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The Role of Thyroxine in Spadefoot Toad Development

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THE ROLE OF THYROXINE IN SPADEFOOT TOAD DEVELOPMENT

By

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This is dedicated

To good friends and good conversation,
To music and band members who rock,
To the Conradi Fishing Team, pound-for-pound the Jack Crevalle,
To Hopkins Eatery, Kitchos and Good Time Charlies,
To night driving for herps,
To night driving with herpers,
To fifty-cent movies on Tuesday night,
To bad music and watered-down drinks at Waterworks,
To fast computers and fast cars,
To Tequila and naked hot tubing,
To professors' open doors and open minds,
To canoeing on lazy Sunday afternoons,
To volleyball teams that play well and play hard,
To teammates that are not afraid to dive,
To weird biological anomalies,
To offices with windows,
To mobile homes with roaches, spiders and cracks in the floor,
To offices with geckos,
To beer and pool on Friday afternoons,
To fellow graduate students who make me laugh,
To fellow graduate students who make me yell,
To challenging advisors,
To supportive advisors,
To eclectic tastes in music,
To the church lot,
To having a song lyric for every-day,
And, without a doubt, to my wife Shonna and my mother Vickie, the two most important people in my life; your strength of soul is infectious

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ABSTRACT

Thyroxine is arguably the most important hormone in anuran development and affects development through exogenous and endogenous means. In this study, I investigate the relationship between exogenous thyroxine and spadefoot tadpole development. Tadpoles of the spadefoot toad *Spea multiplicata* can display either a "typical" omnivorous phenotype or a carnivorous phenotype. Exogenous thyroxine and feeding on conspecific tadpoles was first proposed as the proximate mechanisms for developmental polyphenism in these tadpoles 20 years ago. Recent research on the effects of exogenous thyroxine on anuran development are at odds with the current understanding of the role of exogenous thyroxine on developmental polyphenism is spadefoot toad tadpoles. Understanding the proximate mechanism of ontogenetic polyphenism is the first step in gaining a mechanistic and evolutionary understanding of the factors responsible for the control and evolution of polyphenism. Here, I demonstrate that neither exogenous thyroxine nor feeding on conspecific tadpoles triggers developmental polyphenism in spadefoot toad larvae and show how heterochronic processes, and past reliance on ratios rather than developmental trajectories, mislead us into believing that these proximate mechanisms controlled developmental polyphenism in spadefoot toads. I also investigate comparative development of four species of spadefoot toad with and without thyroxine treatment to speculate on the controlling factors that may be responsible for general differences in development between the species. By controlling the amount of thyroxine that acts on metamorphic tissues during development, I am able to suggest that differences in development between the four species may be due to cell specific differences in receptor or metamorphic gene activity in addition to possible differences in amount of circulating thyroid hormone (thyroxine).

INTRODUCTION

We tend to think of development as a conserved process within related groups of organism and this is especially true for organisms that develop in a buffered environment such as the amniote egg. But, for organisms that undergo at least partial development in an unbuffered external environment, we see an array of different developmental strategies that have evolved to cope with the changing environment even within closely related species.

Spadefoot toads are an example of the second group. There are seven species (Garcia-Paris et.al. in press) of North American spadefoot toads and within this group there is variation in size at metamorphosis, time to metamorphosis (Buchholz and Hayes 2000, 2002), and the presence of developmental polyphenism (Pfennig 1992b). Given this example and numerous other examples within insects (Brakefield and Mazzotta 1995, Nijhout 1999), and amphibians (Van Buskirk and McCollum 2000, Relyea 2001a), the question arises; why do we see the evolution of multiple developmental strategies within related groups of organisms to cope with similar environments rather than one "good" strategy that is converged on, or maintained, repeatedly over evolutionary time? Or more specifically, how and why do organisms evolve multiple developmental strategies to cope with the same environment? The how part of this question addresses the mechanistic level of developmental variation. Have new developmental pathways evolved among related groups of organism; or have developmental programs that are shared among related groups of organisms been co-opted for different developmental functions? The why part of the question addresses the ecological and evolutionary level of developmental variation. Did development type A evolve as an adaptation, and as a consequence species with this developmental type have been able to expand their range into a more diverse set of habitats relative to a related species with development type B?

In this thesis, I investigate the mechanistic control of different developmental programs, both within a species and among species. Specifically, I investigate the mechanistic role of exogenous and endogenous thyroid hormone (thyroxine) in controlling development. In chapter one I investigate the role of exogenous thyroxine as a proximate mechanism controlling developmental polyphenism in the spadefoot toad *Spea multiplicata*. In chapter two, I investigate the mechanistic control of developmental differences between four species of spadefoot toads. This research is important because it provides insights into the environmental and internal control of different developmental strategies. From polyphenism to timing and size at metamorphosis, spadefoot toads have evolved multiple developmental strategies to cope with similar environments. There are many other examples of organisms, such as caudatans, aquatic insects, and crustaceans, in which closely related species have evolved different developmental strategies to cope with the same environment. Understanding how environmental and internal mechanisms control development is important in elucidating how development has evolved within and among groups of organisms.

CHAPTER 1

THYROXINE DOES NOT CONTROL CARNIVORE DEVELOPMENT; A REASSESSMENT OF THE PROXIMATE MECHANISMS OF ONTOGENETIC POLYPHENISM IN SPADEFOOT TOADS

INTRODUCTION

Polyphenism, the development of multiple discrete phenotypes from one genotype, has been implicated in many biological systems, where alternative developmental strategies help organisms cope with changes in community composition (Van Buskirk and McCollum 2000, Relyea 2001a, 2001b), mating-strategies (Emlen 1994, Moczek and Emlen 1999), density (Hoffman and Pfennig 1999, Michimae and Wakahara 2002), habitat longevity (Pfennig 1990, 1992a, 1992b), and season (Brakefield and Mazzotta 1995, Nijhout 1999).

Determining the proximate mechanism(s) controlling the expression of ontogenetic polyphenism is the first step in elucidating the underlying developmental mechanisms of this process and the selective factors that may have been responsible for its evolution. Amphibian metamorphosis is a highly coordinated event in which essentially all tadpole tissues are transformed (Frieden and Just 1970, Kanamori and Brown 1996) and understanding the role of the environment in triggering ontogenetic polyphenism is important in understanding the coordinated evolution of the physiological systems involved in metamorphosis (Denver 1997b).

In many vertebrate and invertebrate systems the proximate control of ontogenetic polyphenism is well understood. In some ambystomatid salamanders the density of conspecifics mediates the development of the broad-headed carnivorous phenotype (Hoffman and Pfennig 1999), and the frequency of that phenotype may be increased by the presence of heterospecific anuran tadpoles (Michimae and Wakahara 2002). Other species of ambystomatid salamanders display facultative paedomorphosis; some individuals metamorphose and move onto land while others remain sexually mature

larvae (Whiteman 1994). The latter retain the ability to metamorphose and may do so under laboratory stress (Brandon 1976). Some anuran tadpoles alter tail morphology and body size in response to the presence of predators (Van Buskirk and McCollum 2000, Relyea 2001a). For example, tadpoles of *Hyla versicolor* may develop brightly colored tail fins in the presence of *Anax* predators (Van Buskirk and McCollum 2000). In some species, effects of these responses may last into adulthood (Relyea 2001b).

In insects, polyphenism is induced by many factors, such as temperature, photoperiod, crowding, pheromones, and diet (Nijhout 1999). In the butterfly *Bicyclus anynana,* ventral eyespot size is determined by larval rearing temperature (Brakefield and Mazzotta 1995), and understanding of this ecological association has led to further studies on the hormonal control of ventral wing-spot formation (Brakefield et.al. 1998). Caterpillars of *Nemoria arizonaria* normally resemble oak twigs, but those developing in the spring feed on and mimic oak catkins (Greene 1989). Finally, horn length in adult dung beetles is determined by the size of the "brood ball" in which larvae are reared (Emlen 1994, Moczek and Emlen 1999). These are just a few examples of proximate mechanisms that govern the expression of ontogenetic polyphenism. Determination of the environmental mechanisms in these systems has been important in continued research into the developmental control and the ecological and evolutionary implications of polyphenism (Brakefield and French 1999, Nijhout 1999, Emlen and Nijhout 2001, Schlichting and Smith 2002).

Spadefoot-toad species have been implicated as a promising model for vertebrate systems that might provide insights into the mechanistic control of ontogenetic polyphenism at the molecular, hormonal, and morphological level (Hall and Larsen 1998, Gilbert 2001, Hall et al. 2002, Michimae and Wakahara 2002), but although the proximate control of polyphenism is well understood in the salamander, frog, and insect groups mentioned above, resolving these mechanisms for spadefoot tadpoles has posed a considerable challenge.

Larvae of the spadefoot toad *Spea multiplicata* show a striking polyphenism in which a "typical" filter-feeding larva is transformed into a carnivore (Fig. 1.1) that actively preys on microcrustaceans and conspecifics. The carnivorous phenotype develops 3–5 days after hatching and is characterized by an enlarged head, enlarged jaw

musculature, shortened intestines, and increased keratinization of the mouth to form a beak (Pomeroy 1981, Pfennig 1990, 1992a, 1992b). This polyphenism is thought to have evolved as an adaptation for survival in temporary pond environments (Pfennig 1990,1992b). Temporary ponds are extremely short-lived; they are filled by rainwater and may dry in as little as a week without regular refilling. In these environments survival depends on the ability to develop and metamorphose rapidly. Individuals of the carnivorous phenotype have been shown to have a competitive advantage in rapidly drying ponds because they metamorphose sooner than omnivores (Pomeroy 1981), thereby avoiding desiccation. Conversely, in long-lived ponds, omnivores have higher survival at metamorphosis because of their greater fat reserves and develop into healthier juvenile toads. Evidence therefore supports fitness trade-offs between becoming carnivorous and remaining omnivorous (Pfennig 1990, Pfennig 1992b).

Carnivores have been shown to metamorphose sooner than omnivores, and because thyroxine is known to accelerate development, thyroxine was proposed to control the development of the carnivorous phenotype. Tadpoles were thought to sequester thyroxine that they acquired by feeding on fairy shrimp (order Anostraca) and/or conspecifics (Pomeroy 1981, Pfennig 1992a). On the basis of three independent studies of the ecological factors controlling carnivore development investigators concluded that carnivore development may be elicited by exogenous thyroxine (Pomeroy 1981, Pfennig 1992a), by feeding on conspecifics (Pomeroy 1981), or by feeding on fairy shrimp (Pomeroy 1981, Pfennig 1990). In the 20 years since these studies, exogenous thyroxine, feeding on conspecifics, and feeding on fairy shrimp has been accepted without reexamination as the proximate trigger for development of carnivorous phenotype (Kupferberg 1997, Alford 1999, Ketterson and Nolan 1999).

Thyroid hormone (thyroxine) acts directly on target tissues by binding to tissue-specific thyroid receptors (TR_α and TR_β) that, when bound, act as transcription factors inducing genes involved in metamorphosis (Das et.al. 2002). Because thyroxine acts directly on target tissues, exogenous thyroxine has been used in many studies to understand the mechanistic association between thyroid hormone and the developmental changes that occur during metamorphosis. Exogenous thyroxine has also been used to investigate tradeoffs between growth and development in anurans. These studies have

universally found that exogenous thyroxine causes somatic growth to slow or stop and induces premature metamorphosis (Kollros 1961, Frieden and Just 1970, Kaltenbach 1970, Beachy 2001, Das et al. 2002). Because naturally developing carnivores show accelerated somatic growth (Fig. 1.1) relative to omnivores, and because thyroxine induces the opposite development pattern ubiquitously in tadpoles, the role of exogenous thyroxine in spadefoot tadpole ontogenetic polyphenism conflicts with our current understanding of thyroxine function across anurans.

Here, the proximate mechanism(s) of spadefoot tadpole polyphenism are brought into question for the first time as I demonstrate that exogenous thyroxine and feeding on conspecifics do not trigger carnivore development in *Spea multiplicata* larvae. In this study (1) I attempted to induce development of the carnivore phenotype in laboratory animals by feeding them conspecifics or thyroxine treated tadpole food; (2) I collected naturally developing carnivores and omnivores from field populations; and (3) I compared the development of laboratory animals and field caught animals. I show that larval development induced by exogenous thyroxine or feeding on conspecifics bears no resemblance to natural carnivore development. Rather, thyroxine-induced tadpoles represent an accelerated development of the normal omnivore morphology, and conspecific-fed tadpoles mimic development of normal omnivores (Fig. 1.1). Lastly, I discuss how heterochronic processes, and the use of ratio statistics, have mislead us into accepting exogenous thyroxine as the proximate mechanisms for carnivore development in spadefoot toads.

METHODS

Breeding Induction

Four male and four female adult *Spea multiplicata* were collected along Portal road (Arizona permit SP710791) in Cochise County, Arizona, between Portal, Arizona, and the Arizona-New Mexico state-line road. The same species and collection area were used in the original studies (Pfennig 1990, 1992a). Animals were transported to the Southwestern Research Station (American Museum of Natural History) and placed in temperature- and light-controlled rooms, where they were maintained at 23–26°C and 12-h light/dark cycles. The following evening, adults were injected with 50 to 100 μl of 1

μg/100 μl GnRH agonist [des-Gly^{10}, (D-His (Bzl)^{6})- luteinizing hormone releasing hormone ethylamide] (Sigma) (Buchholz and Hayes 2000), which stimulated breeding. Animals subsequently mated, and by the following day multiple clutches of eggs were spread out across the aquarium. Hatching occurred 48 h later.

Rearing Conditions

In the original studies, tadpoles were placed in community aquaria and treated with thyroxine (Pomeroy 1981, Pfennig 1992a), and although density has been shown to not be a factor in induction of carnivory in spadefoot toads (Pomeroy 1981), high densities during development may change developmental time, size, and condition in tadpoles of spadefoot toads (Semlitsch and Caldwell 1982, Buchholz and Hayes 2000). In the current study tadpoles were therefore reared individually. On day 5 after hatching, at Gosner stage 27 (early hind limb development) (Gosner 1960)**,** 400 individuals were haphazardly selected from community aquaria and placed individually in 8-ounce plastic drinking cups containing 250 ml of dechlorinated water. Cups were individually labelled according to treatment (150 untreated control, 150 thyroxine-treated, 100 conspecific-fed) and placed in plastic trays in random order. The trays were placed in temperature- and light-controlled rooms (23–26°C, and 12-h light/dark cycle), and the tadpoles were fed according to treatment.

Feeding Regime

Laboratory controls were fed tadpole chow (a finely ground mixture of 3 parts rabbit pellets and 1 part Tetramin ® fish food) (Travis 1980) every other day ad libitum. This diet was developed for ephemeral-pond-inhabiting tadpoles, and *Scaphiopus holbrooki* tadpoles reared on it develop normally relative to naturally developing animals (pers. obs.). Pomeroy (1981) reared *Spea multiplicata* tadpoles in 0.08 mg/l of thyroxine and reported that this concentration produced carnivore-like animals similar but not identical to those observed in the field. Pfennig (1992a) reared tadpoles at a substantially higher concentration of thyroxine (10 mg thryroxine/5 g trout chow in 1 l water) and reported that the full carnivore phenotype was induced. Because the higher dosage was apparently the more effective, I repeated Pfennig's (1992) regime as nearly as possible.

Sigma no longer makes dl-thyroxine, which contains both the active (l) and the inactive (d) isomer of thyroxine, and the active properties of 10 mg of dl-thyroxine (Pfennig 1992a) are equivalent to those of 5 mg of the l-thyroxine now available. In addition, thyroxine is only soluble is a strong alkaline solution (Rugh 1962, pp. 315-322) and not in well water, so I fed tadpoles a mixture of 5 mg of l-thyroxine and 5 g tadpole chow every other day ad libitum, a concentration directly comparable to that used by Pfennig (1992a). Tadpoles in the conspecific-fed treatment were fed conspecific tadpoles that I haphazardly collected every other day from the community aquaria and mortally wounded (as did Pomeroy 1981). Water was changed in all treatments on the same day as feeding.

Laboratory Collections

Ten thyroxine-treated tadpoles and 10 untreated controls were collected daily until thyroxine-treated tadpoles had reached Gosner stage 42 (forelimb emergence). No thyroxine-treated animals lived past stage 42. After that point, untreated controls were collected every 4 days through metamorphic climax (stage 42/43). Five conspecific-fed animals were also collected every 4 days through metamorphic climax, but these animals were relatively unhealthy and only a few lived until metamorphic climax.

Field Collections

Developmental stages 27 through 42 of naturally developing carnivorous tadpoles and naturally developing omnivorous tadpoles were collected from Dearing Pond, approximately 1.6 km north of Portal Road. The summer of 2002 was a severe drought year, and only one breeding aggregation occurred at this pond instead of the usual continuous influx of new tadpoles with each monsoon storm. As a result, all the tadpoles hatched on the same day and could be followed throughout development in a natural setting. The tadpoles were collected throughout the course of development, by dip net or seine, and sorted according to phenotype and stage (Gosner 1960). Phenotype was easily determined by superficial inspection.

Measurements and Analysis

The ratio of the width of the orbitohyoideus jaw muscle (measured at the widest point, as by Pfennig 1992a; OH) and snout-to-vent length (SVL) was determined for all individuals collected. These measurements were used because allometric disparity of the OH character is indicative of the phenotype (Pomeroy 1981, Pfennig 1990, Pfennig 1992a). The comparisons included tadpoles whose developmental stages spanned at least Gosner stage 27 (early hind-limb bud development) through Gosner stage 42 (emergence of forelimbs), and at least 70 tadpoles each were measured for thyroxine-treated tadpoles, untreated controls, field-collected carnivores, and field-collected omnivores. Only 39 conspecific-fed tadpoles were measured. The allometric relationship between OH and SVL was analyzed by linear regression. I tested the relative differences in developmental trajectories for the different phenotypes by sequential ANCOVA (JMPIN version 3.2.6 1998) and Dunn – Sidak corrected significances are listed in Table 2 (Sokal and Rohlf 1995). The statistical differences between developmental trajectories of the treatment groups may be because of different sample sizes (carnivore = 130, omnivore = 136, control = 132, thyroxine treated = 70, and confed = 39) (Sokal and Rohlf 1995), and to address this issue I used a random number generator (JMP version 3.2.6) to select 70 (for comparison to thyroxine treated) and 39 (for comparison with conspecific fed) OH and SVL measurements from carnivore, omnivore, and control data, with replacement. The differences in developmental trajectories of thyroxine-treated and conspecific-fed tadpoles were compared to these data by ANCOVA. This process was replicated five times for all comparisons of thyroxine-treated and conspecific-fed tadpoles.

The OH and SVL measurements were also plotted against Gosner stage in an effort to determine where differences in development arise. Lastly, in order to investigate the effect of lower doses of thyroxine on spadefoot development, I induced *Scaphiopus holbrooki* (a related species of spadefoot toad) tadpoles in the same manner as described above at 0mg thyroxine/5 g tadpole chow, 1.25 mg thyroxine/5 g tadpole chow, 2.5 mg thyroxine/5g tadpole chow, and 5 mg thyroxine/5g tadpole chow and the relationship between SVL and thyroxine dose was plotted.

RESULTS

The relationships between OH and SVL for field-collected carnivores and field-collected omnivores fit linear models (Fig. 1.1, Table 1.1), but carnivores showed positive developmental acceleration with a slope of 0.20, more than twice that for omnivores. This pattern supports the argument that OH growth distinguishes the two phenotypes. The developmental relationships of OH and SVL for thyroxine-treated, conspecific-fed, and control individuals also fit linear models (Figs. 1.1, Table 1.1), and again, growth of OH with SVL in field-collected carnivores was approximately twice that of thyroxine-treated, conspecific-fed, and control individuals. In multiple comparisons, analyses of covariance confirmed significant differences in slope between the field-collected carnivores and all other phenotypes (Table 1.2). F statistics and significances were essentially the same when data was analyzed, by ANCOVA, with equal sample sizes or unequal samples sizes (Table 1.2, 1.3). Therefore, sample size differences did not adversely affect ANCOVA analysis. The slopes for thyroxine-treated animals and field-collected omnivores did not differ significantly, but controls differed significantly from both thyroxine-treated individuals and field-collected omnivores. Differences in slope between control tadpoles and field-collected omnivores and between controls and thyroxine-treated animals may result from the laboratory-rearing environment. Finally, the slope for conspecific-fed animals did not differ significantly from those of field-collected omnivores or controls.

