0.02$ ;  $\bar{x}_{CT} = 0.62 \pm 0.07$ ;  $t$ -test of null hypothesis that true mean = 1,  $t = -33.56$ ,  $df = 23$ ,  $P < 0.0001$ ). However, there was no significant difference between the scaling exponents of the two mating behaviors ( $t$ -test of regression mean analysis - slope differences between groups,  $t = -1.36$ ,  $df = 21.9$ ,  $P = 0.1873$ ); TO males are not more hypoallometric than CT males.

Scaling varied between males and females. Female scaling averages were significantly higher than the male averages (Fig. 4,  $P < 0.0001$ ). Male scaling reflected

hypoallometrically while females scaled isometrically ( $\bar{x}_{TO} = 0.93 \pm 0.03$ ;  $\bar{x}_{CT} = 1.03 \pm 0.15$ ; Fig. 4; t-test of null hypothesis is true mean = 1;  $t = 0.40$ ,  $df = 23$ ,  $P = 0.6947$ ).

## Discussion

The objective of this study was to examine how the length morphology of the gonopodium is influenced by mating behaviors used by species of poeciliids. Male genitalia evolve quickly and can be influenced by several factors, including mating behavior and individual fitness. Here, it is found that gonopodium length is strongly hypoallometric with respect to body size for males of both examined mating types. Also, males that thrust only have on average shorter absolute and relative gonopodia compared to courting and thrusting males.

Hypoallometry in male genitalia with body size has been {Eberhard, 1998 #15} and found to occur in species of insects and spiders {Bertin, 2007 #59; Ramos, 2004 #50}. This study provides an example of hypoallometry of external genitalia variation with male body size for poeciliids, so smaller-sized males possess relatively longer gonopodia than larger males of the same species. Hypoallometry occurred in males of both mating types, which suggests three ideas. First, hypoallometry suggests small-bodied mature males will have relatively longer gonopodia than large-bodied mature males, which may be a hindrance in survival, but is compensated by the advantage in reproduction success. Second, reproductive success for small-bodied males is increased with gonopodium length. Finally, hypoallometry is evidence for supporting the ‘one size fits all’ hypothesis {Eberhard, 1998 #15}.

Long gonopodia may be more of a hindrance than a help for poeciliids. Species where sexual dimorphism involves males with highly exaggerated features results in greater energetic cost to the male {Langerhans, 2005 #23; Moller, 1993 #47; Ramos, 2004 #50}. Particular costs for poeciliids possessing a long gonopodium included reduced swimming speed and maneuverability {Langerhans, 2005 #23}, reducing predator avoidance and increasing the energy requirements for movement. However, elongated gonopodia increase the male's insemination success with evasive females as well as act as an attractive mating cue. Because the findings in this study show hypoallometry in all analyzed poeciliid males, it is suggested that only small-bodied males benefit by having longer gonopodia. It is predicted that small males will have reduced survival rates but increased insemination success. Further investigation on reproductive success rates in males of varying gonopodium length and mating behavior would reveal an ideal gonopodium length for reproductive success in each mating type.

Small males have relatively longer gonopodia than large males, which suggests that they compensate for their reduced body size in male-male competition by spending more time thrusting with a long gonopodium. Intraspecific variation of mature male body size is present in species of poeciliids {Bisazza, 1993 #53; Ptacek, 1996 #26} {Farr, 1986 #16; Hankison, 2006 #46; Pilastro, 1997 #25}. Because large-bodied males are often more attractive to females {Bisazza, 1993 #53} and have a stronger tendency to guard receptive females from male competitors {Bisazza, 1996 #6}, small-bodied males need a way to compete with their large rivals. The solution for small-bodied mating success is to increase the length of the gonopodium in small-bodied mature males. Hypoallometry in all poeciliid species examined supports this notion. This would suggest the small-bodied

males spend more time thrusting rather than courting in species that perform a combination of the two mating behaviors. Further study on how variation of gonopodium length and mating success rate in small-bodied males would support this conclusion.

