THE EFFECTS OF DIEL WATER QUALITY FLUCTUATIONS ON REPRODUCTION AND GROWTH IN THE SAN MARCOS SALAMANDER

THESIS

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ABSTRACT

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SAN MARCOS SALAMANDER

by

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The effects of diel pH fluctuations and upwelling flow on growth and reproduction in the San Marcos Salamander (*Eurycea nana*) were examined in culture. Paired individuals were measured monthly for growth in length and weight during a 9-month experiment. Seven clutches of eggs resulted, with an average of 34.7 eggs deposited during each reproductive event. The average hatching success for each clutch was 22.9%. One breeding pair reproduced three times during a 5-month period, with a total of 132 eggs deposited and an average hatching success of 35.9%. Egg production in *E. nana* was not sufficient to determine if any of the pH or upwelling treatments affected reproduction. During the last month of the study, male salamanders with access to upwelling tubes were significantly greater in weight ($P < 0.05$) than those without access.

INTRODUCTION

The San Marcos salamander (*Eurycea nana*) is a federally listed threatened species (United States Department of the Interior, 1980) endemic to the headwaters of the San Marcos River, Hays County, Texas (Bishop, 1941; Chippindale et al., 1998). This species inhabits the constantly flowing waters of the San Marcos Springs that issue from the Edwards Aquifer through limestone faults along the Balcones Fault Zone. These springs fill Spring Lake, which forms the headwaters of the San Marcos River (Brune, 1981).

Eurycea nana is one of several species that rely solely on the Edwards Aquifer and its outflow to sustain an adequate water supply. Excessive pumping of the aquifer may cause a reduction in spring outflow, limiting the water resource for aquatic species found in central Texas. Rapid population growth and urbanization in this area increased water demands on the Edwards Aquifer, and contributed to the need for listing several endangered and threatened species that occur in the San Marcos River. These include the San Marcos gambusia (*Gambusia georgei*), the Texas blind salamander (*Typhlomolge rathbuni*), and Texas wild rice (*Zizania texana*). Other threats to these species as well as the San Marcos salamander are pollution of the spring system, increased recreational activity, and habitat destruction (United States Fish and Wildlife Service, 1996). Because of its limited distribution, maintenance of the habitat of *E. nana* is crucial to its survival.

The range of *E. nana* includes Spring Lake and extends 150-m downstream of Spring Lake Dam (Nelson, 1993). Salamanders often have been collected from the aquatic moss (*Amblystegium riparium*) that grows along the concrete walls in front of Aquarena Springs Hotel and also have been reported occupying the dense, floating mats of filamentous algae (*Lyngbya* sp.) that occur in Spring Lake (Tupa and Davis, 1976; Nelson, 1993). *Eurycea nana* has been observed in all the spring openings in the lake as well as in the gravel and rocks just below the dam (Nelson, 1993).

A diverse community of aquatic macrophytes, over 49 species (Bruchmiller, 1973), in Spring Lake provides cover that houses many organisms such as amphipods, insect larvae and pupae, and small aquatic snails that compose the diet of the *E. nana* (Tupa and Davis, 1976). The metabolic processes of this productive macrophyte community may be largely responsible for driving diel fluctuations of water quality within Spring Lake and the upper portion of the San Marcos River (Groeger et al., 1997).

The water quality of the San Marcos Springs is influenced by the water quality within the underground aquifer. The springs are remarkably stenothermic, exhibiting temperatures from 21.0 to 21.5°C (Tupa and Davis, 1976), with temperature variations increasing as water moves downstream from the springs (Hannan and Dorris, 1970; Groeger et al., 1997). The water around the spring openings has a dissolved oxygen content of approximately 4.0 mg/L, and pH levels ranging from 6.9 to 7.8 (United States Fish and Wildlife Service, 1996). Water flowing from the San Marcos Springs is neutral to slightly alkaline as a result of passing through the underground limestone aquifer (Groeger et al., 1997).