Development of SVL and OH over Gosner stage for thyroxine-treated individuals, conspecific-fed individuals, and natural carnivores demonstrates important differences among the three phenotypes. Figure 1.2 shows that development of field-collected carnivores followed an accelerated trajectory; somatic size increased to approximately Gosner stage 40 and then decreased through metamorphic climax. Aside from the acceleration, its pattern is the same as those of field-collected omnivores and controls. In thyroxine-treated tadpoles, however, growth in SVL stops entirely. That in conspecific-fed animals follows essentially the same pattern as that in laboratory controls. OH continues to grow in thyroxine-treated animals (Fig. 1.3) but shows neoteny (depression) and progenesis (truncation) (Alberch et al 1979) relative to those of field-collected

carnivores, field-collected omnivores, and controls; in thyroxine-treated animals, OH growth peaked at stage 34 (1.2 mm) and decreased through metamorphic climax. Forelimb emergence (Gosner stage 42) occurred in thyroxine-treated animals while hind limb development was at stage 35, which explains why thyroxine-treated animals "jump" from stage 35 to 42 on figures 1.2 and 1.3. Conspecific-fed tadpoles, again, followed essentially the same pattern of growth as laboratory controls for OH development, but the growth peak could not be determined because animals died before completion of premetamorphic development. In field-collected carnivores, OH growth was accelerated, peaking at stage 40 (4.8 mm), and decreased through metamorphic climax, a developmental pattern similar (aside from acceleration) to that of controls and field-collected omnivores. The acceleration of carnivore development and neotony/progenesis of thyroxine-treated tadpoles, relative to controls and field-collected omnivores, are opposite developmental patterns and do not support the conclusion that exogenous thyroxine is the proximate trigger of carnivore development. Conspecific-fed tadpoles, other than being smaller, essentially mimicked the developmental patterns of untreated controls, a finding that does not support the conclusion that feeding on conspecifics induces carnivore development.

Plotting the relationship between SVL and different concentrations of thyroxine for *Scaphiopus holbrooki* reveals a predictable pattern of developmental response that is ubiquitous amongst anurans. As the concentration of thyroxine that tadpoles are fed increases, the somatic growth of the tadpoles declines until tadpole SVL growth asymptotes (Fig. 1.4).

DISCUSSION

Clearly, neither exogenous thyroxine nor feeding on conspecifics induces the carnivorous phenotype under the conditions of the experiments reported here. In the original experiments by Pfennig (1990, 1992a), the size-corrected OH values (OH-to-SVL ratios) for field-collected carnivores and field-collected omnivores were plotted together on a frequency histogram and formed a bimodal distribution. The carnivores constituted the upper mode and the omnivores the lower one, and their means differed significantly (Pomeroy 1981, Pfennig 1990, Pfennig 1992a). Because developmental

studies suggested that the carnivore phenotype was developmentally accelerated with respect to omnivores (Pomeroy 1981, Pfennig 1990, 1992a, 1992b), and because exogenous thyroxine accelerates metamorphosis, Pomeroy and Pfennig hypothesized that thyroxine was involved in triggering development of the carnivore phenotype. When they tested this hypothesis with induction assays (Pomeroy 1981, Pfennig 1992a) and plotted size-corrected OH values for thyroxine-treated and control animals together on a frequency histogram (Pfennig 1992a), the data again formed a bimodal distribution; thyroxine-treated tadpoles constituted the upper mode and laboratory controls the lower (Pfennig 1992a). Again, the means differed significantly (Pomeroy 1981, Pfennig 1992a). From these results, it was concluded that exogenous thyroxine is linked with the development of the carnivore phenotype.

The field-collected carnivores and omnivores in the present study showed the same statistical difference in mean OH-to-SVL ratio (Fig. 1.5), and in fact the means fall within previously reported distributions (Pfennig 1992a). The thyroxine-treated animals and controls also differed significantly in mean OH-to-SVL ratio (Fig. 1.6), and again the means fall within previously reported distributions (Pfennig 1992a). As Fig. 1.7 shows, however, this similarity in ratios does not accurately reflect similarities in phenotype.

In thyroxine-treated animals, SVL growth stopped entirely (Fig. 1.2). OH continued to grow but more slowly than in laboratory controls (Fig. 1.3). In the natural carnivores, on the other hand, SVL grew faster than in controls, and OH grew even faster than SVL, more than doubling in relative size (Figs. 1.1 - 1.3). The high OH-to-SVL ratio in field-collected carnivores arose from increases in the numerator, whereas for thyroxine-treated individuals it arose mainly because the denominator remained so small.

Pomeroy (1981) also found a significantly higher OH-to-SVL ratio in tadpoles fed a combination of fairy shrimp and conspecifics than in tadpoles fed a control diet (trout chow). In the present study conspecific-fed tadpoles did not differ significantly from controls in OH-to-SVL ratio (Fig. 1.6), nor did they resemble field-collected carnivores in development (Fig. 1.1).

Both the proximate and the underlying hormonal mechanisms for the switch from omnivore phenotype to carnivore phenotype are probably far more complex than previously understood. Normal tadpole development requires highly coordinated

interactions among multiple hormones and phenotypic-character-specific titers of those hormones (Frieden and Just 1970, Shi 1994, Das et al. 2002). The transformation from omnivore to carnivore may be just as specialized, and although thyroxine may be involved in the hormonal pathway at some point, the data presented here do not support the conclusion that exogenous thyroxine is a proximate trigger of carnivore development. Thyroxine-treated animals showed depressed and/or truncated development, whereas field-collected carnivores developed with accelerated positive allometry twice that of any other phenotype.

The thyroxine-treated tadpoles metamorphosed in as little as 6 days, faster than any anuran is known to do so in nature (Buchholz and Hayes 2000), rather than in the 13–23 days of field-collected carnivores, which suggests an unnatural phenotypic response is occurring rather than a mimic of the actual natural carnivore phenomenon. Because the increase in OH with SVL is similar for thyroxine-treated tadpoles, controls, and field-collected omnivores, exogenous thyroxine is probably not causing a developmental shift in OH growth similar to the one shown by field-collected carnivores. Rather, it crowds the entire somatic growth and development into a time span unknown for naturally developing anurans. It causes depressed developmental curves of tail length (TL), hind limbs (HLL), head width (HW), and anterior/posterior head growth (SME) in *Spea multiplicata* (Figs. 1.7, 1.8) and rapid, uncoordinated development; for example, forelimb emergence and head transformation occur prior to full hind-limb development. These results are similar to those of classic thyroxine-treatment assays on both ranid and bufonid tadpoles (Frieden and Just 1970). In addition, the thyroxine-treated tadpoles do not survive past Gosner stage 42 and in no way mimic the behaviors of the field-collected carnivores; they are almost completely inactive and show erratic swimming when perturbed.

The present study does not rule out the possibility that higher or lower thyroxine concentrations produce different effects, but the effects of exogenous thyroxine on developing tadpoles have been investigated for approximately 90 years; it universally stimulates early metamorphosis and slows or stops growth in tadpoles (Kaltenbach 1970, Kollros 1961, Frieden 1961, Das et al. 2002), regardless of level of environmental stress or initial growth history (Beachy 2001). In a related species of spadefoot toad

(*Scaphiopus holbrooki*), the same concentration of exogenous thyroxine used on *Spea multiplicata* tadpoles also causes somatic growth to stop. Even at lower concentrations, *Scaphiopus holbrooki* somatic growth of tadpoles slows (1.25 mg/5 g tadpole chow) or stops (2.5 mg thyroxine/5 g tadpole chow) (Fig. 1.4). The universality of the response to exogenous thyroxine in anurans suggests that this response evolved very early in anuran evolution. Tadpoles of *Spea multiplicata* seem highly unlikely to have lost the primitive response and simultaneously to have evolved the alternative response of developmental polyphenism.

Besides being smaller, the conspecific-fed tadpoles showed the same developmental patterns as untreated laboratory controls and did not survive past Gosner stage 36. Feeding on conspecifics did not induce the carnivore phenotype. Rather, it produced small, unhealthy, short-lived tadpoles (Fig. 1.7). This result may be due to the high-protein diet; although diet type has been suggested to have relatively little effect on growth and development in *Spea multiplicata* (Buchholz and Hayes 2000).

Pfennig (1990) reported a positive correlation between the presence of fairy shrimp and the presence of carnivorous *Spea multiplicata* in a field study, and I too found that carnivores are absent from ponds without fairy shrimp (pers. obs.). Pomeroy (1981) and Pfennig (1992a) hypothesized that a diet of fairy shrimp supplied the thyroxine they believed to induce the carnivore phenotype. Assays using fairy shrimp as a food source produced size-corrected OH values similar to those from the exogenous thyroxine assays (Pfennig 1990) but suffer from the same difficulties of interpretation. Fairy shrimp may induce the carnivore phenotype directly as a diet component (Pomeroy 1981, Pfennig 1990, Pfennig 1992a), or through other biotic or abiotic factors associated with their presence. The relationship between fairy shrimp presence and expression of *Spea multiplicata*'s developmental polyphenism remains to be explored.

Because there is debate among anuran biologists concerning which spadefoot toads display polyphenism (pers. comm.), this study started as an attempt to investigate the taxonomic distribution of polyphenism, by way of thyroxine induction assays, and the general habitat correlates that may have been associated with the evolution of polyphenism. Because exogenous thyroxine does not induce polyphenism, I was not able to resolve these evolutionary questions. Future studies should focus on the proximate

mechanisms of developmental polyphenism because understanding the environmental mechanisms that elicit developmental polyphenism in spadefoot toads is the first step in initiating evolutionary and developmental studies of broad implications.

Table 1.1. Results of regression of orbitohyoideus width on snout-to-vent length with a linear model.

Phenotype	Slope	Y-intercept	R^2	*F* ratio,	D. F.	*P*
Control	0.13	0.01	0.96	3238.88	1, 130	< 0.0001
Omnivore	0.09	0.34	0.76	402.19	1, 134	< 0.0001
Carnivore	0.20	-0.09	0.86	781.16	1, 128	< 0.0001
Thyroxine-treated	0.09	0.49	0.29	28.44	1, 68	< 0.0001
Conspecific-fed	0.12	0.14	0.78	129.57	1, 37	< 0.0001

Table 1.2. Results of ANCOVA comparing slopes of orbitohyoideus jaw muscle width with snout-to-vent length. Dunn – Sidak significance shown (*).

	Treatment-by-SVL				
Phenotype	Sum of squares	*F* ratio, D.F.	*P* , Significance, Dunn – Sidak Corrected Sig.		
Carnivore/Omnivore	11.28	120.53, 1	< 0.0001	*	*
Carnivore/Thyroxine-treated	0.41	3.97, 1	0.05	*	
Carnivore/Control	3.95	49.12, 1	< 0.0001	*	*
Omnivore/Control	0.70	37.81, 1	< 0.0001	*	*
Thyroxine-treated/Omnivore	0.002	0.07, 1	0.79		
Thyroxine-treated/Control	0.05	7.63, 1	0.01	*	*
Conspecific-fed/Control	0.01	0.75, 1	0.39		
Conspecific-fed/Omnivore	0.05	1.95, 1	0.16		
Conspecific-fed/Carnivore	0.50	4.11, 1	0.04	*	

Table 1.3. F-ratio ranges and significance ranges of ANCOVA comparing slopes of orbitohyoideus jaw muscle width with snout-to-vent length. 70 (thyroxine-treated comparison) and 39 (conspecific-fed comparison) OH and SVL measurements were randomly selected for carnivores, omnivores, and controls. Process was replicated 5 times per comparison.

	Treatment-by-SVL		
Phenotype	F ratio range	Significance Range, Sig.	
Carnivore/Thyroxine-treated	4.52 – 8.13	0.005 – 0.04	*
Thyroxine-treated/Omnivore	0.04 – 0.52	0.47 – 0.85	
Thyroxine-treated/Control	7.82 – 8.60	0.004 – 0.006	*
Conspecific-fed/Control	0.95 – 1.63	0.21 – 0.33	
Conspecific-fed/Omnivore	1.57 – 3.55	0.06 – 0.21	
Conspecific-fed/Carnivore	3.63 – 10.57	0.002 – 0.06	*

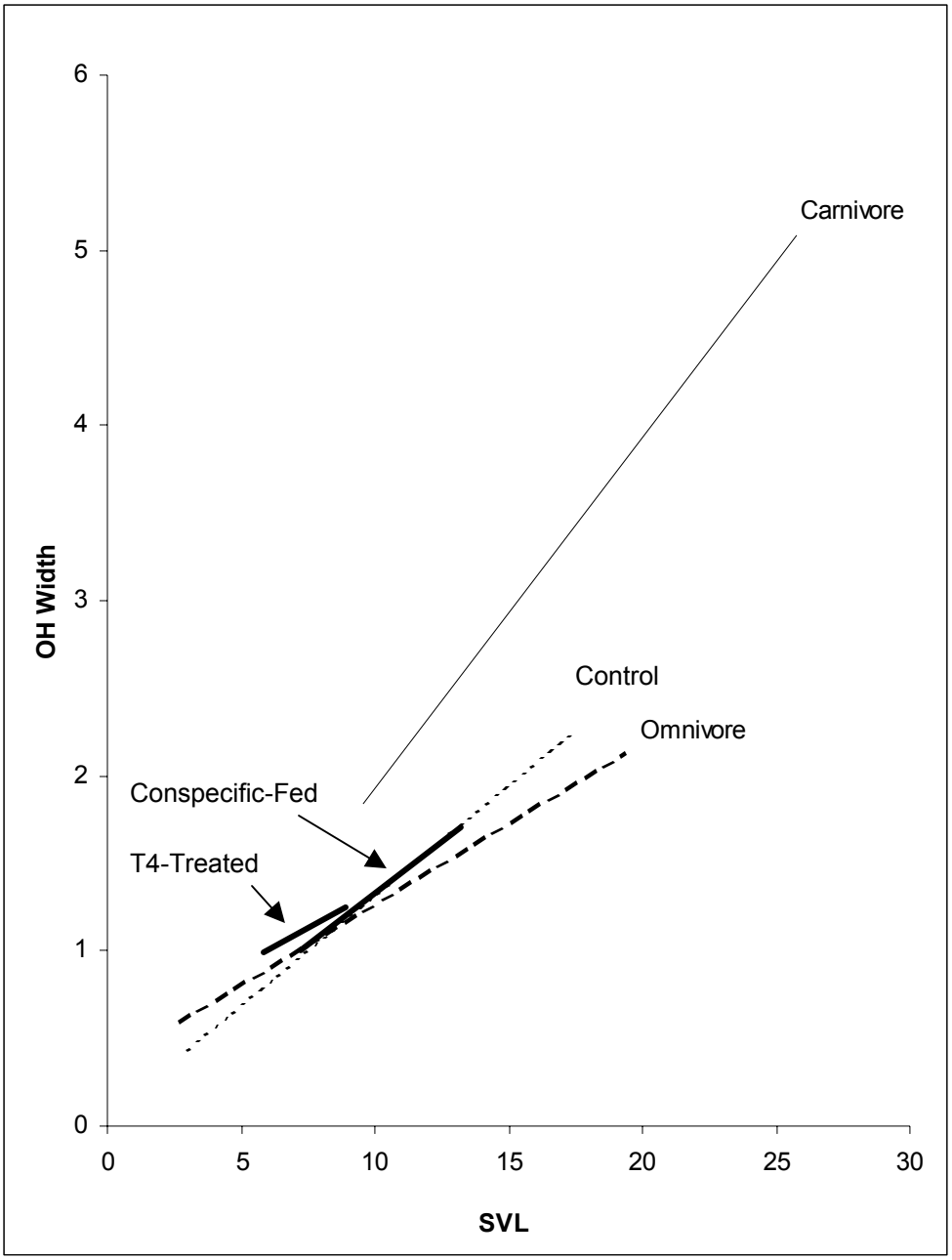

Fig. 1.1. Simple linear regression of orbitohyoideus (OH) jaw muscle width (mm) on snout-to-vent length (SVL) (mm) for field-collected carnivores, field-collected omnivores, thyroxine-treated tadpoles (T4), conspecific-fed tadpoles, and laboratory controls. Field-collected carnivores and omnivores are represented by Gosner developmental stages 25–42. Thyroxine-treated and control animals are represented by Gosner stages 27–42. Conspecific-fed animals are represented by Gosner stages 27–36. Only the slope for field-collected carnivores differs significantly from the others.

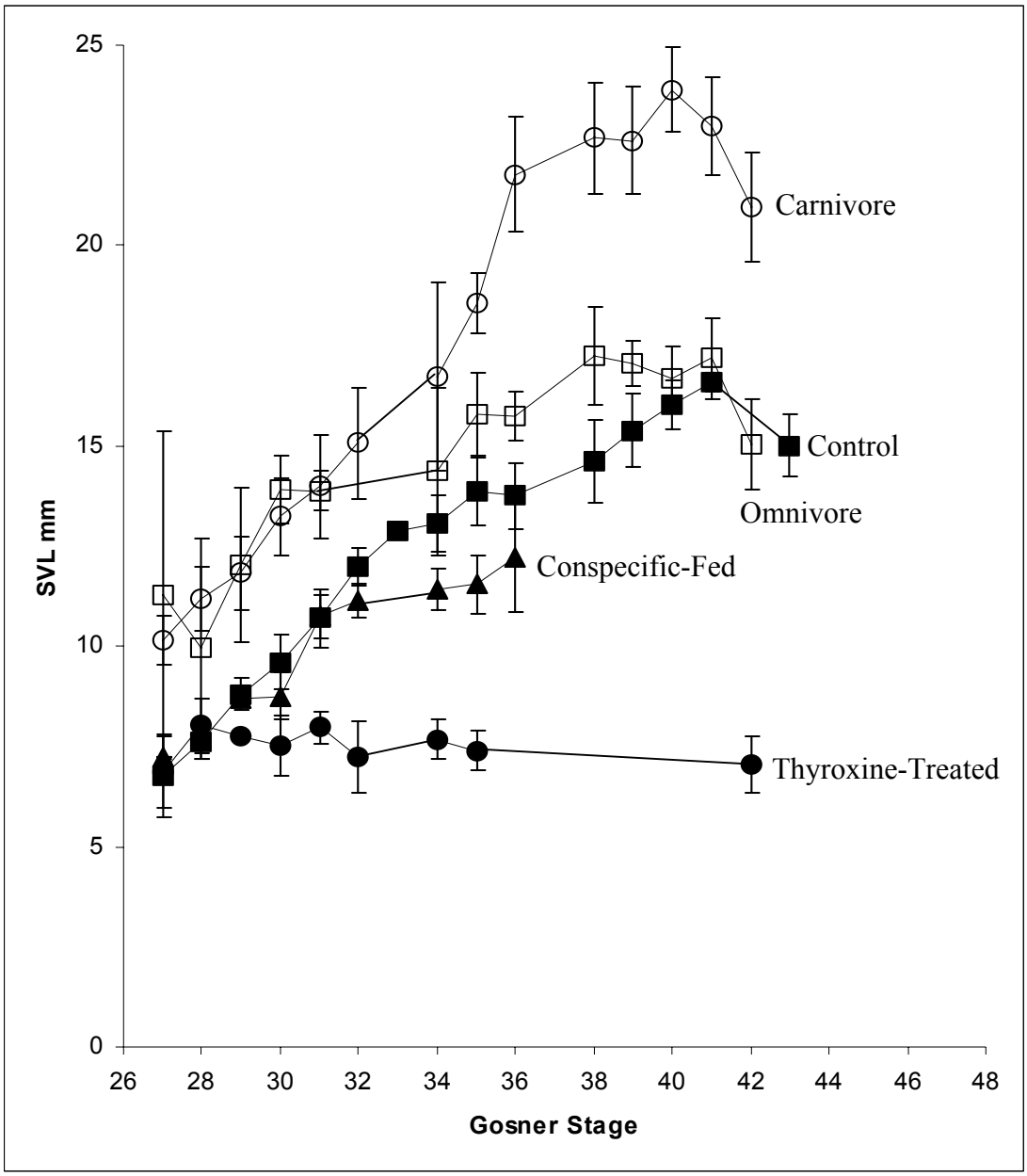

Fig. 1.2. SVL (mm) development of the five phenotypes as a function of Gosner developmental stage; mean and standard deviations represented. The curves for field-collected omnivores, laboratory controls, and conspecific-fed tadpoles do not differ significantly. That for field-collected carnivores is similar in shape to that for controls but is significantly higher. That for thyroxine-treated (T4) tadpoles is significantly lower and essentially flat.