It has been suggested that the length of male genitalia conforms to the ‘one size fits all’ hypothesis {Eberhard, 1998 #15}, where the ideal length of the male genitalia is a size that mechanically fits into the widest range of female genitalia. The scaling exponent between genital length and body length in this hypothesis would be equal to zero. Here, males of both mating types have scaling exponents significantly less than 1 (Fig. 4). Because of the significant difference between the scaling of the anal fin and gonopodium, it seems that hypoallometry in the gonopodium is an evolutionary step towards the ‘one size fits all’ hypothesis. An ancestral gonopodium may have scaled similarly to the anal fin and shifted towards hypoallometry over time. One way to investigate this idea would be to compare scaling relationships of the male and female anal fin in extant fishes that are closely related to species with gonopodia.

Mating behavior in poeciliids is a factor in relative gonopodium size. Mating behavior can influence features of male poeciliids {Pilastro, 1997 #25}, such as body color, body size, and caudal fin morphology {Brooks, 1995 #10}{Rosenthal, 1998 #54}. The length of the male gonopodium is another feature of male poeciliids that can be influenced by the mating systems used by poeciliids. Here, TO males were found to have a smaller mean and relative gonopodium size than CT males (Figs. 1, 2, and 3). This suggests that there is a benefit to having a longer gonopodium if courtship is a part of the mating system. Long gonopodia act as a cue in female mate choice; females of *Gambusia* have a preference for males with long gonopodia {Langerhans, 2005 #23}. An increase in

gonopodium length would therefore increase male attractiveness. Future studies on this material should attempt to quantify male attractiveness based on specific features, which would determine how significant a long gonopodium is in comparison to other features deemed attractive to females. Trait enhancement for attractiveness seems to be common in poeciliid fishes {Brooks, 1995 #10; Langerhans, 2005 #23; Rosen, 1961 #35}. Since female mate choice is reduced in non-courting mating systems {Bisazza, 1993 #53}, gonopodium elongation for increased attractiveness is not as beneficial for TO males. However, investigation on female mate choice in TO species is may reveal some female mate choice, since it is suggested that females in species only using forced copulation have a passive choice (Bisazza 1995), which may only influence gonopodium size if the gonopodia is used in interactions between males.

Male and female poeciliids vary in functionality of the anal fin. Median fins evolved before paired fins over 400mya {Coates, 1994 #33}. A morphologically unique anal fin noted to resemble the gonopodium is found in fossils from the Triassic, though its functionality in the fossil fish is unknown {Lombardo, 1999 #31}. Because the anal fin appeared before the modified anal fin resembling the gonopodium, the ancestral function of the anal fin was for swimming {Arreola, 1996 #3; Standen, 2005 #28}. The gonopodium is primarily used in copulation {Hubbs, 1957 #32; Rosen, 1953 #25} and acts as a hindrance to swimming ability {Langerhans, 2005 #14}, deterring the possibility of its use for swimming. However, Langerhans {2005 #14} observes the gonopodium assisting in braking, hinting that some mobility functionality remains in this derived anal fin. Because the scaling relationships of the two fins significantly varied (Fig. 4), it can be concluded that variation in anal fin scaling reflects variation in fin functionality, and

that functionality influences organ morphology. Further investigation on the topic would require quantifying poeciliid anal fin functionality to examine how variations in functionality between species influence morphology.

## Tables and Figures

Table 1 - totals for number of lots, females, and mature males representing each species. Colors distinguish sexes (F – pinks; M – blues) and mating type (CT – darker shades; TO – lighter shades).