Previous studies addressing the natural history of the San Marcos salamander provided limited information about reproduction in this species. Bishop (1941) reported that both sexes become reproductively mature upon reaching 41 mm in total length. Tupa and Davis (1976) ascertained that male *E. nana* reach sexual maturity or possess at least one fully pigmented lobe in each testis after attaining 35 mm in total length. They also hypothesized that females could not carry more than 20 eggs, 1.5 to 2.0 mm in diameter, in the abdominal region at one time. Nelson (1993) later found egg number ranging from 23 to 37. Eggs of *E. nana* have never been observed in Spring Lake, although juveniles and gravid females have been observed there in every month of the year (Bogart, 1967; Tupa and Davis, 1976). These observations indicate that *E. nana* likely breeds throughout the year. The environmental requirements necessary for reproduction in this species are not yet known.

In 1982, the U. S. Fish and Wildlife Service developed a recovery plan for the endangered and threatened species of the San Marcos and Comal springs. Part of this recovery plan called for the initiation of a captive-breeding program for *E. nana* at the San Marcos National Fish Hatchery and Technology Center (SMNFH&TC). A successful captive-breeding program would ensure the continued existence of the San Marcos salamander if a catastrophic event such as a drought or chemical spill destroyed the wild population. Identification of factors that stimulate reproduction in *E. nana* is necessary to establish a program in which salamanders reproduce on a predictable basis.

Effects of pH on reproduction have been documented for many aquatic species. Weiner et al. (1986) attributed the impairment of oogenesis and spermatogenesis in rainbow trout (*Salmo gairdneri*) to low pH. Craig and Baksi (1977) demonstrated a reduction in egg number and embryo survival as a result of exposing adult female flagfish (*Jordanella floridae*) to waters with a pH at or below 6.0. Acidity also has been identified as a cause of egg and hatchling mortality among many amphibian species (Gosner and Black, 1957; Pough and Wilson, 1977; Dunson and Connell, 1982; Clark and Lazerte, 1985).

Spring openings provide the source for upwelling flow in the habitat of *E. nana*. Schleser et al. (1994) documented several reproductive events occurring from a closely related salamander in the Comal River (c.f., *E. neotenes*) within artificial upwelling tubes. It also has been hypothesized that *E. nana* deposit their eggs within spring upwellings (Nelson, 1993). The objective of this study was to examine the effects of diel pH fluctuations and upwelling flow on reproduction and growth in the San Marcos salamander.

METHODS

Test Salamanders

Salamanders used in this experiment were collected in the San Marcos River approximately 30 m downstream from Spring Lake Dam on 10 June 1998 and 28 April 1999. Snorkeling equipment and small, hand-held dip nets were used to capture individuals at a water depth of no greater than 0.5 m.

Depending on the date of collection from the river, salamanders were kept in captivity at the SMNFH&TC for 9 to 20 months prior to the experiment. Because various stages of maturity were represented in the collections, this time allowed for maturation as well as acclimation to captive conditions. Individuals were separated according to sex and placed in a large reservoir tank which received recirculated water maintained at a temperature of approximately 22°C. A screen prevented contact between the two sexes. To identify the sex of each individual, salamanders were examined for eggs monthly. Those carrying visible eggs were categorized as sexually mature females (Tupa and Davis, 1976). By April 1999, any non-gravid salamanders greater than 41 mm in total length (Bishop, 1941) collected in June 1998 were assumed to be sexually mature males and were the only males used in the study.

Spring Lake Data Collection

A series of diel water quality measurements were taken in areas occupied by *E. nana* in Spring Lake. These measurements were performed over a 6-week period during May-

July 1999. A hydrolab probe (H2O Multiprobe®, Hydrolab Corporation, Austin, Texas) and data display unit (Scout 2®, Hydrolab Corporation) were used to record temperature, pH, and dissolved oxygen twice each week at 0700, 1500, and 2300 hours at six different sites in Spring Lake. Three of these sites were located near spring openings along the walkway near Aquarena Springs Hotel while three sites were 10 to 20 m from spring openings at the northern-most region of the lake. All sampling sites were less than 5 m from one another. Measurements were taken at depths no greater than 0.5 m. Data were collected to determine levels of diel water quality fluctuations in the natural environment of *E. nana*.

Aquarium Set-up

Three experimental culture systems (EX1, EX2, and EX3) were housed in a semi-open, covered building at the SMNFH&TC. Each system consisted of 12, 6-L flow-through aquaria above a 442-L recirculation tank (MT-700, Frigid Units, Inc., Toledo, Ohio). The dimensions of each aquarium were 30.2 X 15.0 X 20.5 cm which have been adequate for *E. nana* reproduction at SMNFH&TC (J. N. Fries, pers. comm.). Standpipes were set in the aquaria to allow for a volume of approximately 4.8 L of water. Each standpipe was fitted with a mesh screen to prevent salamander escape. Plastic, rectangular aquarium covers also were installed to prevent escape. Openings were cut in the covers to allow the addition of water and food.