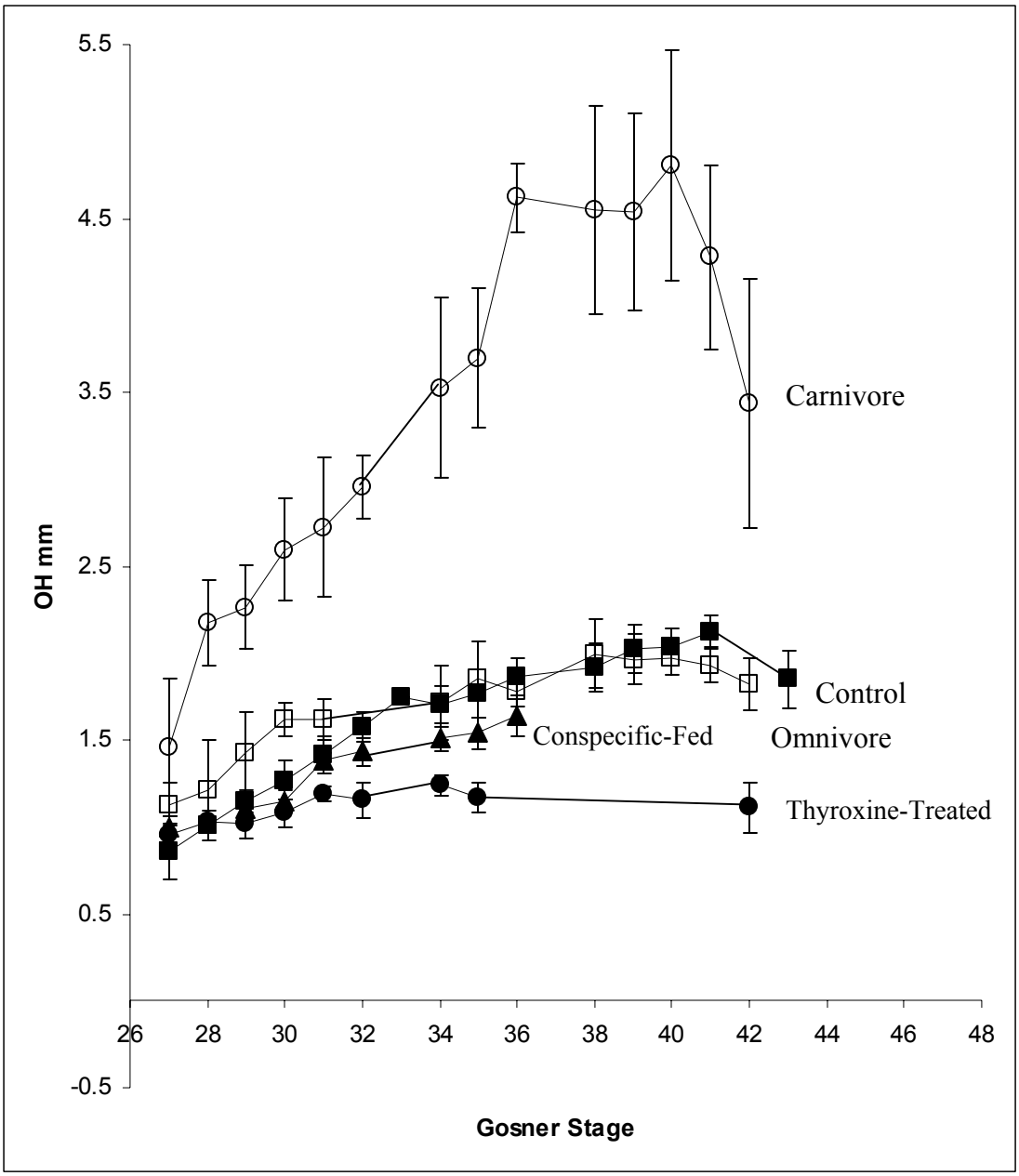

Fig. 1.3. OH (mm) development of the five phenotypes as a function of Gosner developmental stage; mean and standard deviations represented. The curves for field-collected omnivores, laboratory controls, and conspecific-fed tadpoles do not differ significantly. That for field-collected carnivores is similar in shape to that for controls but is significantly higher. That for thyroxine-treated (T4) tadpoles is significantly lower and essentially flat.

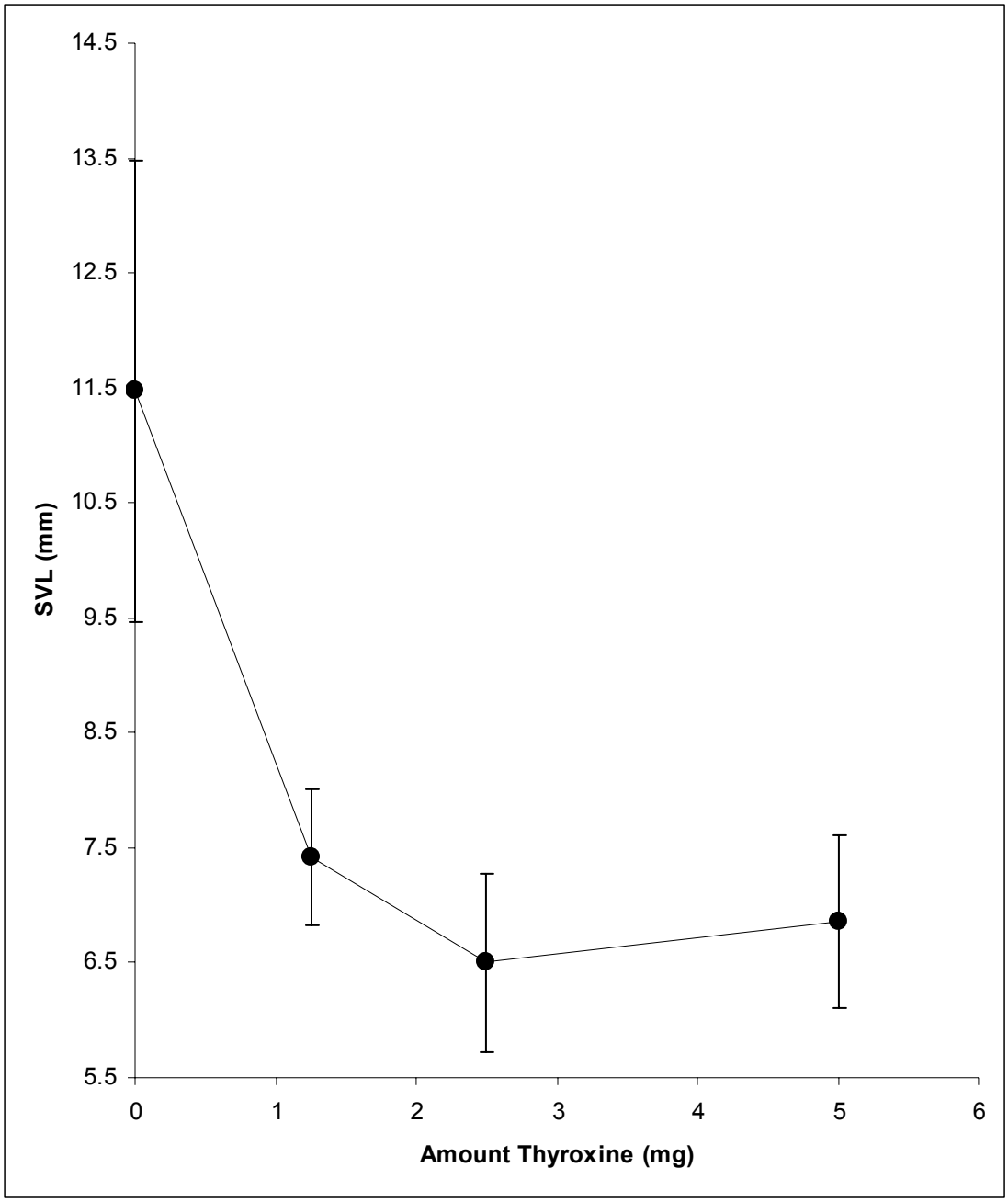

Fig. 1.4. SVL (mm) of the thyroxine-treated *Scaphiopus holbrooki* tadpoles as a function of thyroxine concentration (mg per 5 g tadpole chow); mean and standard deviations represented.

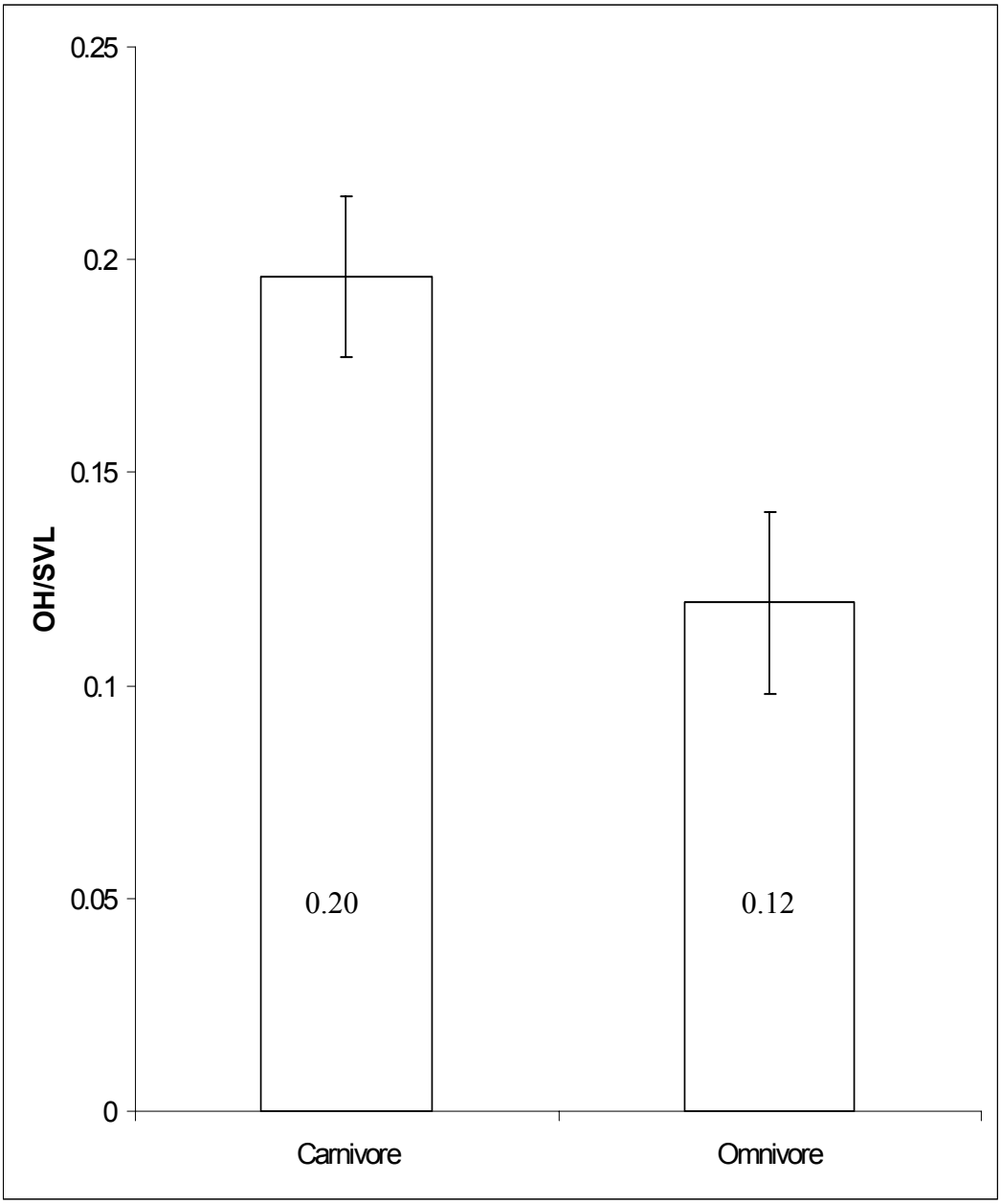

Fig. 1.5. Mean ratio of OH to SVL and standard deviation for field-collected carnivores (0.20 ± 0.019) and field-collected omnivores (0.12 ± 0.021). The mean ratio for natural carnivores is significantly larger than that for natural omnivores ($P < 0.0001$).

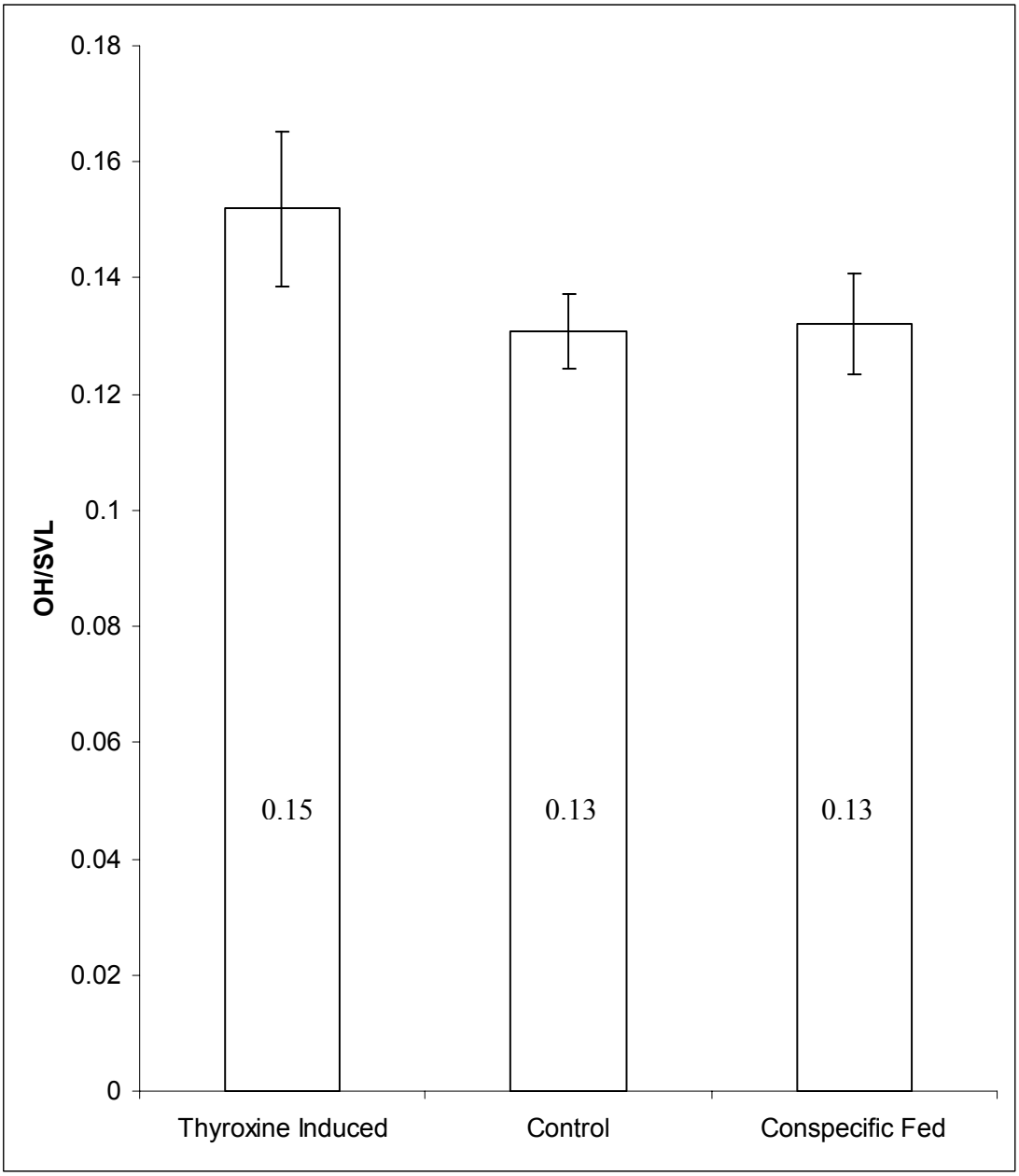

Fig. 1.6. Mean ratio of OH to SVL standard deviation for treated (0.15 ± 0.013), laboratory controls (0.13 ± 0.006), and conspecific-fed tadpoles (0.13 ± 0.009). The mean ratio for thyroxine-treated tadpoles is significantly larger than that for laboratory controls ($P < 0.0001$); mean ratios for conspecific-fed tadpoles and laboratory controls do not differ significantly.

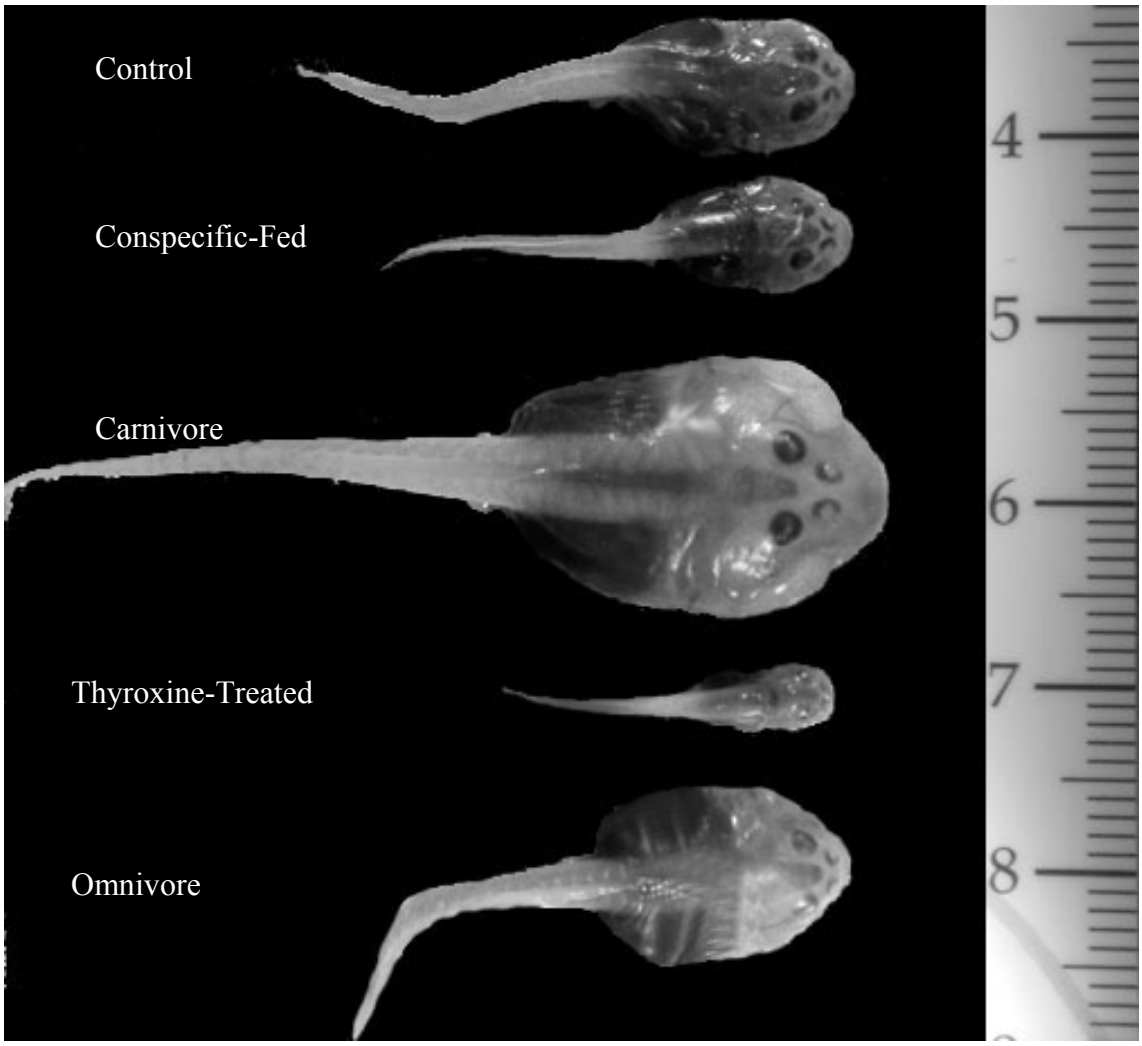

Fig. 1.7. This figure shows the five *Spea multiplicata* phenotypes used in this study. All tadpoles shown are at Gosner stage 36 (middle hind-limb development), and the scale to the right is in millimeters. Size and shape differences among the phenotypes are apparent.

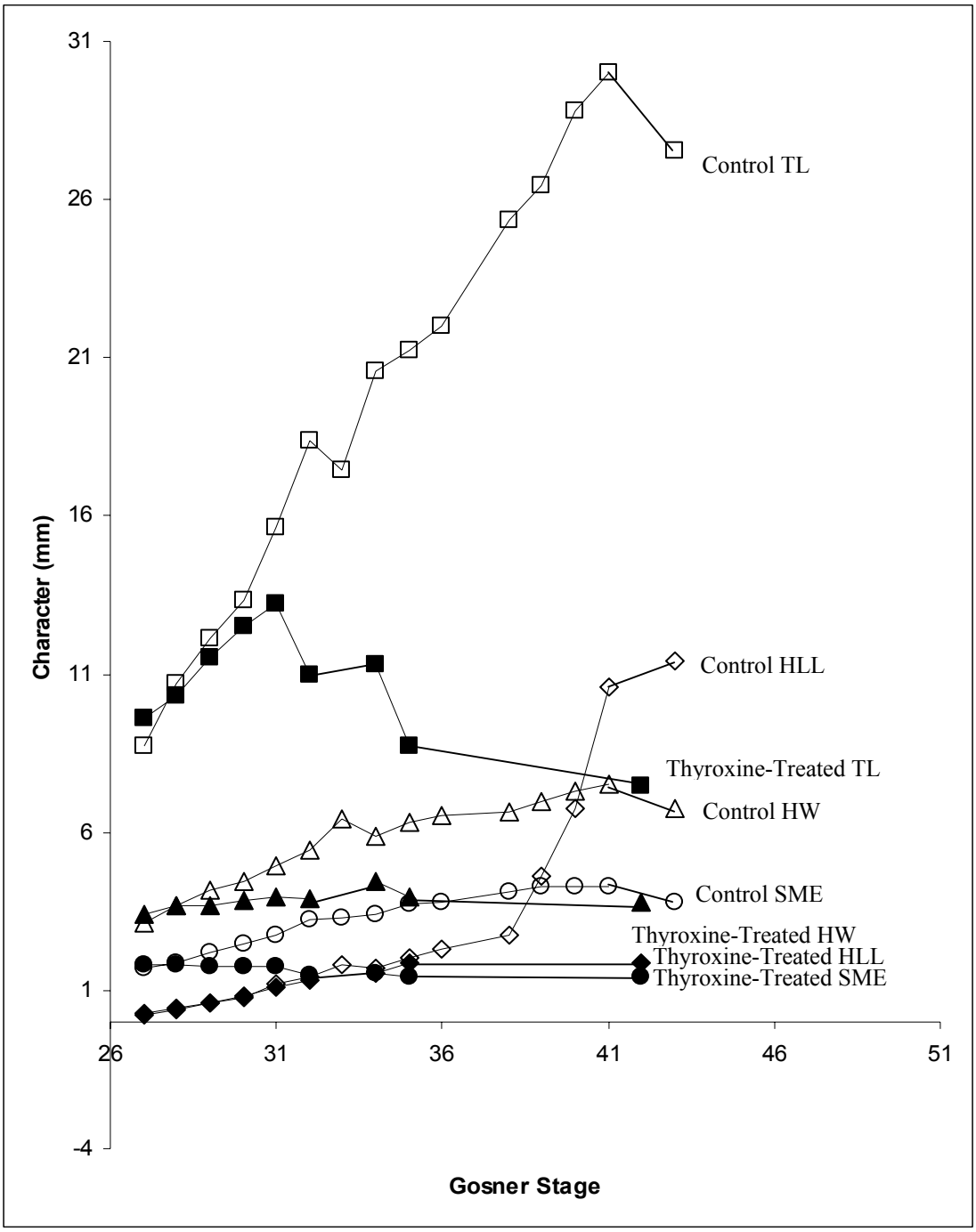

Fig. 1.8. Tail length (TL), head width (HW), snout-to-mideye length (SME), and hindlimb length (HLL) (mm) development for untreated controls and thyroxine treated tadpoles of *Spea multiplicata* as a function of Gosner developmental stage; means represented. The TL, HW, SME, and HLL curves for thyroxine-treated tadpoles are depressed relative to untreated controls.