<b>Species</b>	<b>Number of lots</b>	<b>Number of females</b>	<b>Number of males</b>
<i>latipinna</i>	4	120	180
<i>latipunctata</i>	4	119	129
<i>mexicana</i>	3	90	152
<i>orri</i>	5	149	189
<i>petenensis</i>	4	102	37
<i>velifera</i>	4	119	120

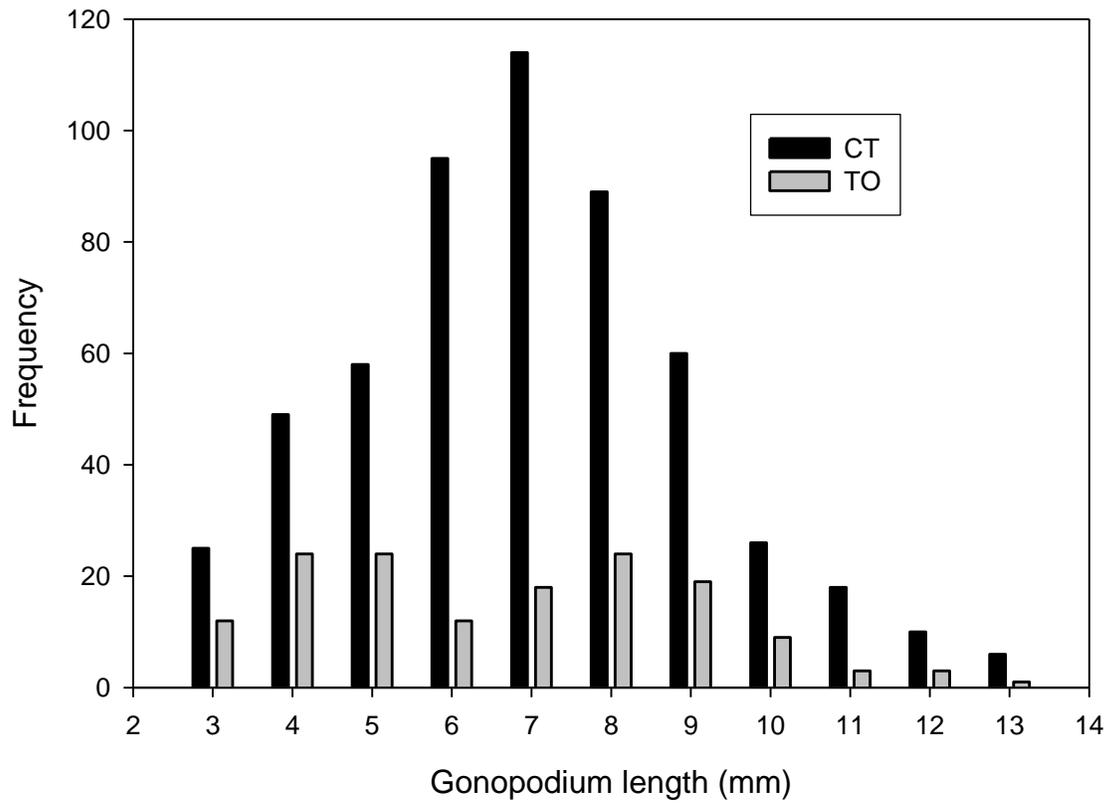


Figure 1 – Absolute gonopodium size in courting and thrusting (CT) and thrusting only (TO) species. The lowest and highest classes on the x-axis include some individuals with extreme values.