CHAPTER 2

COMPARATIVE DEVELOPMENT OF FOUR SPECIES OF SPADEFOOT TOADS WITH AND WITHOUT EXOGENOUS THYROXINE

INTRODUCTION

Thyroid hormone (TH) is arguably the most important hormone in anuran metamorphosis. TH controls coordinated morphological changes of the majority of tissues (Kaltenbach 1957,1970, Kollros 1958,1961, Frieden 1961, Frieden and Just 1970, Shi 1994, Kanamori and Brown 1996, Das et.al. 2002) that are associated with changing from a water dwelling organism to a terrestrial form. TH is maintained at very low levels in early prometamorphic larvae (early hind-limb bud development), increases in the blood stream as larvae get closer to metamorphosis, reaches its highest concentration at metamorphic climax (reviewed in Denver 1997b), and then declines. The release of endogenous thyroid hormone into a developing anuran is the final stage of a complicated hormonal cascade involving positive and negative feedback loops among the hypothalamus, pituitary gland, and thyroid gland (Denver 1997a, 1997b). Three major acting hormones control metamorphosis. Developmental timing and/or external environmental stimuli (Denver 1997a) may induce release of corticotropin-releasing hormone (CRH) from the hypothalamus. CRH acts on the pituitary gland and stimulates the release of thyroid stimulating hormone (TSH). TSH acts on the thyroid gland, which in turn produces thyroid hormones T_4 (thyroxine) and T_3 (Denver 1997a, 1997b). The thyroid hormones act directly on target tissues by binding to tissue-specific thyroid hormone receptors (TR_α and TR_β). When bound, the receptors act as transcription factors inducing genes involved in metamorphosis (Shi 1994, Yaoita and Brown 1990, Huang and Brown 2000, Das et.al. 2002).

Researchers have exploited the fact that thyroid hormones act directly on target tissues in order to understand the mechanistic association between thyroid hormone and

tissue specific changes during metamorphosis. To understand this relationship, researchers have used thyroid hormone injections (Frieden 1961, Kaye 1961), immersions (Kollros 1958, 1961, Van Stone 1960, Frieden 1961, Kaye 1961, Gona 1969), pellet implants (Kaltenbach 1957, 1970), and feeding assays (Pomeroy 1981, Pfennig 1992a). From these studies, an understanding has emerged regarding the tissue-specific thyroid hormone titers, timing of these titers, and the thyroid hormone/thyroid-hormone receptor binding relationship required for metamorphic transformation (Shi 1994, Kanamori and Brown 1996).

Virtually nothing is know about the natural endogenous thyroid hormone cycles in spadefoot toads, but spadefoot toads display striking differences in development (Buchholz and Hayes 2000, 2002) even between closely related species. This makes spadefoot toads an ideal system to investigate the mechanistic control of developmental variation among species. New world spadefoot toads consist of two genera and seven species (Garcia-Paris et.al. in press). Within the spadefoot toad group, different species display different patterns of development (e.g. size, time to metamorphosis) that are phylogenetically conservative (Buchholz and Hayes 2000, 2002). For example, species within *Scaphiopus* metamorphose in 16 – 19 days at 8 – 12 mm in the laboratory, but *Spea* species metamorphose in 28 – 36 days at 20 –22 mm (Buchholz and Hayes 2002). Differences in time to metamorphosis and size at metamorphosis among these species are most likely in part due to differences in the timing of thyroid hormone release. Differences in development may also be due to differences in concentration of circulating endogenous thyroid hormone, differences in thyroid hormone/thyroid- hormone receptor activity, or differences in activity of the activated metamorphic genes.

In the present study I induced four species of spadefoot toads (*Spea multiplicata, Spea bombifrons, Scaphiopus couchii, Scaphiopus holbrooki*) with the same amount of exogenous thyroid hormone (T_4) and investigated the patterns of growth and development. Variation in circulating endogenous thyroid hormone exists across anurans before, during, and after metamorphosis (Mondou and Kaltenbach 1979, Suzuki and Suzuki 1981, Weil 1986, Weber 1994, Gancedo et.al. 1997). By keeping the concentration of thyroid hormone constant at much higher levels than that found in normally developing anurans, I can theoretically remove the differences in development

due to differences in amount of circulating endogenous thyroid hormone. If differences in development among the spadefoot species are due to plasma thyroid hormone concentration, I would expect thyroxine-treated tadpoles to display more similar development, when comparing among thyroxine-treated species, than among controls.

Among the four species investigated, I expected species that are more closely related to display more similar developmental patterns (Buchholz and Hayes 2000, 2002) with and without thyroxine treatment. This is because the species that are more closely related have had less evolutionary time to diverge from one another in development, or rather, they display phylogenetic conservation of development (Alberch et. al. 1979, Oster and Alberch 1982).

This was a pattern-based study and the actual, endogenous thyroid concentrations, hormone-receptor variation, or metamorphic-gene variations among the species were not investigated. The results of this study can only provide speculation regarding the hormonal and cellular differences among the species. Nonetheless, the results may provide insights into understanding whether natural differences in spadefoot toad development are controlled at the thyroid hormone titer level or at the cellular level. This study is also a detailed comparison of ontogenies among four related species of spadefoot toads and is important for understanding the patterns of developmental evolution across anurans.

MATERIALS AND METHODS

Breeding Induction

Adults of *Sc. couchii* and *Sp. multiplicata* were collected in the summer of 2002 in Cochise County, Arizona, (Arizona permit SP710791); Adults of *Sp. bombifrons* were collected during the same summer in Hutchinson County, Texas, (Texas permit SPR-0902-244) and assays for the three species were conducted at the Southwestern Research Station (American Museum of Natural History). *Sc. holbrooki* were collected during the spring of 2003 in the Apalachicola National Forest (permit not required) and transported to Florida State University where assays were conducted in the amphibian research facility (ACUC protocol #0020). Both facilities are temperature and light-controlled, and animals were maintained at 23–26°C and 12-h light/dark cycles.

Breeding was induced by injection with 50 to 100 µl of 1 µg/100 µl GnRH agonist [des-Gly^{10}, (D-His (Bzl)^{6})- luteinizing hormone releasing hormone ethylamide] (Sigma) (Buchholz and Hayes 2000). Animals subsequently mated, and by the following day multiple clutches of eggs were spread out across the aquarium. Larvae hatched 48 hours later; at 72 hours yolk was fully absorbed and larvae began feeding.

Induction Assay

On day 5 after hatching, at Gosner stage 27 (early hind limb development) (Gosner 1960)**,** 300 individuals were haphazardly selected from community aquaria and placed individually in 8-ounce plastic drinking cups containing 250 ml of dechlorinated water. Cups were individually labeled according to treatment (150 untreated control, 150 thyroxine-treated) and placed in plastic trays in random order. The trays were placed in temperature and light-controlled rooms (23–26°C, and 12-h light/dark cycle), and the tadpoles were fed according to treatment.

Feeding Regime

Laboratory controls were fed tadpole chow (a finely ground mixture of 3 parts rabbit pellets and 1 part Tetramin ® fish food) (Travis 1980) every other day ad libitum. This diet was developed for ephemeral-pond-inhabiting tadpoles, and *Sc. holbrooki* tadpoles reared on it develop normally relative to naturally developing animals (pers. obs.). Treatment larvae were fed approximately 5mg of a mixture of 5 mg of l-thyroxine (T_4) and 5 g tadpole chow every other day, which is approximately a dose of 5000ng of thyroxine. The typical thyroxine-treated tadpole in this study weighs 0.04 g, which means that if tadpoles are ingesting all the food provided the measurable amount of thyroxine in thyroxine-treated tadpoles should be 125,000 ng per tadpole gram. This dose far exceeded normal plasma levels in anurans, which at their highest point during development (metamorphic climax) range from 1.95 ng/g for *Xenopus laevis* (Gancedo et.al. 1997), to 3.45 ng/g for *Rana perezi*, to 8.4 ng/g in *Bufo marinus* (Weber et.al. 1994). Also, in a dose/response study, using *Sc. holbrooki*, the amount of thyroxine used in this study was found to be the maximum amount at which tadpoles could survive through metamorphic climax (Gosner stage 42, forelimb emergence) and the

developmental response of tadpoles for this concentration lies on the asymptote of the dose/response curve (Fig. 2.1) (B.L. Storz submitted). This means that increased concentrations of thyroxine would not elicit a different developmental response in *Sc. holbrooki* and this concentration is the highest possible that could be used. I chose this amount of thyroxine because if natural differences in endogenous thyroxine titers exist among the different species of spadefoot toads, than an enormous amount of exogenous thyroxine would swamp any natural circulating hormonal variation and the different species of spadefoot toads would be effectively equal for thyroid hormone concentration. By removing the effect of different concentrations of circulating thyroid hormone, differences in development with thyroxine-treatment may be attributable to differences at the cellular level, such as differences in thyroid hormone receptor activity or tissue specific metamorphic genes. Water was changed in all treatments on the same day as feeding.

Because the different species of spadefoot toads develop at different rates (Buchholz and Hayes 2002), I used the Gosner staging system (Gosner 1960) rather than age of the larvae to determine the start of the experiments. Therefore, although the species may have been different ages when the assays were started, they were at equivalent developmental stage. All assays were started at Gosner stage 27 (early hind limb bud development) immediately after feeding begins.

Ten thyroxine-treated tadpoles and 10 controls were collected daily until thyroxine-treated tadpoles had reached Gosner stage 42 (forelimb emergence). No thyroxine-treated animals lived past stage 42. After that point, controls were collected every 4 days through metamorphic climax (stage 42/43), which ranged from 18 days for *Sc. holbrooki,* to 32 days for *Sp. muliplicata*, and 33 days for *Sp. bombifrons*. *Sc. couchii* controls did not reach metamorphic climax and died at stage 38 for unknown reasons.

Measurements and Analysis

Larvae were euthanized with MS-222 and preserved in 10% formalin. All morphological measurements were made using an ocular micrometer or digital image analysis (Image J NIH version 1.29X). Tadpoles were sorted into Gosner stage classes, and average orbitohyoideus width (OHW) (measured at the widest point, as by Pfennig

1992a), interhyoideus length (IHL) or head width, snout-to-mid-eye length (SME), snout-to-vent length (SVL), tail length (TL) (Fig. 2.2) were measured. Intestines (IL) were removed from tadpoles and length was measured from the beginning of the mid-gut to the end of the rectum (at point of attachment to cloaca). Thyroid hormone transforms essentially all tissues of the anuran larvae, and these characters were chosen because they display dramatic changes during the metamorphic cascade. The oribitohyoideus jaw muscle widens and lengthens during development and rotates from a dorsal/ventral to a more lateral orientation. The interhyoideus jaw muscle extends across the ventral side of the tadpole and attaches to the ventral end of the orbitohyoideus on either side. This muscle lengthens as the tadpole develops and measuring this muscle gives an estimate of lengthening of the muscle and widening of the head. The tadpole head shortens dorsal/ventrally during metamorphic climax and snout-to-mid-eye is a measurement of this head shortening. Snout-to-vent length is a ubiquitous measurement in tadpole developmental studies; SVL lengthens throughout premetamorphosis and prometamorphosis and then dramatically shortens during metamorphic climax. A similar pattern of lengthening during pre and prometamorphosis and shortening during metamorphic climax is also observed for tail length and intestine length, and these characters were measured accordingly.

Allometry is the change of shape or proportion with increase in size (Alberch et. al. 1979). There was no detectable difference in head shape (log head width – log head length) at metamorphic climax between thyroxine-treated tadpoles and controls (*Sp. bombifrons* $T_{14} = 1.97$, $P = 0.07$, *Sc. holbrooki* $T_{19} = 0.53$, $P = 0.61$), and rather than focus on shape, which is the ratio of characters, I focus on proportions or the comparative relationship between size of specific characters and body size (SVL). I do this because I am interested in the relative change in growth, rather than shape, between control and thyroxine-treated tadpoles in order to make clear the effects of standardized thyroxine on development. Multiple studies have suggested that individual morphological characters grow and degenerate autonomously during premetamorphosis and metamorphic climax (Wang and Brown 1993, Shi 1994, Kanamori and Brown 1996, Das et.al. 2002), and by focusing on ratios of autonomously developing characters I lose valuable information

regarding developmental of the characters and I also may not be able to tease out the specific reason why shape change occurs.

The allometric relationship between log transformed SME, HL, IHL, OHW, TL and IL and log transformed SVL were analyzed by linear regression. The slope of isometric growth is 1.0, and regressions of the characters on SVL are isometric if the slope is not significantly different from 1.0. Alternatively, if the slope of the regression line is significantly greater or less than 1.0 (isometry), development of the character relative to SVL shows positive allometry or negative allometry respectively. The slope of log/log development was tested against isometry (slope = 1) by two-sided t-test (Ramsey and Schafer 1997). Allometry slopes of different species were tested against each other, to determine significance of magnitude, by multiple comparisons in ANCOVA (JMPIN version 3.2.6 1998) and probability values were compared to Dunn-Sidak multiple comparison-adjusted critical values to determine significance (Sokal and Rohlf 1995). All tadpoles grew, although only slightly for thyroxine-treated tadpoles, until metamorphic climax, at which point all measured traits except HL decreased. I assume that allometric relationships of characters and SVL remain the same whether tadpoles are growing or reducing in size during metamorphic climax and all data is plotted together. Evidence suggests this is accurate; for instance, when total-data and growth-only data are plotted separately for *Sc. holbrooki* log SME by log SVL, growth-only data points envelop the total-data data points. The morphological measurements were also plotted against Gosner stage to determine if differences occurred in the position of developmental curves, for thyroxine-treated tadpoles relative to controls, for each character.

RESULTS

Allometry

For both control larvae and thyroxine-treated larvae, the log transformed relationships between the six measured characters and log SVL, with the exception of HL for *Sp. multiplicata* and *Sc. couchii*, fit linear models (Tables 2.1-2.6).

Allometry of thyroxine-treated larvae varied among species and did not show that more closely related species have more similar developmental patterns (phylogenetic

conservation in development). SME development was isometric for *Sp. bombifrons*, but was significantly positive for both *Sp. multiplicata* and *Sc. holbrooki* and significantly negative for *Sc. couchii* (Table 2.1, Fig. 2.3). As discussed above, HL development for two of the species did not fit a linear model (*Sp. multiplicata* and *Sc. couchii*). Interestingly, HL development for *Sp. bombifrons* fit a linear model and showed significantly positive allometry, but HL development for thyroxine-treated *Sc. holbrooki* showed negative allometry (Table 2.2, Fig. 2.4). Development of IHL for *Sp. multiplicata*, *Sc. holbrooki*, and *Sc. couchii* showed significantly negative allometry for thyroxine-treated larvae, but the magnitude varied significantly between *Sp. multiplicata* and *Sc. holbrooki* ($F = 9.97$, $P = 0.002$). *Sp. multiplicata* IHL development had the largest slope (0.68), while IHL slope for *Sc. holbrooki* was 0.23 (Table 2.3, Fig. 2.5). Unlike the other species, development of IHL for *Sp. bombifrons* was isometric. OHW showed a pattern of development similar to IHL (Fig. 2.6). The slope of OHW development for *Sp. multiplicata*, *Sc. holbrooki*, and *Sc. couchii* was significantly negative, and again, development of OHW for *Sp. bombifrons* was isometric (Table 2.4, Fig. 2.6). The slopes of TL development for thyroxine-treated tadpoles were isometric for all species (Table 2.5, Fig. 2.7), and finally, the slopes of IL development showed significantly positive allometry for three of the species treated with thyroxine (Table 2.6, Fig. 2.8). IL development for *Sp. bombifrons* was isometric.

Control larvae also differed among the species in allometric development. In addition, there is conflicting evidence for phylogenetic conservation of development among the species. For instance, *Sp. bombifrons, Sp. multiplicata*, *Sc. couchii* all displayed significantly positive allometry of SME growth, but the slope of SME development for *Sc. holbrooki* showed negative allometry (Table 2.1, Fig. 2.9). If development is conserved phylogenetically, I would have expected *Sc. couchii* to have shown development more similar to *Sc. holbrooki* rather than the *Spea* species. HL allometry was significantly positive for all controls and slopes of some species differed significantly in magnitude. The slopes for HL development for *Sp. bombifrons* and *Sp. multiplicata* did not differ significantly ($F_1 = 0.1035$, $P = 0.75$), which may represent phylogenetic conservatism of hind limb development. In contrast, the slope of HL development for *Sc. holbrooki* was significantly different from *Sp. bombifrons* and *Sp.*

multiplicata ($F_1 = 6.76$, $P = 0.01$, $F_1 = 7.41$, $P = 0.007$ respectively). *Sc. couchii* was significantly different from *Sp. bombifrons* ($F_1 = 19.48$, $P < 0.0001$) and *Sc. couchii* and *Sc. holbrooki* were also significantly different from one another ($F_1 = 30.66$, $P < 0.0001$) (Table 2.2, Fig. 2.10). *Sc. couchii* showed isometry for IHL development, while both *Sp. multiplicata* and *Sc. holbrooki* showed significantly negative allometry and *Sp. bombifrons* significantly positive allometry (Table 2.3, Fig. 2.11). OHW development was isometric for all species (Table 2.4, Fig. 2.12) except *Sp. bombifrons*, which showed significantly positive allometry. TL development for all the species was significantly positive (Table 2.5, Fig. 2.13) and lastly, the slope of IL development for *Sc. holbrooki* was isometric, but *Sp. bombifrons*, *Sp. multiplicata*, and *Sc. couchii* all showed significantly positive allometry in the slopes of IL development (Table 2.6, Fig. 2.14).

Development with Gosner Stage

Plotting development of the different morphological characters over Gosner Stage showed, in general, a conserved ranking of species development curves, regardless of treatment, for most characters analyzed (SVL, HL, IHL, OHW, IL) (Fig. 2.15). *Sp. bombifrons* was the uppermost curve (rank 1), *Sp. multiplicata* just below (rank 2), and *Sc. holbrooki* and *Sc. couchii* were rank 3 and rank 4 respectively. For two characters, thyroxine treatment caused a shift in rank of developmental curves for SME and TL (Fig. 2.16, 2.17). For SME, treatment with thyroxine caused the developmental curves of *Sp. multiplicata* and *Sc. holbrooki* to reverse (*Sp. multiplicata* = rank 3, *Sc. holbrooki* = rank 2) (average SME over curve $T_{14} = 3.37$, $P = 0.005$, SME at Gosner stage 42 $T_{15} = 2.39$, $P = 0.03$). TL development also showed a reversal of *Sp. multiplicata* and *Sc. holbrooki* when tadpoles were treated with thyroxine. *Sc. holbrooki* became rank 2 and *Sp. multiplicata* became rank 3 (TL average over curve $T_{14} = 1.40$, $P = 0.18$, TL at Gosner stage 42 $T_{15} = 2.70$, $P = 0.02$).

DISCUSSION

If differences in development among the species were due solely to differences in plasma thyroid hormone concentrations, I would expect thyroxine-treated tadpoles of the different species to converge on a common pattern of development. This is not what was

observed, and in fact, there is essentially just as much (if not more) difference in thyroxine-treated development patterns as controls among the species. This suggests that thyroid hormone concentration may not be the only mechanistic difference controlling development among the species. By comparing development of controls among the species, it is clear that there has been evolutionary modification in the development of the spadefoot toads. But it is unclear whether these differences are due to differences in plasma thyroid hormone concentration, thyroid hormone receptor activity, or expression and activity of metamorphic genes.

For controls, most characters (except TL) developed isometrically or are close to isometry, but treatment with thyroxine broke down this similarity among species. I expected treatment with thyroxine to alter development in the same manner across the species (i.e. all species would show either positive or negative allometry when treated with thyroxine) or that there would be phylogentic conservation of development (more closely related species would show more similar development). Instead, for all of the characters there was some variation in developmental pattern across the species for controls or tadpoles treated with thyroxine. For instance, snout-to-mideye, which describes head anterior/posterior growth and later reduction in size, showed developmental differences among species within thyroxine-treated and control tadpoles. In controls, three of the species (*Sp. bombifrons, Sp. multiplicata, Sc. couchii*) showed a conserved pattern of positive allometry in development, but *Sc. holbrooki* (although weakly) demonstrated negative allometry in development (Table 2.1, Fig. 2.4). Under thyroxine treatment, SME development for *Sc. holbrooki* and *Sp. multiplicata* was positively allometric, but the rank order for the two species was reversed relative to controls (Figs. 2.3, 2.16). *Sp. bombifrons* and *Sc. couchii* showed divergent patterns; *Sp. bombifrons* developed isometrically, but *Sc. couchii* converged on negative allometry and had a slope similar to *Sc. holbrooki* control (Table 2.1, Figs. 2.3).

In another example, IL development showed positive allometry in controls for all species except *Sc. holbrooki.* Treatment of tadpoles with exogenous thyroxine resulted in positive allometry in all species except *Sp. bombifrons*, in which IL development was isometric (Table 2.6, Figs. 2.14, 2.15).

Multiple studies have provided evidence for the specificity of thyroid hormone activity on individual tissues and individual cells within those tissues (Shi 1994, Kanamori and Brown 1996). Research also shows that different metamorphic genes are activated by thyroxine in individual tissues (Wang and Brown 1993, Kanamori and Brown 1996). In fact, fast tail muscle and slow cord tail muscle, which degenerate during metamorphosis, are not broken down in the same manner (Das et.al. 2002). Given that there has been a considerable amount of evolutionary modification of how tissues respond to thyroid hormones during metamorphosis within an organism, it is not surprising that there should be evolutionary modification in how morphological characters change during metamorphosis even between closely related species. Besides differences in timing of thyroid hormone release, there are at least three additional mechanistic reasons why these species of spadefoot toads differ in development, as discussed earlier. Here I investigated one possibility (differences in TH concentration). There were changes in development among the species of spadefoot toads when thyroxine was standardized, but thyroxine-treated tadpoles did not show more similar development than controls among the species. This suggests that differences in TH concentration in addition to other mechanistic differences may be involved in controlling the differences in development among the species of spadefoot toads. The next step towards understanding would be a thorough examination of the thyroid hormone receptor variation in sequence and mechanistic function, the plasma levels of thyroid hormone during development, and the different metamorphic genes that are expressed in similar tissues across the species of spadefoot toads.

Table 2.1. Results of linear regression of log SME on log SVL for thyroxine-treated and controls of *Spea bombifrons (Sp. b.), Spea multiplicata (Sp. m.), Scaphiopus holbrooki (Sc. h.), and Scaphiopus couchii (Sc. c.)* and results of nonisometry test, comparing slopes of thyroxine-treated and controls to one (significance = *).

Treatment/Species	Slope	Y-intercept	R^2	*F* ratio	D.F.	*P*	Test of nonisometry
T4 *Sp. b.*	1.05	-0.70	0.65	96.61	1,51	<0.0001	$T_{51} = 0.50$, $P > 0.5$
Control *Sp. b.*	1.17	-0.85	0.88	709.24	1,97	<0.0001	$T_{97} = 3.96$, $P < 0.001$ *
T4 *Sp. m.*	1.45	-1.07	0.55	84.22	1,68	<0.0001	$T_{68} = 2.84$, $P = 0.01$ *
Control *Sp. m.*	1.14	-0.73	0.94	2154.97	1,130	<0.0001	$T_{130} = 5.62$, $P < 0.001$ *
T4 *Sc. h.*	1.28	-0.77	0.80	192.29	1,48	<0.0001	$T_{48} = 3.00$, $P = 0.005$ *
Control *Sc. h.*	0.78	-0.32	0.90	1094.30	1,118	<0.0001	$T_{118} = 9.34$, $P < 0.001$ *
T4 *Sc. couchii*	0.51	-0.27	0.35	23.02	1,42	<0.0001	$T_{42} = 4.54$, $P < 0.001$ *
Control *Sc. couchii*	1.15	-0.70	0.94	1432.00	1,88	<0.0001	$T_{88} = 5.06$, $P < 0.001$ *

Table 2.2. Results of linear regression of log HL on log SVL for thyroxine-treated and controls of *Spea bombifrons (Sp. b.), Spea multiplicata (Sp. m.), Scaphiopus holbrooki (Sc. h.), and Scaphiopus couchii (Sc. c.)* and results of nonisometry test, comparing slopes of thyroxine-treated and controls to one (significance = *).

Treatment/Species	Slope	Y-intercept	R^2	*F* ratio	D.F.	*P*	Test of nonisometry
T4 *Sp. b.*	2.08	-1.96	0.31	22.83	1,51	<0.0001	T_{51} = 2.48, P = 0.02 *
Control *Sp. b.*	3.32	-3.59	0.80	380.46	1,97	<0.0001	T_{97} = 13.64, P < 0.001 *
T4 *Sp. m.*	-0.14	0.19	0.0006	0.04	1,68	0.83	----------
Control *Sp. m.*	3.39	-3.43	0.86	824.64	1,130	<0.0001	T_{130} = 20.24, P < 0.001 *
T4 *Sc. h.*	-1.65	1.41	0.31	21.25	1,48	<0.0001	T_{48} = 7.41, P < 0.001 *
Control *Sc. h.*	4.05	-3.89	0.74	330.10	1,118	<0.0001	T_{118} = 13.69, P < 0.001 *
T4 *Sc. couchii*	-0.71	0.25	0.02	1.03	1,42	0.32	----------
Control *Sc. couchii*	2.74	-2.41	0.92	1071.83	1,88	<0.0001	T_{88} = 20.80, P < 0.001 *

Table 2.3. Results of linear regression of log IHL on log SVL for thyroxine-treated and controls of *Spea bombifrons (Sp. b.), Spea multiplicata (Sp. m.), Scaphiopus holbrooki (Sc. h.), and Scaphiopus couchii (Sc. c.)* and results of nonisometry test, comparing slopes of thyroxine-treated and controls to one (significance = *).

Treatment/Species	Slope	Y-intercept	R^2	*F* ratio	D.F.	*P*	Test of nonisometry
T4 *Sp. b.*	0.85	-0.16	0.63	87.21	1,51	<0.0001	T_{51} = 1.65, P = 0.4
Control *Sp. b.*	1.07	-0.41	0.96	2059.73	1,97	<0.0001	T_{97} = 2.86, P = 0.01 *
T4 *Sp. m.*	0.68	-0.002	0.33	34.13	1,68	<0.0001	T_{68} = 2.76, P = 0.01 *
Control *Sp. m.*	0.95	-0.29	0.96	3022.61	1,130	<0.0001	T_{130} = 2.70, P = 0.01 *
T4 *Sc. h.*	0.23	0.36	0.16	9.01	1,48	0.004	T_{48} = 10.17, P < 0.001 *
Control *Sc. h.*	0.92	-0.27	0.94	1769.91	1,118	<0.0001	T_{118} = 3.52, P = 0.002 *
T4 *Sc. couchii*	0.37	0.11	0.14	6.59	1,42	0.01	T_{42} = 4.41, P < 0.001 *
Control *Sc. couchii*	1.04	-0.39	0.96	2002.54	1,88	<0.0001	T_{88} = 1.53, P = 0.2

Table 2.4. Results of linear regression of log OHW on log SVL for thyroxine-treated and controls of *Spea bombifrons (Sp. b.), Spea multiplicata (Sp. m.), Scaphiopus holbrooki (Sc. h.), and Scaphiopus couchii (Sc. c.)* and results of nonisometry test, comparing slopes of thyroxine-treated and controls to one (significance = *).

Treatment/Species	Slope	Y-intercept	R^2	*F* ratio	D.F.	*P*	Test of nonisometry	
T4 *Sp. b.*	0.88	-0.74	0.63	88.60	1,51	<0.0001	T_{51} = 1.27, P = 0.3	
Control *Sp. b.*	1.07	-0.98	0.94	1644.18	1,97	<0.0001	T_{97} = 2.64, P = 0.01	*
T4 *Sp. m.*	0.59	-0.47	0.32	31.48	1,68	<0.0001	T_{68} = 3.84, P < 0.001	*
Control *Sp. m.*	0.99	-0.88	0.96	2985.57	1,130	<0.0001	T_{130} = 0.41, P > 0.5	
T4 *Sc. h.*	0.33	-0.32	0.19	11.19	1,48	0.002	T_{48} = 6.85, P < 0.001	*
Control *Sc. h.*	0.97	-0.91	0.95	2139.46	1,118	<0.0001	T_{118} = 1.31, P = 0.2	
T4 *Sc. couchii*	0.20	-0.33	0.10	4.68	1,42	0.04	T_{42} = 8.91, P < 0.001	*
Control *Sc. couchii*	1.06	-0.96	0.93	1194.44	1,88	<0.0001	T_{88} = 1.99, P = 0.1	

Table 2.5. Results of linear regression of log TL on log SVL for thyroxine-treated and controls of *Spea bombifrons (Sp. b.), Spea multiplicata (Sp. m.), Scaphiopus holbrooki (Sc. h.), and Scaphiopus couchii (Sc. c.)* and results of nonisometry test, comparing slopes of thyroxine-treated and controls to one (significance = *).

Treatment/Species	Slope	Y-intercept	R^2	*F* ratio	D.F.	*P*	Test of nonisometry
T4 *Sp. b.*	0.65	0.47	0.08	4.34	1,51	0.04	T_{51} = 1.12, P = 0.3
Control *Sp. b.*	1.08	0.13	0.92	1026.39	1,97	<0.0001	T_{97} = 2.32, P = 0.04 *
T4 *Sp. m.*	1.57	-0.36	0.30	29.60	1,68	<0.0001	T_{68} = 1.98, P = 0.1
Control *Sp. m.*	1.37	-0.21	0.90	1127.37	1,130	<0.0001	T_{130} = 9.02, P < 0.001 *
T4 *Sc. h.*	1.11	0.13	0.42	34.70	1,48	<0.0001	T_{48} = 0.58, P > 0.5
Control *Sc. h.*	1.42	-0.19	0.91	1187.96	1,118	<0.0001	T_{118} =10.16, P < 0.001 *
T4 *Sc. couchii*	1.29	-0.05	0.46	34.31	1,42	<0.0001	T_{42} = 1.33, P = 0.2
Control *Sc. couchii*	1.31	-0.04	0.92	963.87	1,88	<0.0001	T_{88} = 7.30, P < 0.001 *

Table 2.6. Results of linear regression of log IL on log SVL for thyroxine-treated and controls of *Spea bombifrons (Sp. b.), Spea multiplicata (Sp. m.), Scaphiopus holbrooki (Sc. h.), and Scaphiopus couchii (Sc. c.)* and results of nonisometry test, comparing slopes of thyroxine-treated and controls to one (significance = *).

Treatment/Species	Slope	Y-intercept	R^2	*F* ratio	D.F.	*P*	Test of nonisometry
T4 *Sp. b.*	1.39	0.16	0.09	5.33	1,51	0.03	T_{51} = 0.65, P > 0.5
Control *Sp. b.*	1.34	0.54	0.67	194.63	1,97	<0.0001	T_{97} = 3.52, P < 0.001 *
T4 *Sp. m.*	3.50	-1.87	0.25	23.07	1,68	<0.0001	T_{68} = 3.43, P = 0.002 *
Control *Sp. m.*	1.54	0.29	0.61	202.09	1,130	<0.0001	T_{130} = 5.01, P < 0.001 *
T4 *Sc. h.*	4.79	-2.85	0.72	124.52	1,48	<0.0001	T_{48} = 8.83, P < 0.001 *
Control *Sc. h.*	0.97	0.71	0.39	76.28	1,118	<0.0001	T_{118} = 0.27, P > 0.5
T4 *Sc. couchii*	2.98	-1.02	0.37	24.58	1,42	<0.0001	T_{42} = 3.30, P = 0.005 *
Control *Sc. couchii*	1.64	0.12	0.71	211.23	1,88	<0.0001	T_{88} = 5.68, P < 0.001 *

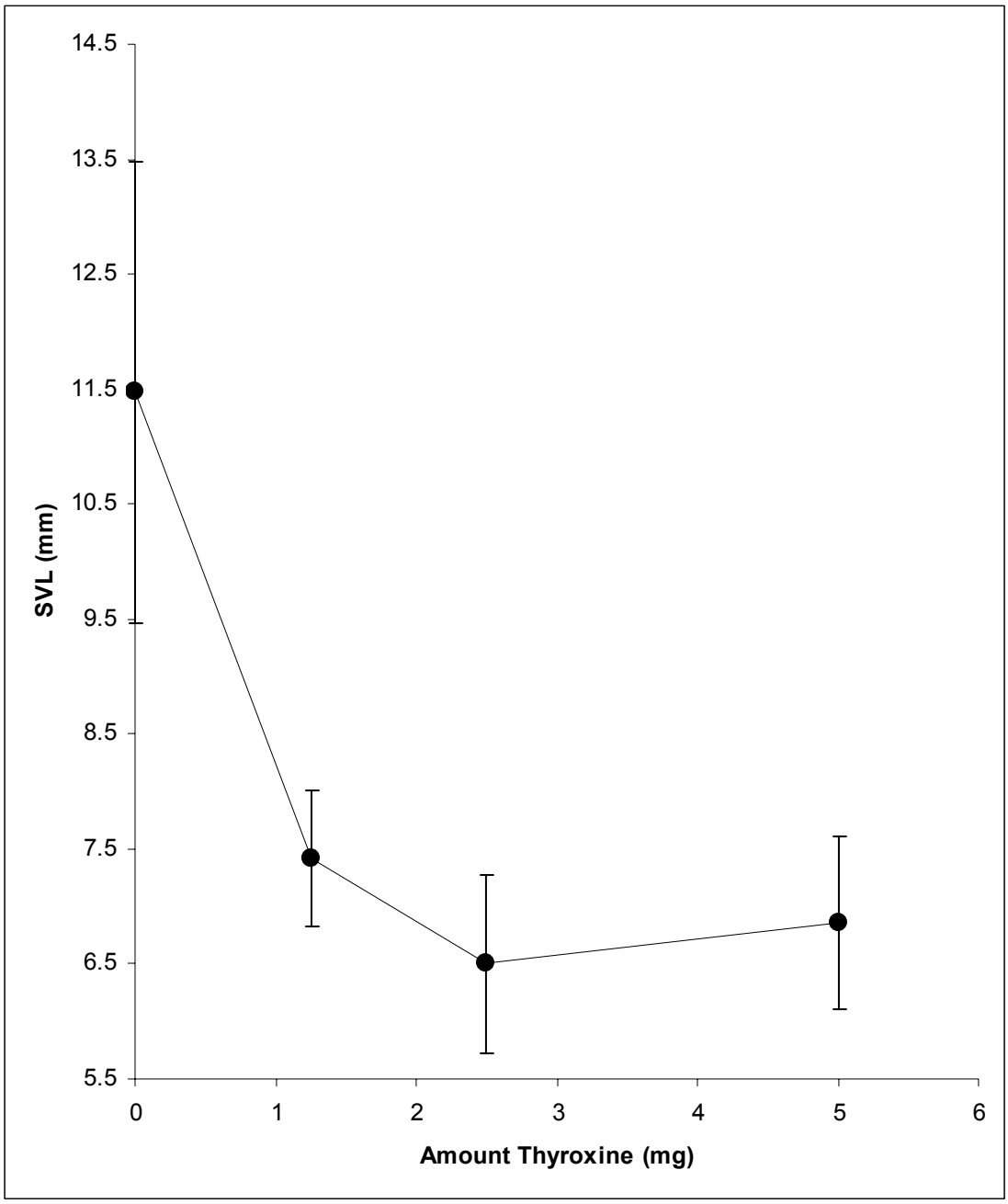

Fig. 2.1. SVL (mm) of the thyroxine-treated *Scaphiopus holbrooki* tadpoles as a function of thyroxine dose; mean and standard deviations represented.

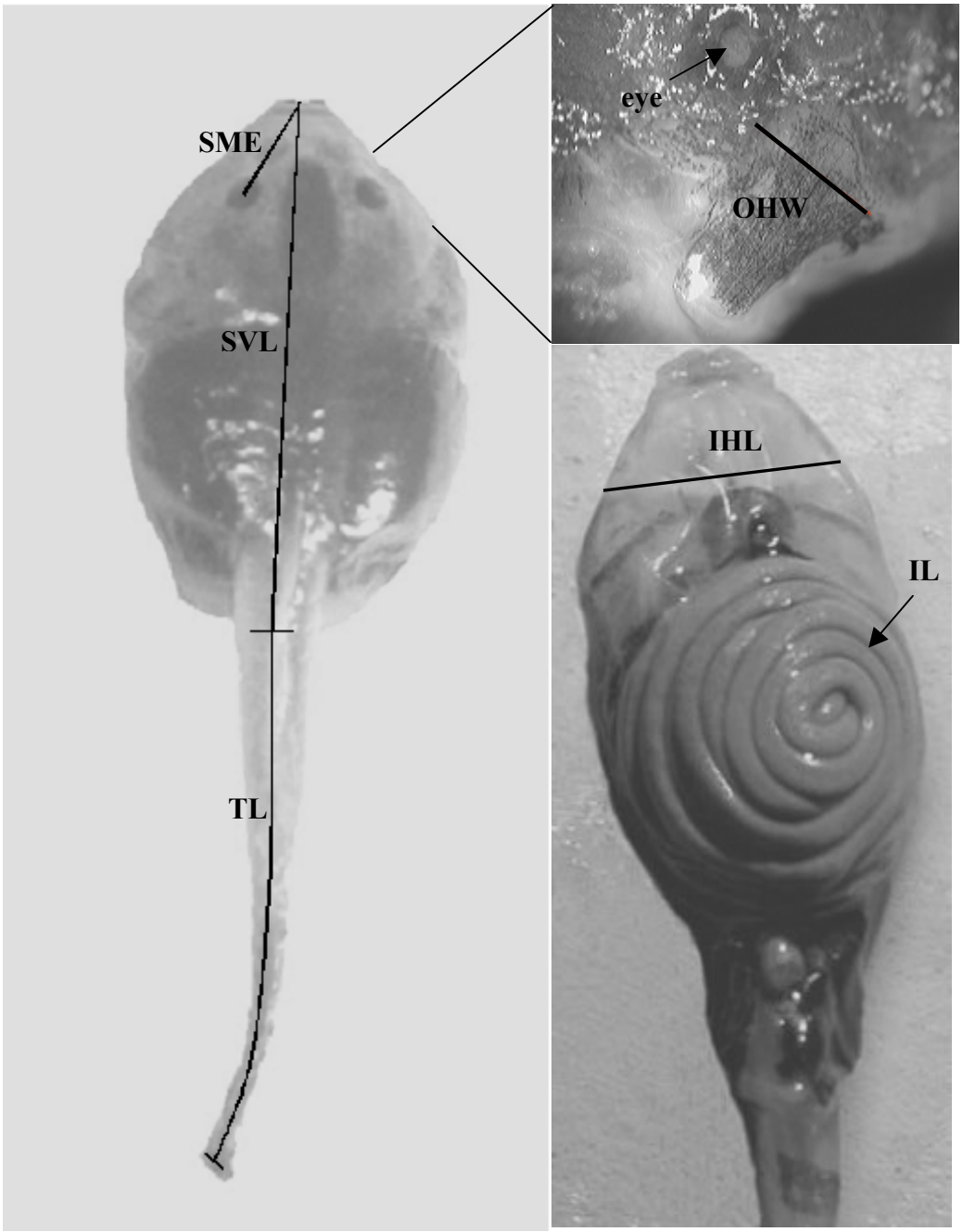

Fig. 2.2. Morphological measurements taken for *Spea bombifrons*, *Spea multiplicata*, *Scaphiopus holbrooki*, and *Scaphiopus couchii*