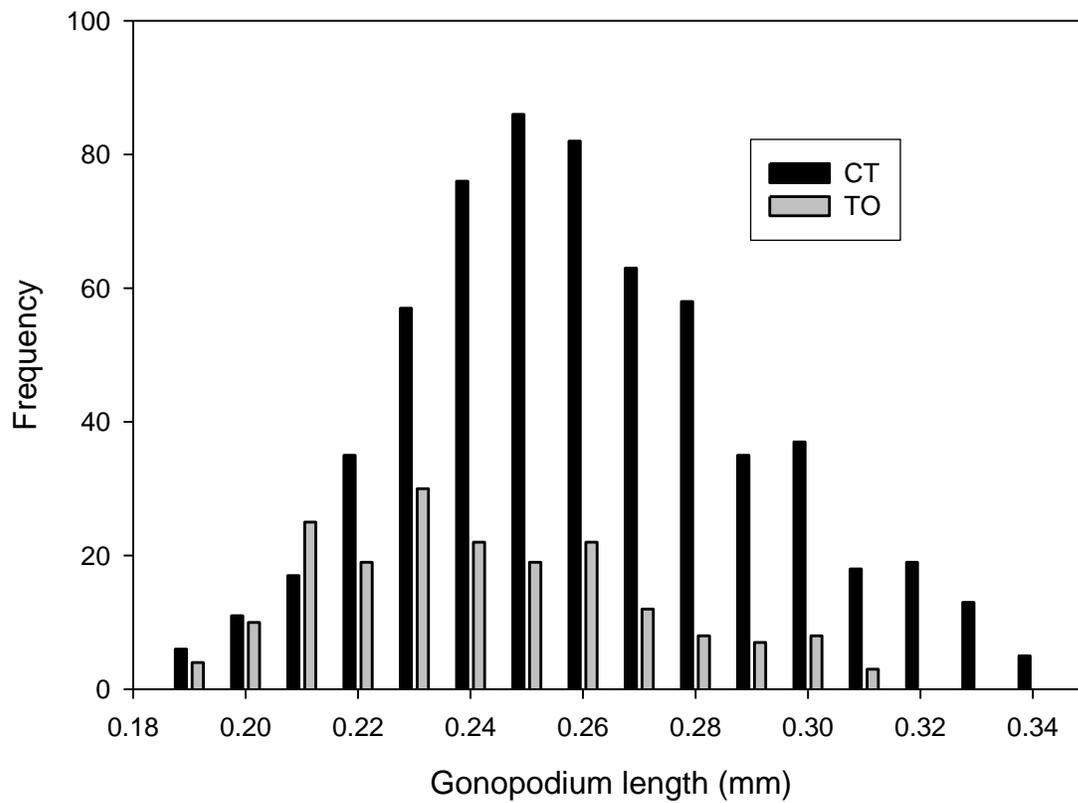


Fig. 2 – Relative gonopodium size in CT and TO males. The lowest and highest classes on the x-axis include some individuals with extreme values.