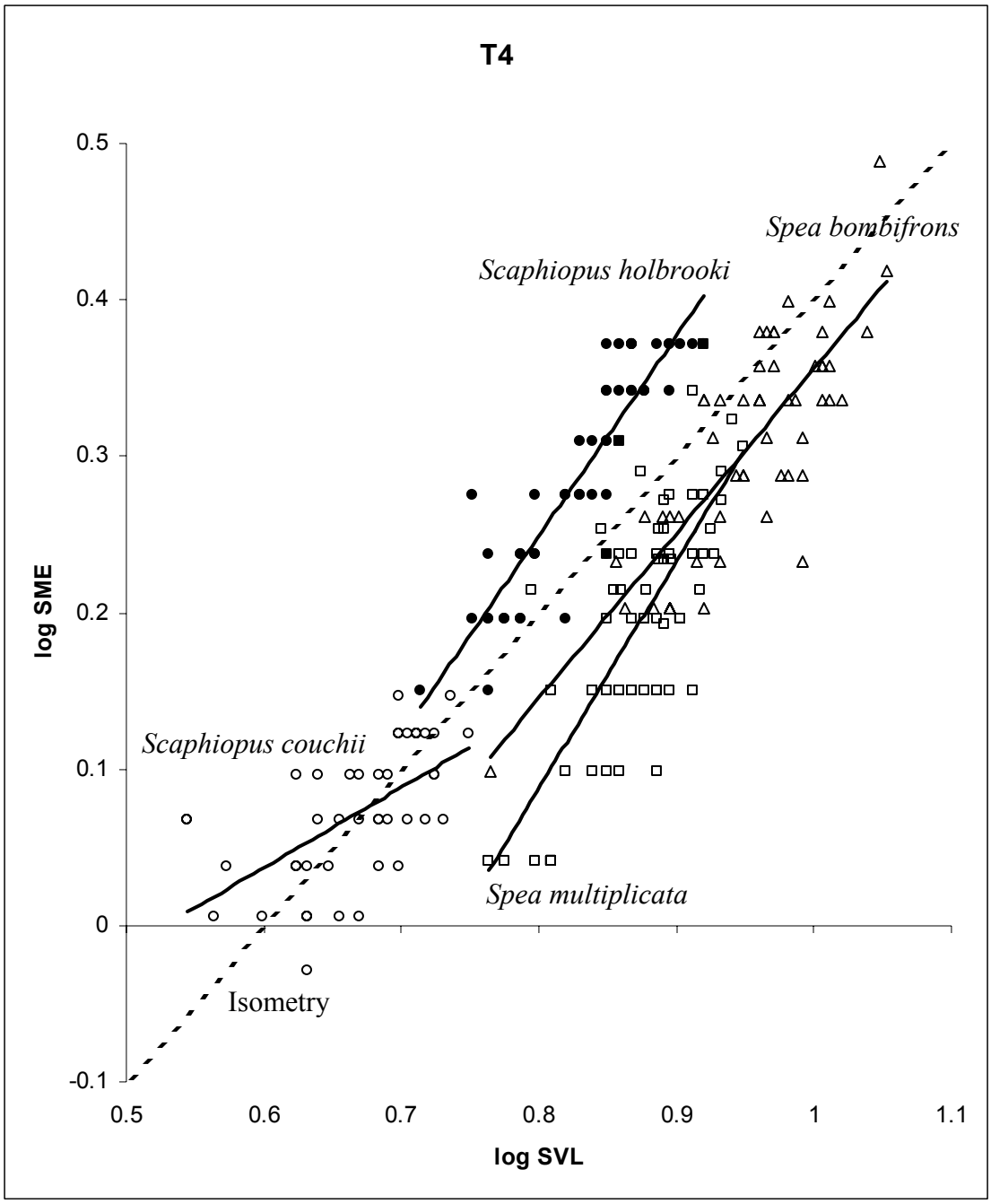

Fig. 2.3. Simple linear regression of log snout-to-mideye (log SME) on log snout-to-vent length (log SVL) for thyroxine-treated tadpoles (T4) of *Spea bombifrons* (Δ), *Spea multiplicata* (□), *Scaphiopus holbrooki* (●), *Scaphiopus couchii* (○).

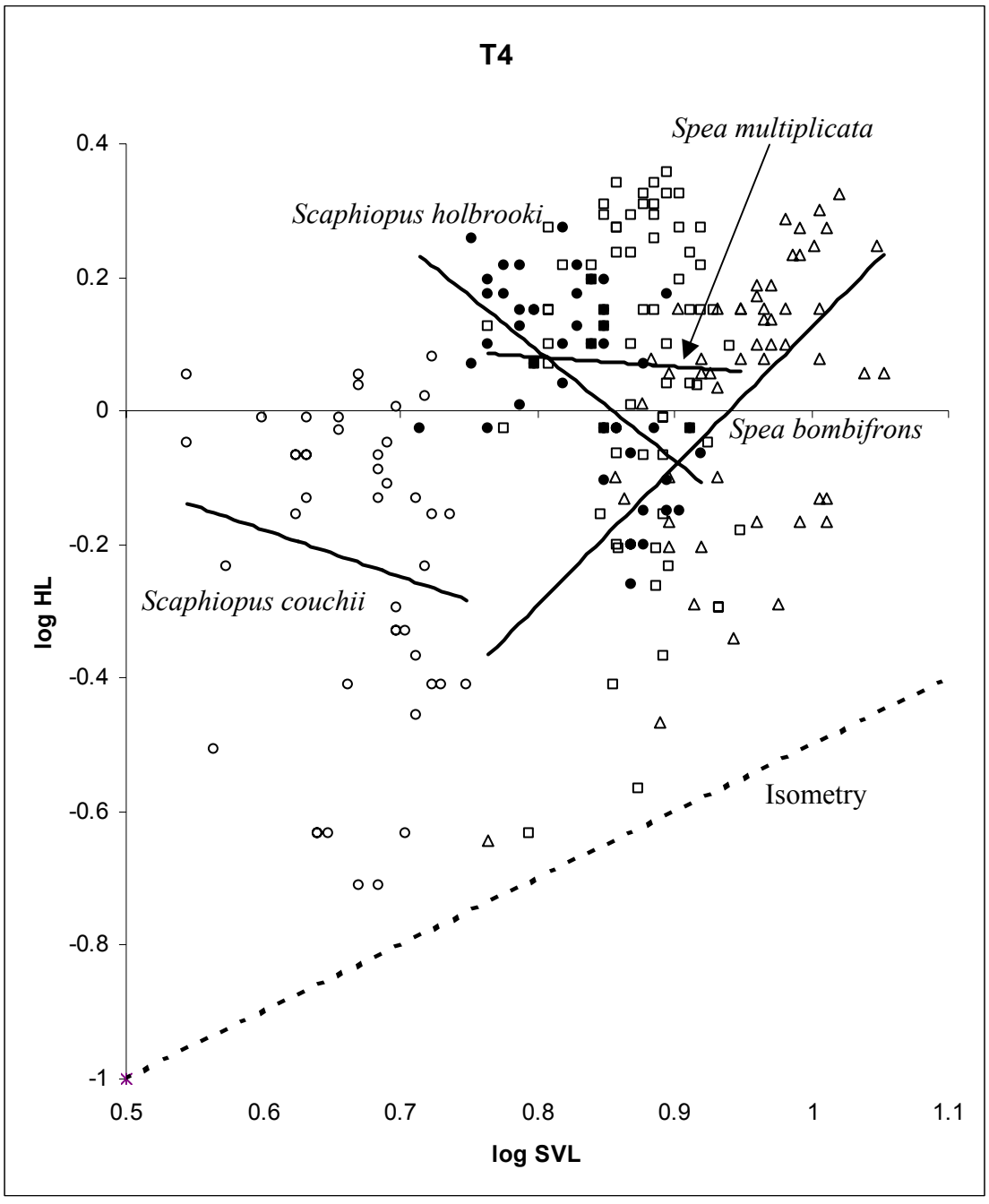

Fig. 2.4. Simple linear regression of log hind limb length (log HL) on log snout-to-vent length (log SVL) for thyroxine-treated tadpoles (T4) of *Spea bombifrons* (Δ), *Spea multiplicata* (□), *Scaphiopus holbrooki* (●), *Scaphiopus couchii* (○).

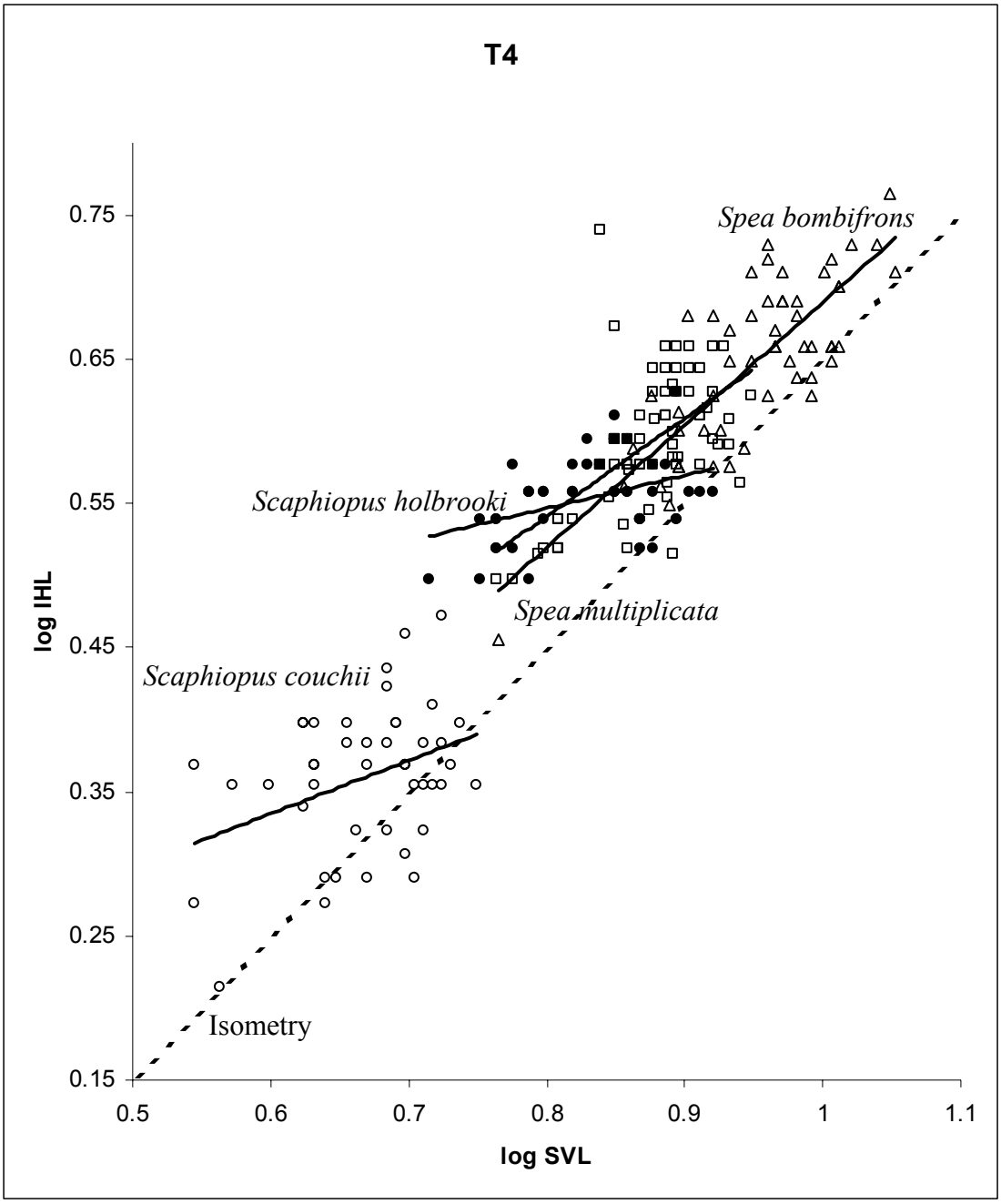

Fig. 2.5. Simple linear regressionof log interohyoideus length (log IHL) on log snout-to-vent length (log SVL) for thyroxine-treated tadpoles (T4) of *Spea bombifrons* (Δ), *Spea multiplicata* (□), *Scaphiopus holbrooki* (●), *Scaphiopus couchii* (○).

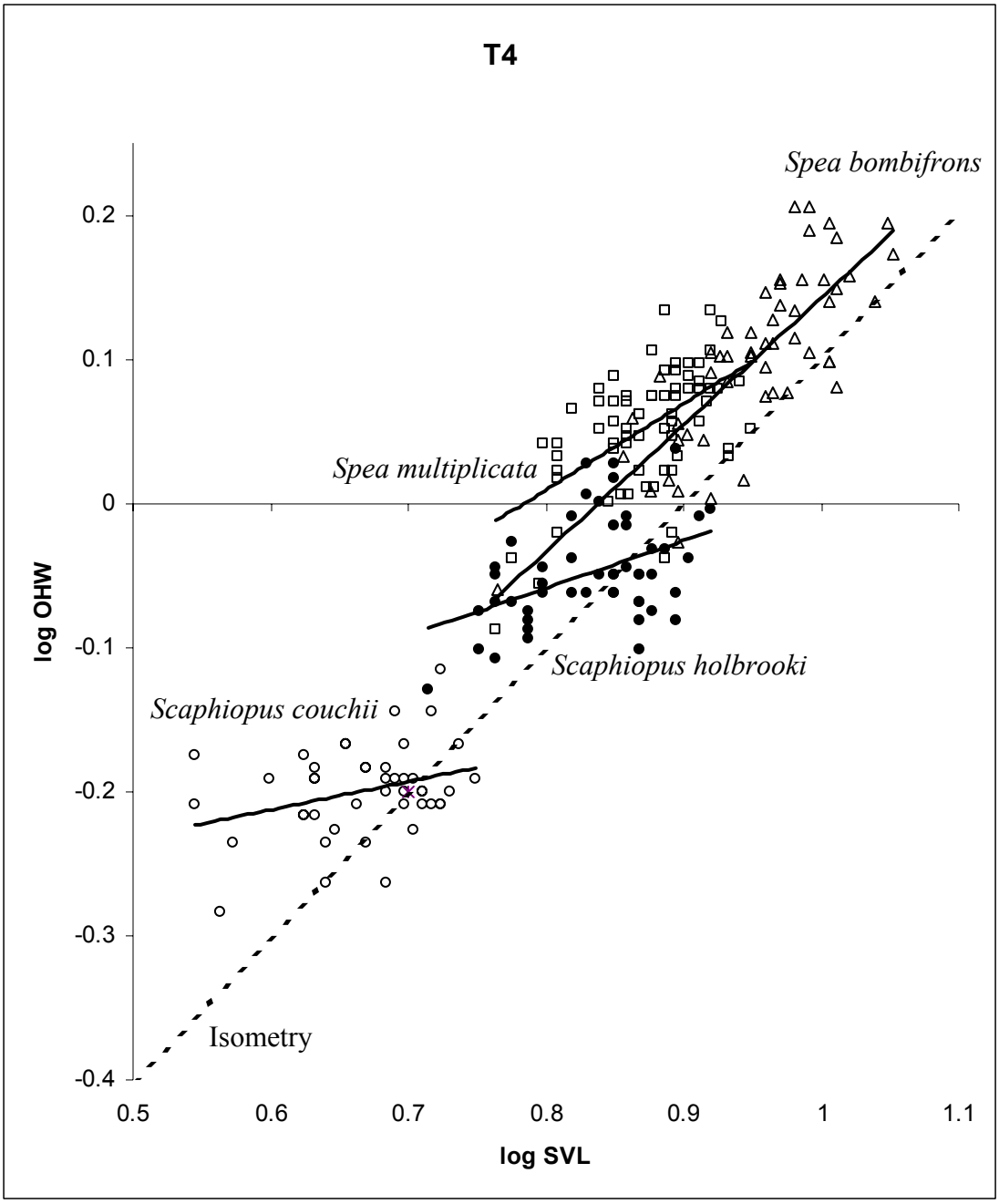

Fig. 2.6. Simple linear regressions of log orbitohyoideus width (log OHW) on log snout-to-vent length (log SVL) for thyroxine-treated tadpoles (T4) of *Spea bombifrons* (Δ), *Spea multiplicata* (□), *Scaphiopus holbrooki* (●), *Scaphiopus couchii* (○).

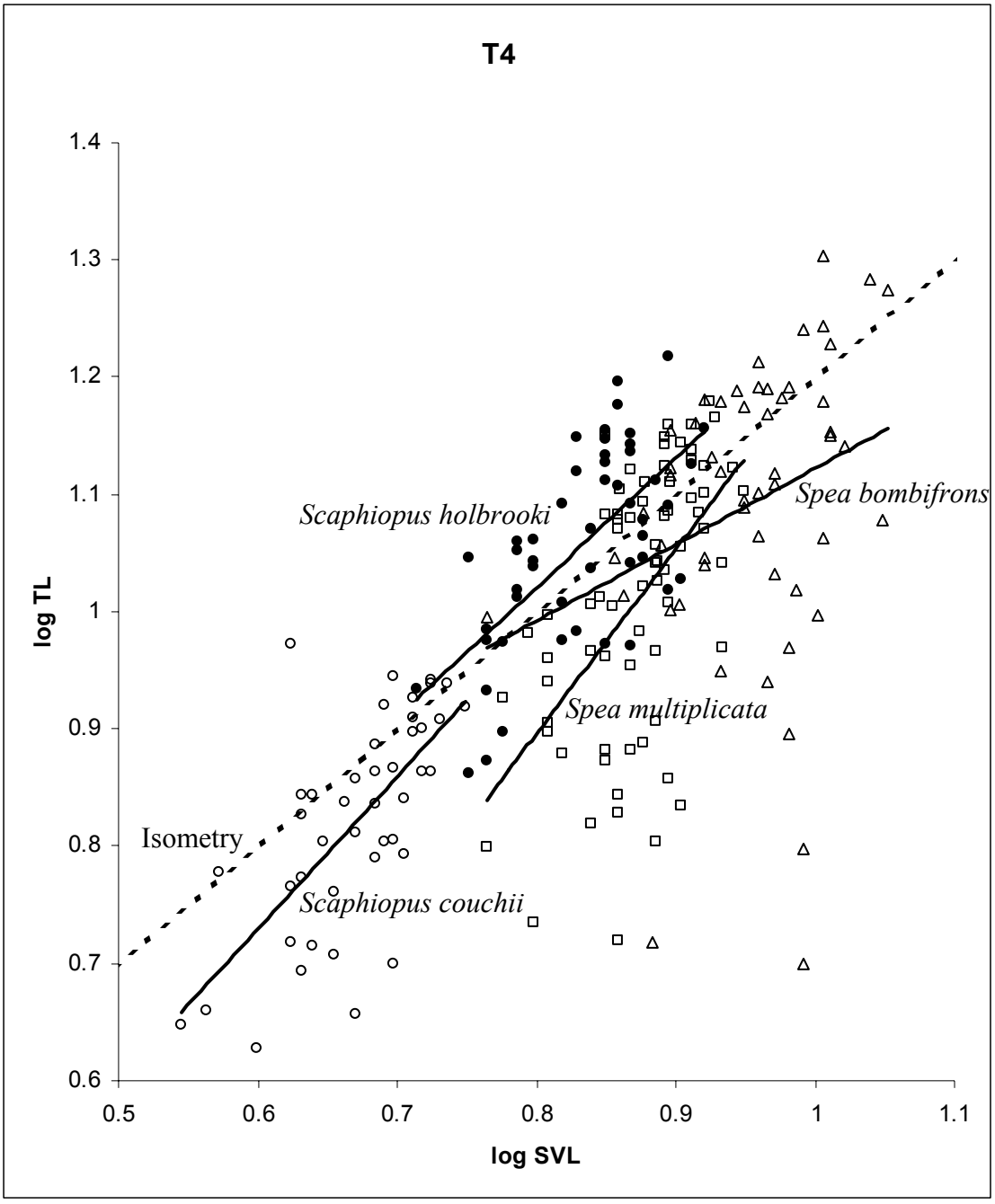

Fig. 2.7. Simple linear regressions of log tail length (log TL) on log snout-to-vent length (log SVL) for thyroxine-treated tadpoles (T4) and laboratory controls, *Spea bombifrons* (Δ), *Spea multiplicata* (□), *Scaphiopus holbrooki* (●), *Scaphiopus couchii* (○).