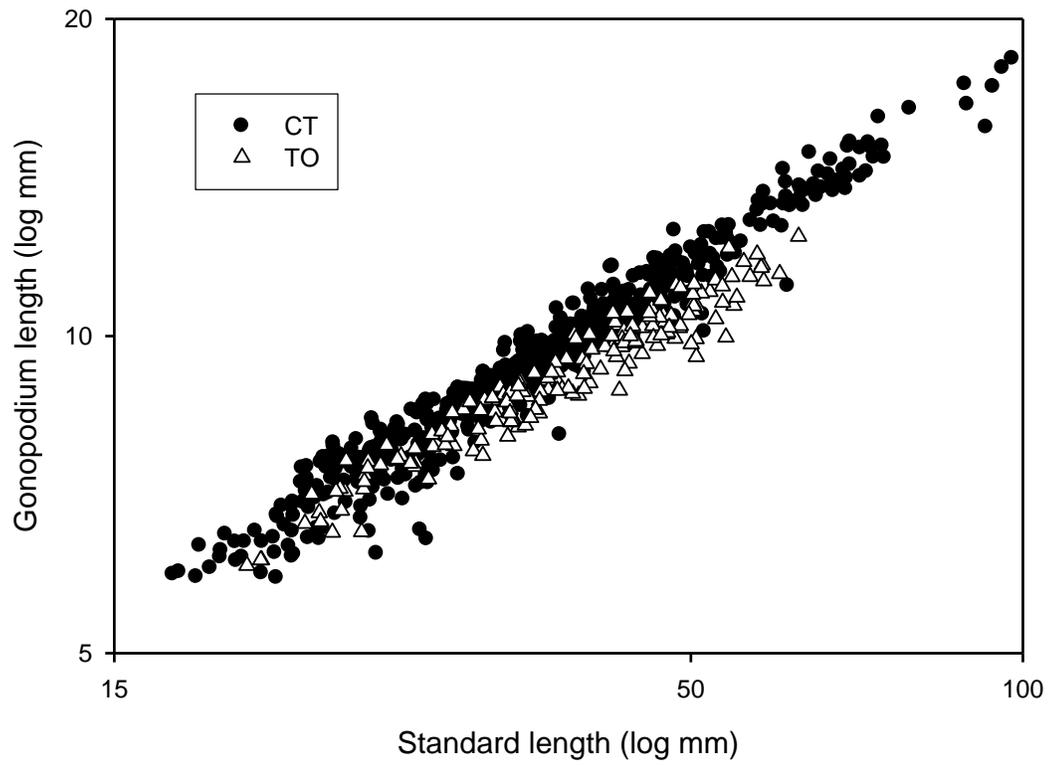


Fig. 3 – Comparison between the rate of change in anal fin length at body length increases for CT and TO mature males.

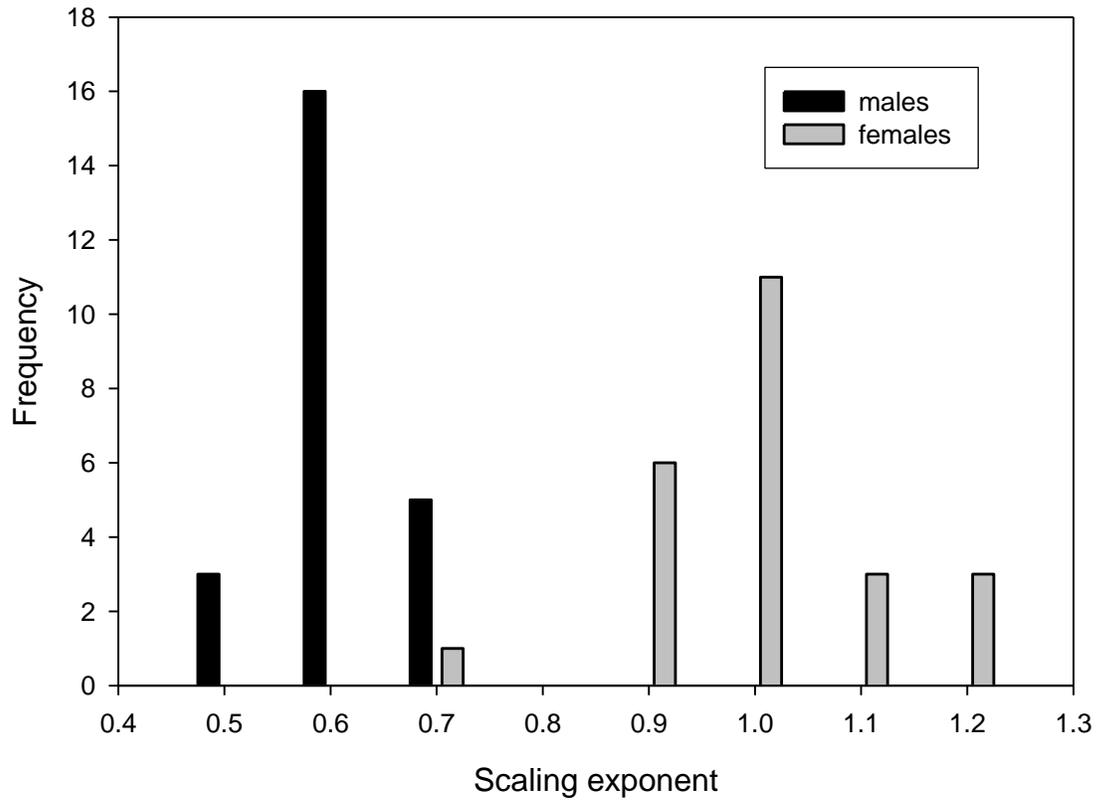


Fig. 4 – Comparison of the scaling relationship exponent between anal fin length and body for males and females. The lowest and highest classes on the x-axis include some individuals with extreme values. The distinction of scaling exponents for mating behavior is excluded because there is no significant difference.

## Literature Cited