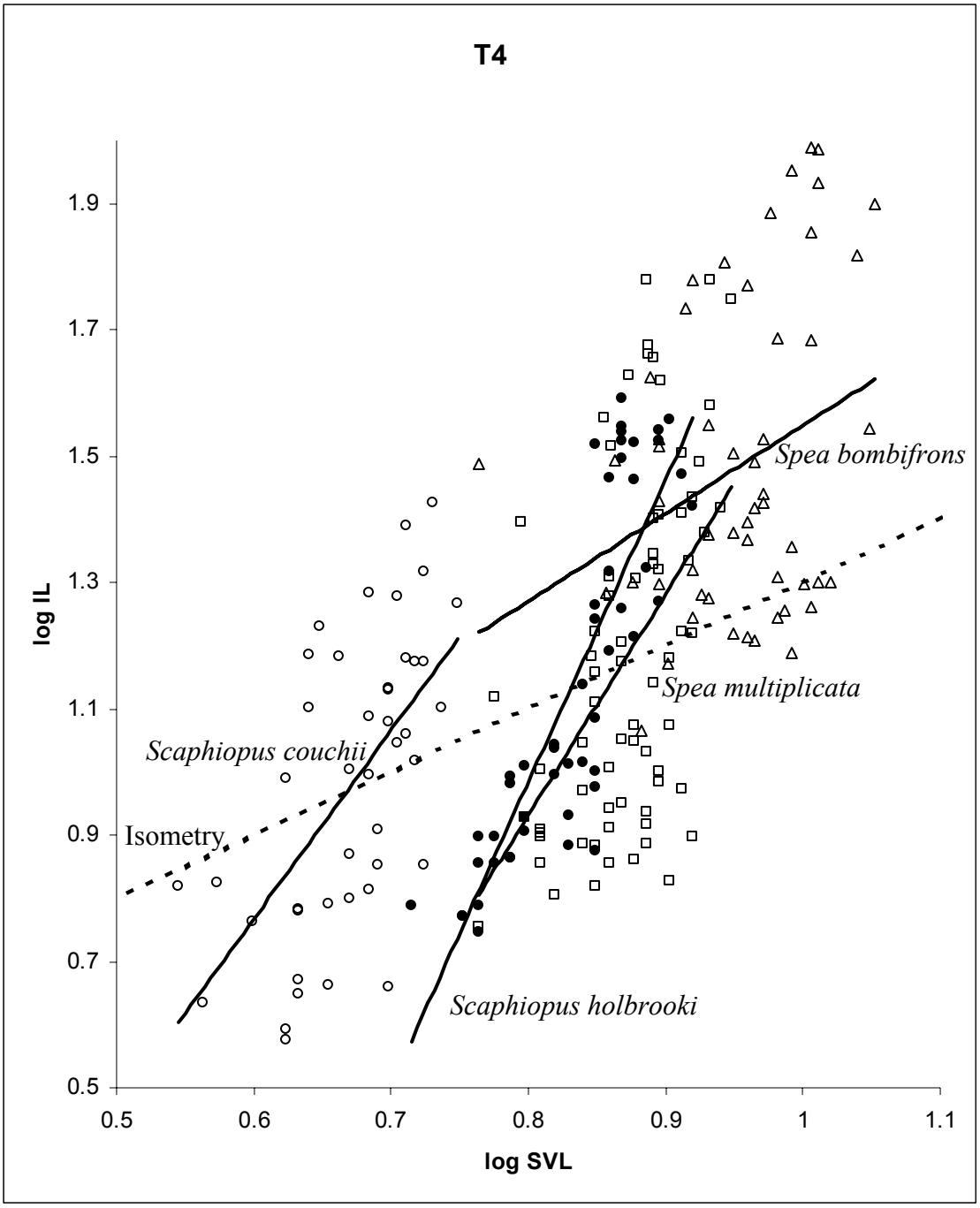

Fig. 2.8. Simple linear regressions of log intestine length (log IL) on log snout-to-vent length (log SVL) for thyroxine-treated tadpoles (T4) of *Spea bombifrons* (Δ), *Spea multiplicata* (□), *Scaphiopus holbrooki* (●), *Scaphiopus couchii* (○).

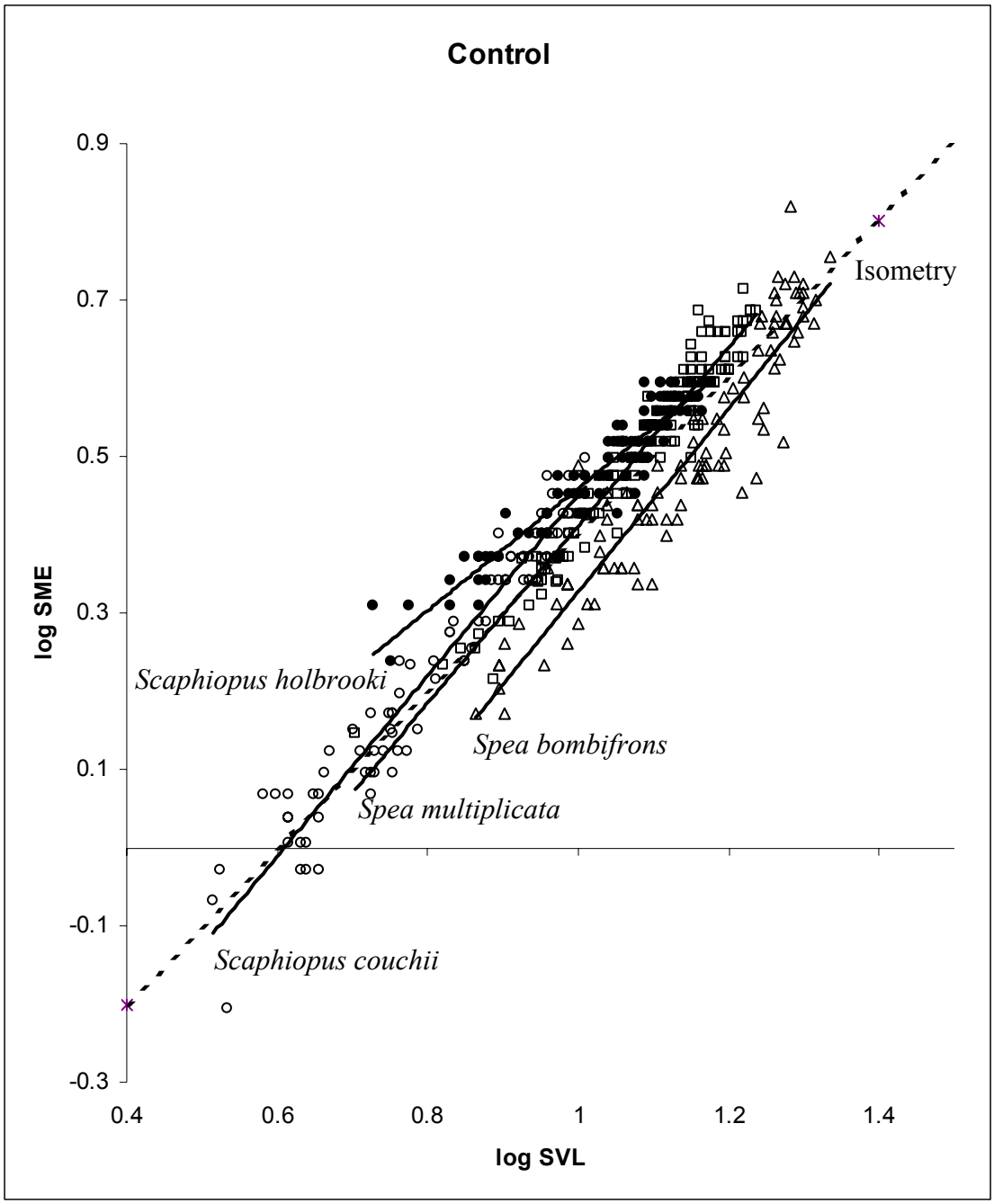

Fig. 2.9. Simple linear regression of log snout-to-mideye (log SME) on log snout-to-vent length (log SVL) for laboratory controls of *Spea bombifrons* (Δ), *Spea multiplicata* (□), *Scaphiopus holbrooki* (●), *Scaphiopus couchii* (○).

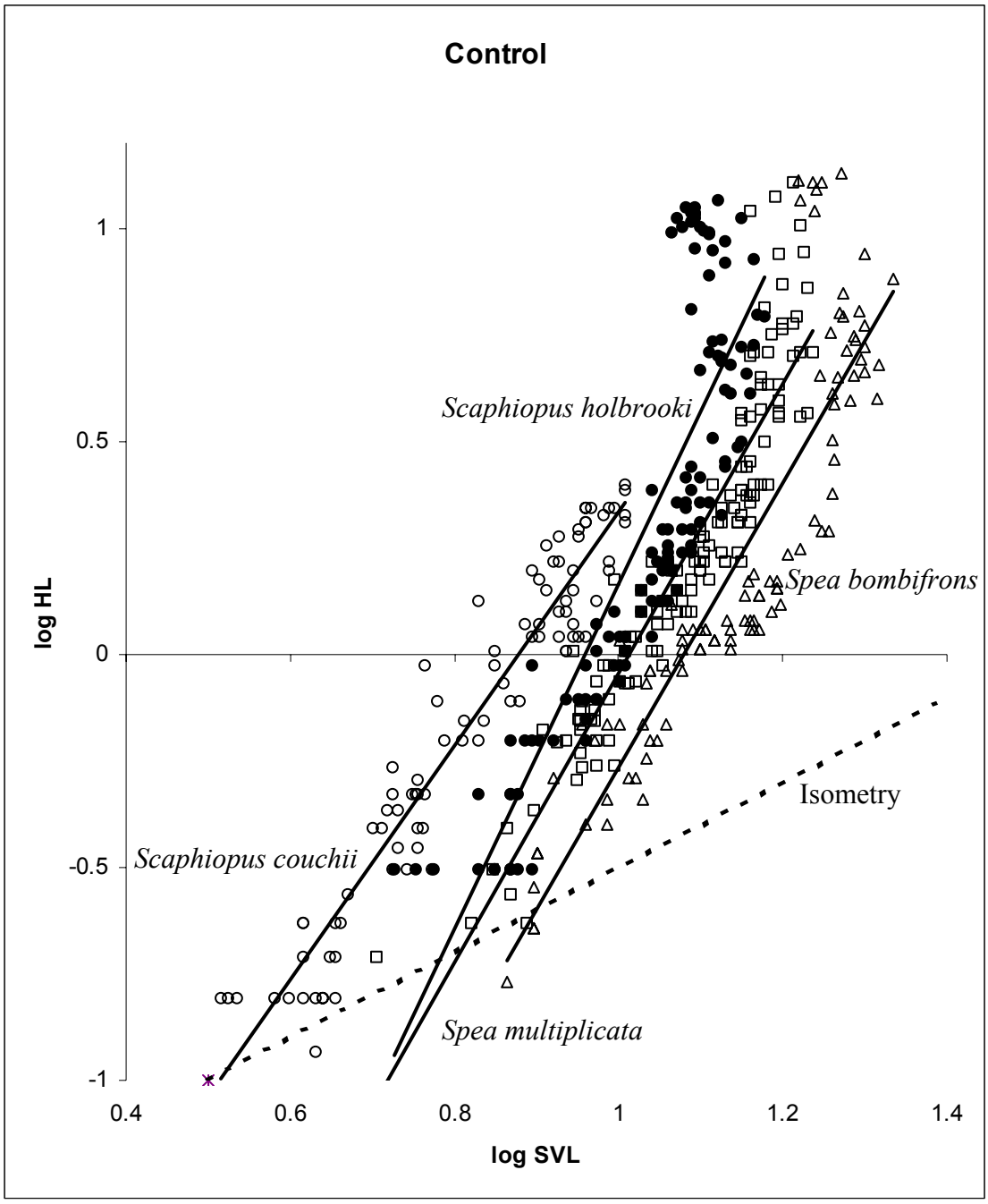

Fig. 2.10. Simple linear regression of log hind limb length (log HL) on log snout-to-vent length (log SVL) for laboratory controls of *Spea bombifrons* (Δ), *Spea multiplicata* (□), *Scaphiopus holbrooki* (●), *Scaphiopus couchii* (○).

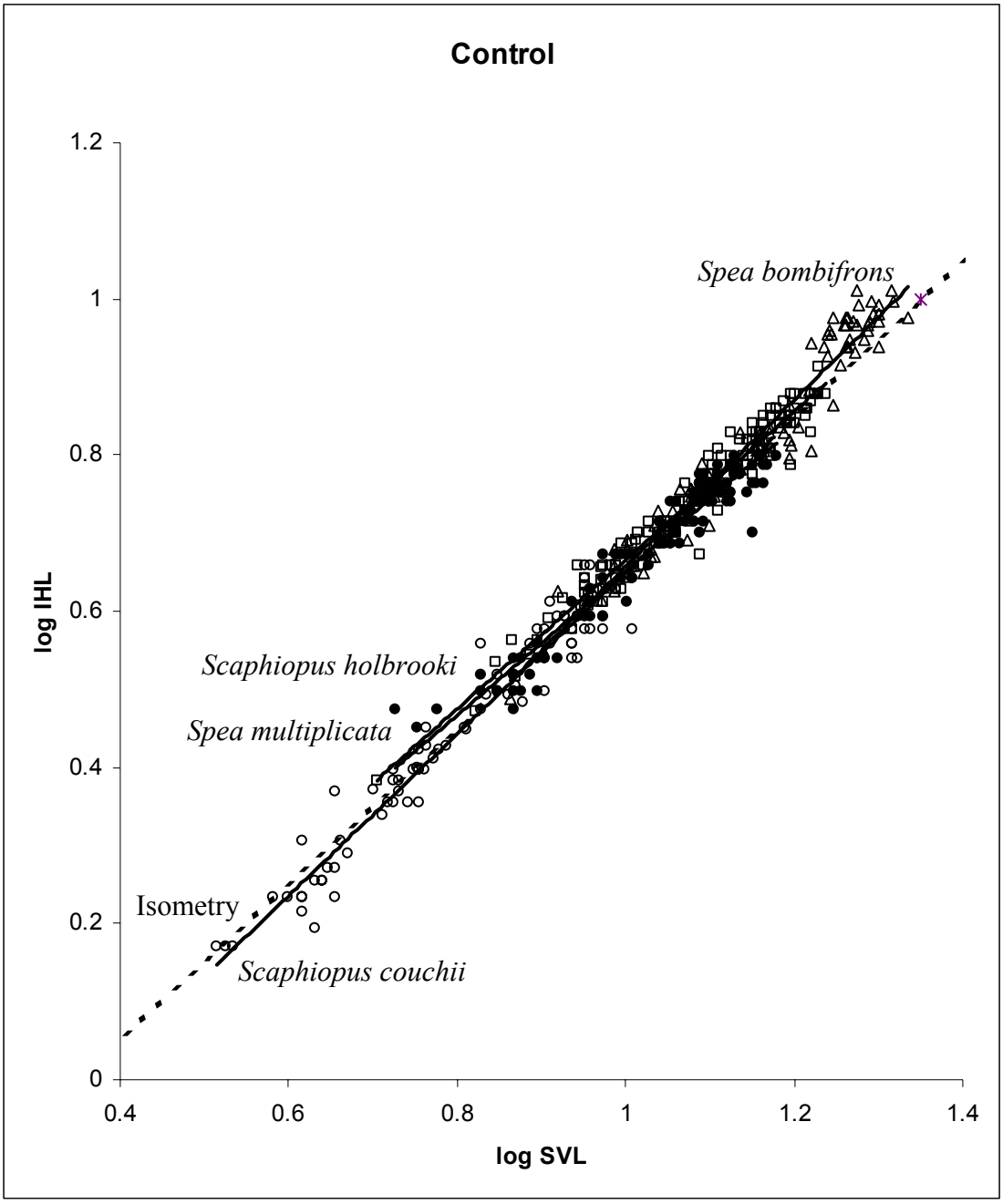

Fig. 2.11. Simple linear regressions of log interohyoideus length (log IHL) on log snout-to-vent length (log SVL) for laboratory controls of *Spea bombifrons* (Δ), *Spea multiplicata* (□), *Scaphiopus holbrooki* (●), *Scaphiopus couchii* (○).

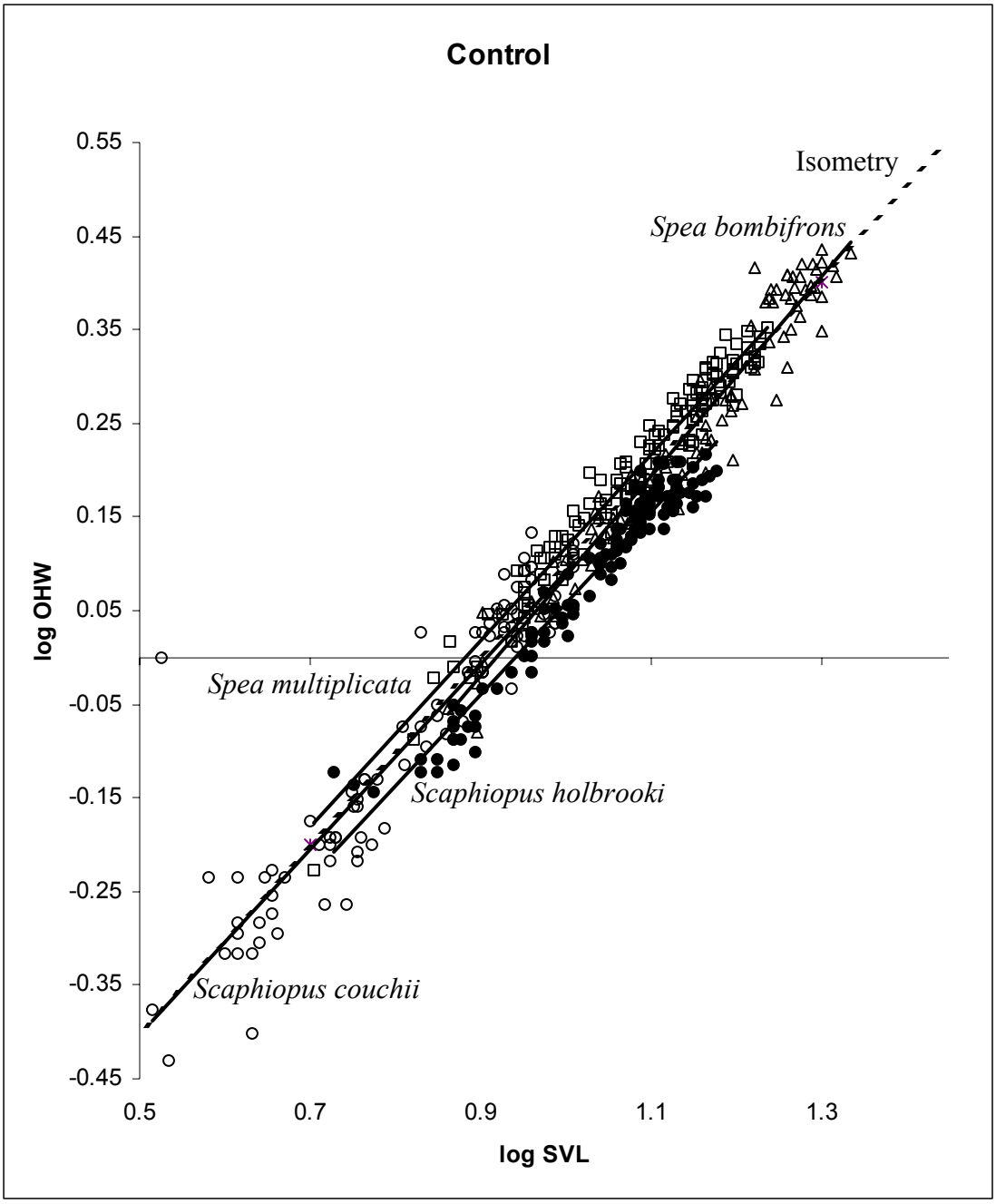

Fig. 2.12. Simple linear regressions of log orbitohyoideus width (log OHW) on log snout-to-vent length (log SVL) for laboratory controls of *Spea bombifrons* (Δ), *Spea multiplicata* (□), *Scaphiopus holbrooki* (●), *Scaphiopus couchii* (○).

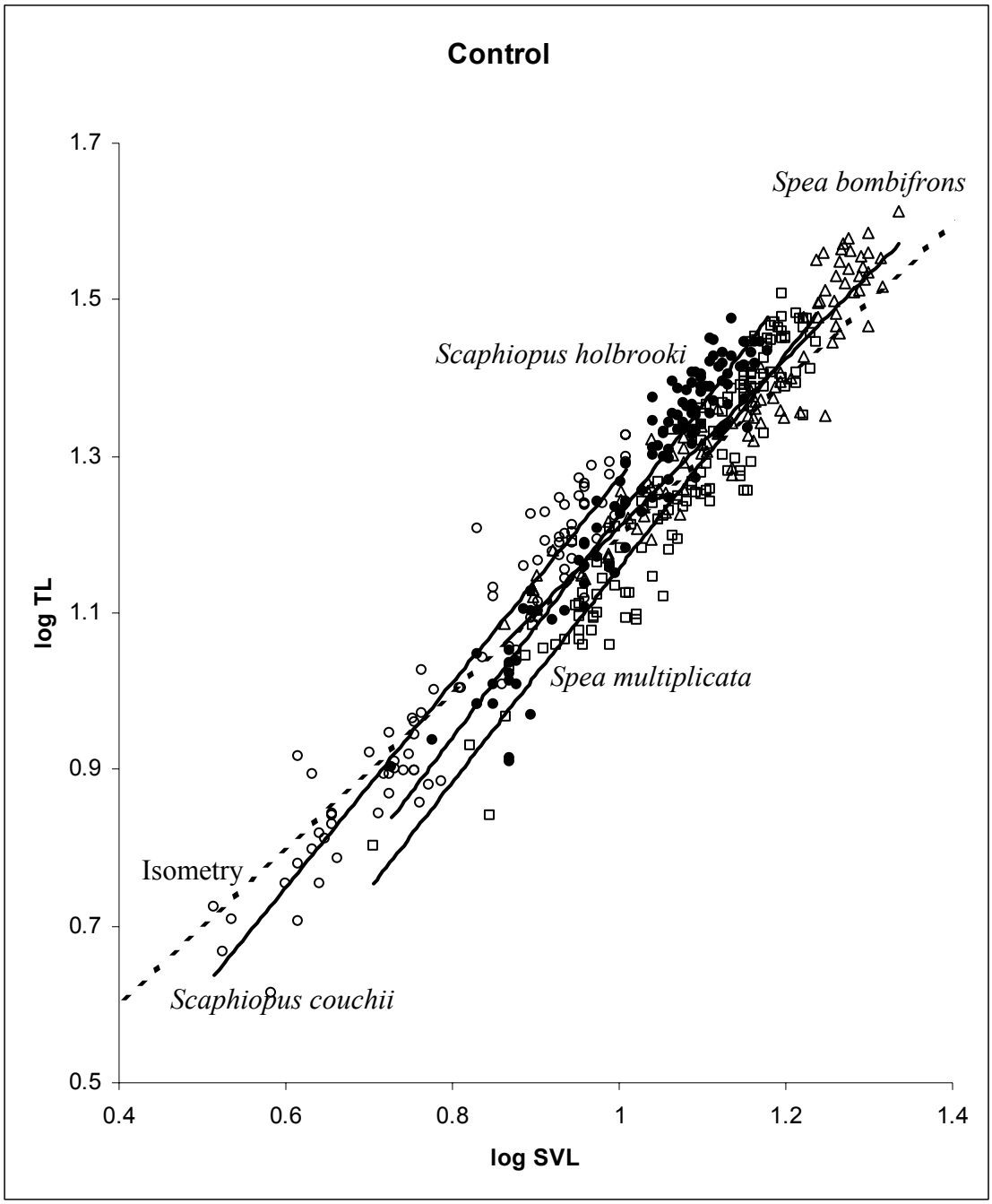

Fig. 2.13. Simple linear regressions of log tail length (log TL) on log snout-to-vent length (log SVL) for laboratory controls of *Spea bombifrons* (Δ), *Spea multiplicata* (□), *Scaphiopus holbrooki* (●), *Scaphiopus couchii* (○).

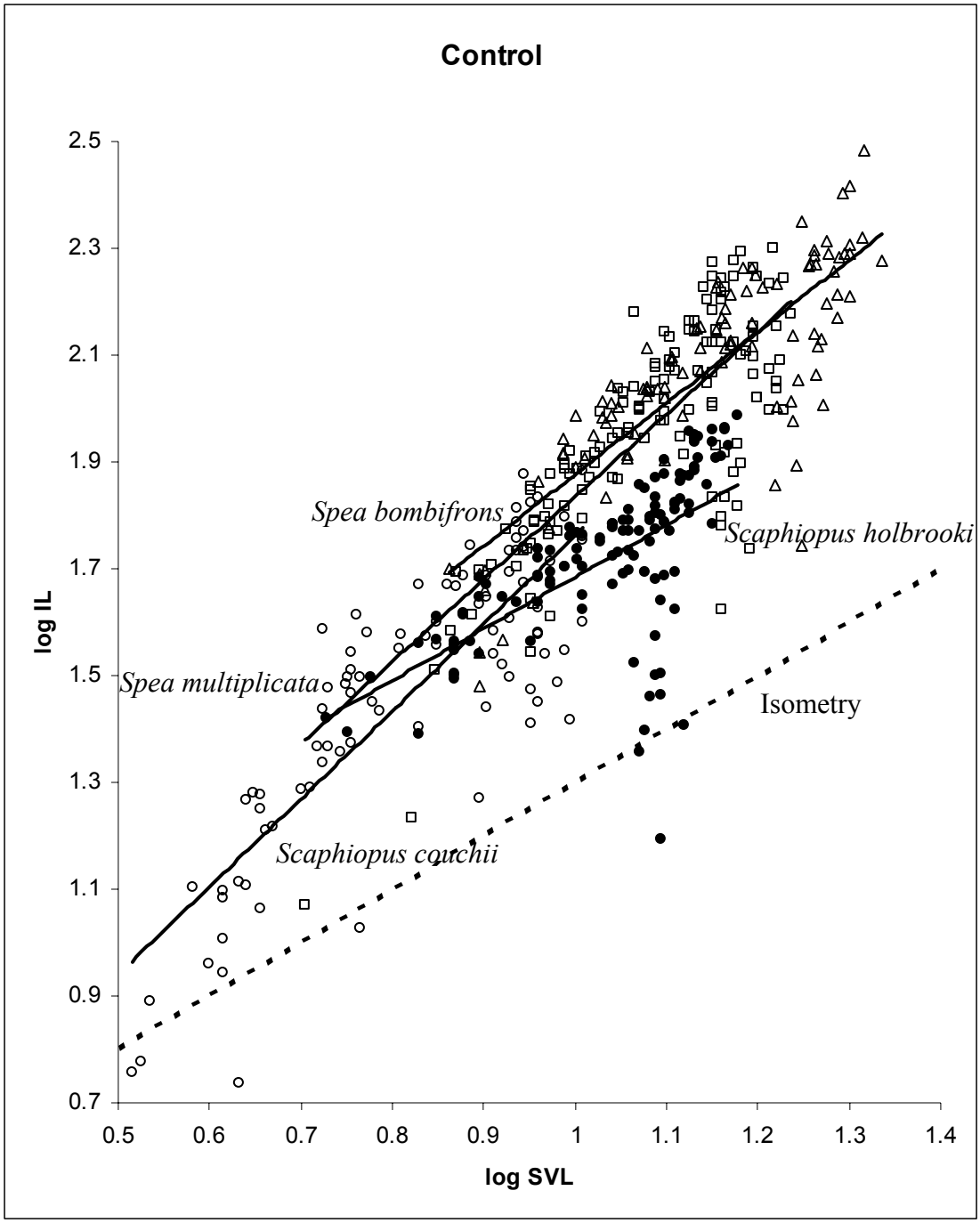

Fig. 2.14. Simple linear regressions of log intestine length (log IL) on log snout-to-vent length (log SVL) for laboratory controls of *Spea bombifrons* (Δ), *Spea multiplicata* (□), *Scaphiopus holbrooki* (●), *Scaphiopus couchii* (○).

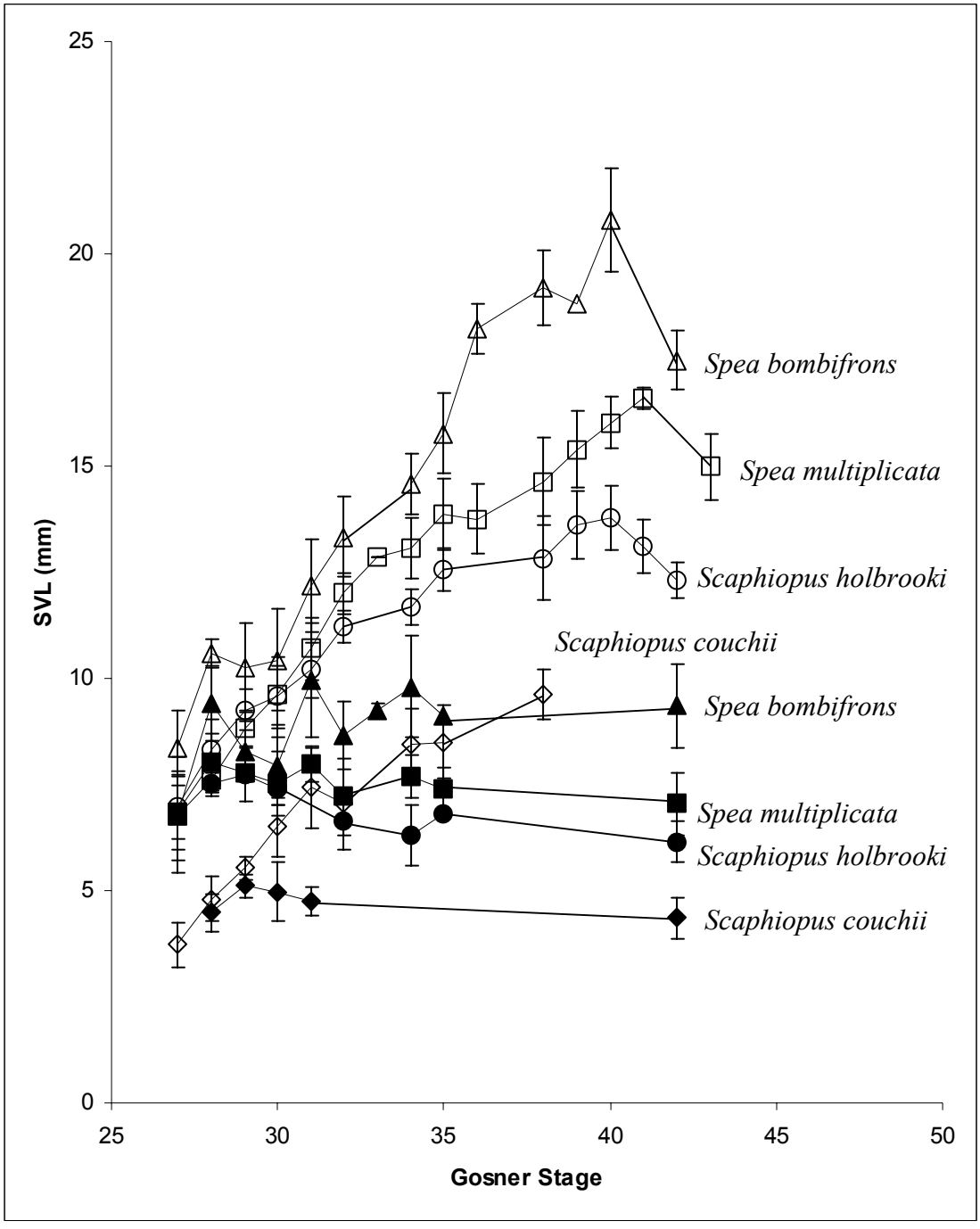

Fig. 2.15. SVL (mm) development of the thyroxine-treated and untreated controls as a function of Gosner developmental stage; mean and standard deviations represented.

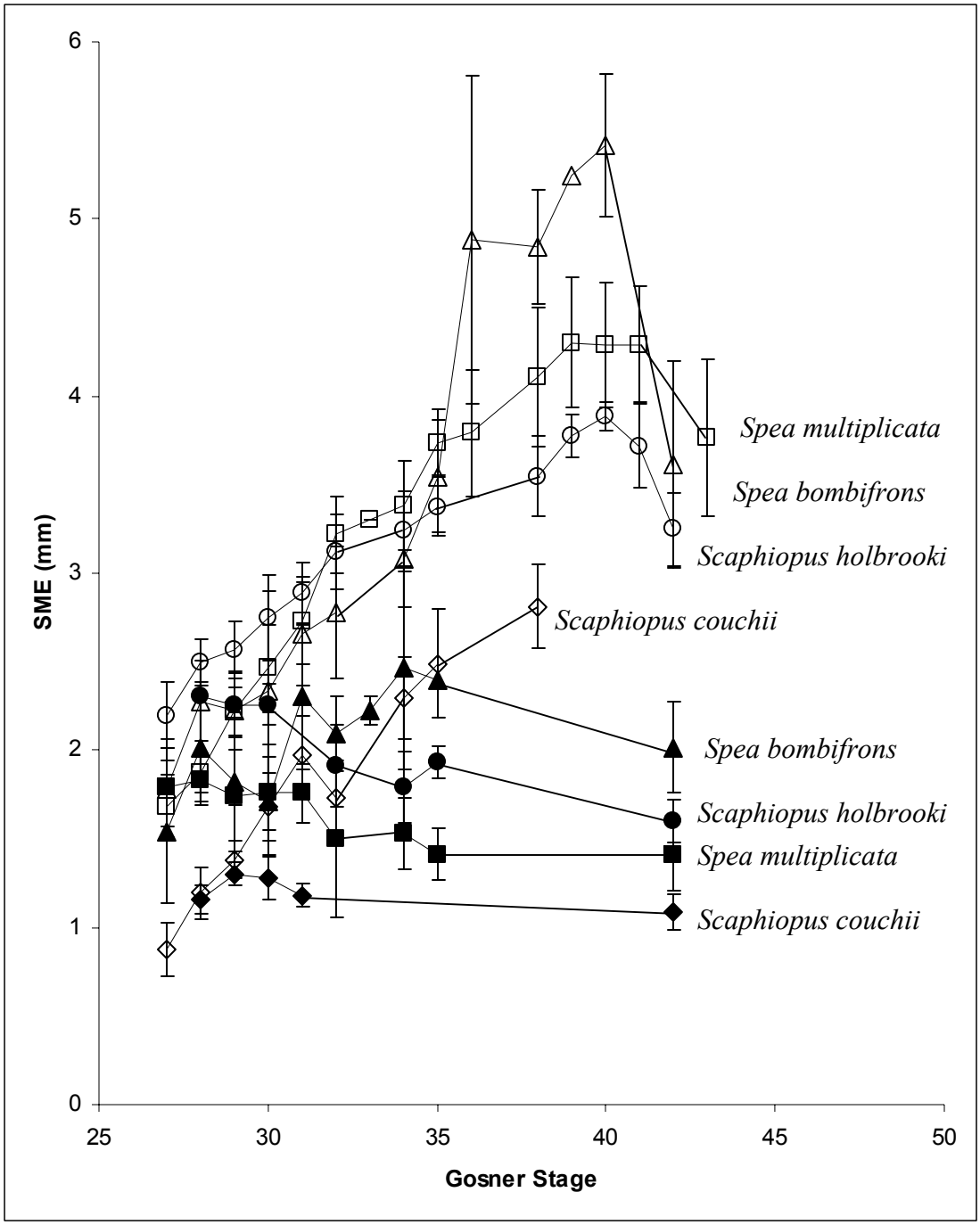

Fig. 2.16. SME (mm) development of the thyroxine-treated and untreated controls as a function of Gosner developmental stage; mean and standard deviations represented.

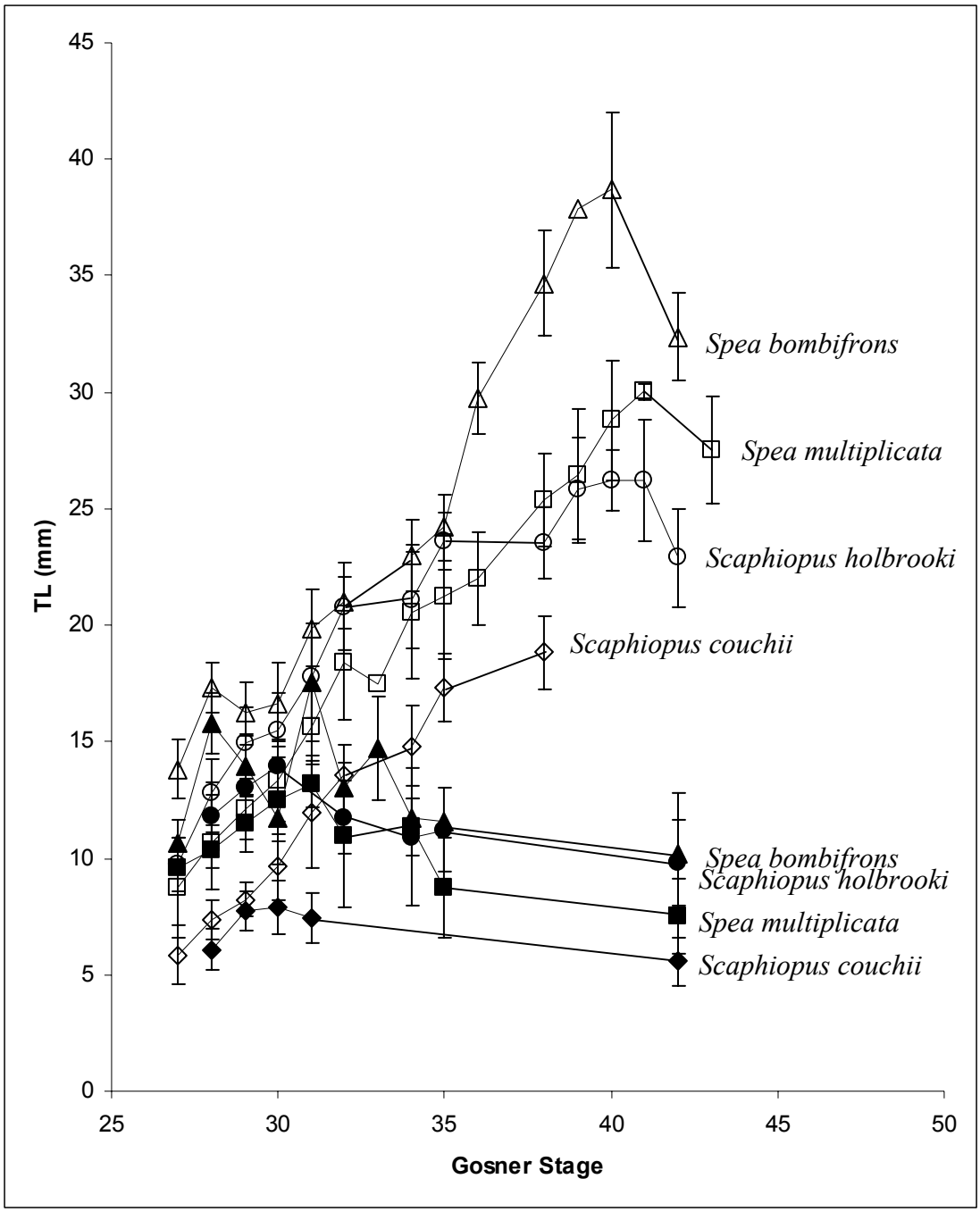

Fig. 2.17. TL (mm) development of the thyroxine-treated and untreated controls as a function of Gosner developmental stage; mean and standard deviations represented.

CONCLUSION

In chapter one I show that thyroxine and feeding on conspecifics is not the proximate mechanism of developmental polyphenism in the spadefoot toads *Spea multiplicata*. Rather, feeding on fairy shrimp, or biotic and/or abiotic factors that may be associated with fairy shrimp probably controls developmental polyphenism.

In chapter two I attempted to elucidate possible mechanistic reasons that may be responsible for differences in development between four species of spadefoot toads. Tadpoles treated with thyroxine show more difference in development than controls, which is the opposite pattern than what was expected if differences in the concentration of plasma thyroxine controls differences in development between the species. This study does not eliminate or support differences in thyroxine as the mechanistic reason for developmental differences. Concentration differences may be a controlling factor, but other factors such as thyroid hormone receptor differences or metamorphic differences may also be involved.

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BIOGRAPHICAL SKETCH

I cannot believe I have made it this far. Growing up in a border-line poverty household in which the evening movie on ABC was far more important than home work, I consider myself lucky to have finished highschool let alone a Bachelors of Science at UC Davis and a Masters of Science at Florida State University. I was born in Eugene, Oregon and most of my family still lives in Oregon, although I did not get the chance to live there long. When I was seven years of age the family packed up and moved to Fruitland, Idaho and I had to leave my first love, Tanya, she wore overalls every day and we used to play hawks on the playground (she would guard the nest and I would forage to feed our pretend hawk-lets). My ex-stepfather decided it would be a great idea to start a lawn care service in Fruitland, but unfortunately, it is a very small town and there is snow on the ground for five months of the year. This must explain why he is my ex-stepfather. After five years of snow and ice-filled fun we moved to sunny California, not Malibu, San Diego, San Francisco, or anywhere nice. No, we moved to Visalia, situated in the central valley between lovely Bakersfield and even lovelier Fresno. At eighteen, armed with a fresh high school diploma, I started up my 1964 Dodge Dart and got the hell out of Visalia. I moved to Eureka, California, rented a 26-foot travel trailer, on the beach, and started taking classes at the local junior college, College of the Redwoods. My first two years at of junior college were all remedial classes to get me up to college level and most of my classes did not even count for college credit. Thank god the junior college system exists! College of the Redwoods is where I first developed an interest in biology. It is a beautiful campus situated on a hillside, surrounded by redwoods, and overlooking Humboldt Bay. Probably because of the location, C.R. has a large proportion of highly educated professionals. Most of these professors were retired or overstressed from academia, and ran away to enjoy the low stress job of teaching at a junior college. I was fortunate to learn chemistry from a former N.A.S.A. rocket scientist and biology from a former UCLA human anatomy professor, etc. After three years in Eureka, I packed up

my 1983 Dodge Colt (the Dart died) and moved to Davis, California to start my upper division work. My first semester, I took Organic Chemistry 1A at 8 a.m. in the morning. Every morning, I was completely annoyed by a group of three overly cheery girls. They would come in every morning just before class and talk and laugh loudly while I was trying desperately to wake up on my second cup of coffee. The following semester I took Organic Chemistry 1B, which had a laboratory section in addition to the lecture. During the first lab one of the annoying girls from the previous semester waltzed in and took a seat next to me and we were paired up as laboratory partners. She was coughing and hacking all over me the entire class and I promptly got sick two days later. Well, that annoying girl with the hacking cough turned out to be Shonna Compton (now Shonna Storz). Our own little chemical reaction occurred during that semester; I guess you could say it was chemistry from the start. At the beginning of my second year at UC Davis, I started working with Dr. H. Bradley Shaffer on the phylogeography of Australian chelid turtles and finally graduated in 1999 with a Bachelors of Science. The rest is history; to continue this epic saga please see my curriculum vitae.