Aquaria had two water supply sources: well water pumped from the Edwards Aquifer at approximately 7.6 L/h and recirculated water from the reservoir tank at approximately 102.1 L/h. Water in each recirculation tank was circulated with a pump (Power-Flo, Hayward Pool Products, Inc., Elizabeth, New Jersey) through a 0.5-hp heater/chiller unit

(UTCH-3, Universal Marine Industries, San Leandro, California), which was programmed to maintain a water temperature of 21±1 °C in all systems. A pair of 500-W, submersible heaters (VT-500, Aquatic Ecosystem Inc., Apoka, Florida) was suspended near the center of the recirculation tanks. They were used during winter months when heater/chiller units could not maintain temperatures above 19 °C. The pump sent water, which had been heated or cooled, to each of the aquaria. Water in the reservoir tank passed through a screened filter containing biofiltration media (BF64, Biobarrel, Aquatic Ecosystems, Inc.). The media served as substrate for bacteria needed to break down nitrogenous wastes in the systems. To enter the pump inlet, water flowed through a mesh screen surrounding a ball valve. The screen prevented the entrance of snails, amphipods, and plant material into the pump. The ball valve allowed for the reduction of total dissolved gases for each system (modified from Herman, 1995). A standpipe was set in each recirculation tank to drain the system of overflow water. The pathway of water flow and arrangement of aquaria are illustrated in Figure 1a.

Four limestone rocks (longest axis 5-15 cm) were placed in all aquaria and stacked in a pyramid arrangement. Aquatic moss (*Amblystegium riparium*) collected from Spring Lake was added to each aquarium in similar amounts. The rocks and moss provided cover and oviposition substrate. Snails native to the San Marcos River were placed inside each aquarium to help reduce algal growth and provide a potential food source for salamanders.

Experimental Design

The experimental design consisted of two factors: (1) three levels of diel pH fluctuation (±0.03, ±0.04, and ±0.05) and (2) two treatments of upwelling flow (presence and absence of upwelling-flow tubes). Each of the three recirculation systems had a different level of diel pH fluctuation. Four of the aquaria on each system were equipped with upwelling-flow tubes and four lacked upwelling-flow tubes. Four auxiliary aquaria on each recirculation system were used for taking water quality measurements and housing salamanders used as replacements for salamander mortality during the experiment.

Experiment Set-up

The upwelling-flow tubes were constructed of clear plastic 30 cm in length with an inner diameter of 5 cm and secured to the base of each aquarium with a bulkhead fitting which connected the tube. This design enabled salamanders to travel easily between the aquarium and the tube. Clear glass marbles, 2.5 cm in diameter, filled each tube. Marbles were chosen as the substrate for the tubes because of their uniform shape and transparency, allowing for observations of salamanders within the tubes. Foam pipe insulation covered each tube to simulate the dark environment of natural spring upwellings. The foam insulation on each tube was removed approximately twice each week for less than 15 s to note egg production and record observations of salamander behavior. Recirculated water entered the bottom of the upwelling tube through a hose attached to a reducer fitting. The fitting was held in place by a flexible coupling and a pair of pipe clamps. Perforated plastic and a mesh cloth were situated in the bottom of the upwelling-flow tube to prevent salamanders from entering the recirculation hose. A small piece of PVC pipe was inserted just above the perforated plastic to hold the marbles in the tube during removal of the coupling to collect salamanders for monthly recording of their lengths and weights. A pair of tie-down straps (PS12 polyflex straps, Aquatic

Ecosystems, Inc.) was installed on every aquarium equipped with a tube to guard against the loss of the coupling if the pipe clamps failed. Upwelling flow within the tubes was approximately 2.4 cm/s.

Diel pH fluctuations were manipulated using aquatic plants to remove carbon dioxide and elevate pH during daylight hours (Wetzel, 1975). *Vallisneria americana*, an abundant and native aquatic plant of the San Marcos River (Hannan and Dorris, 1970; Bruchmiller, 1973), was collected from Spring Lake, isolated in a reservoir tank, and grown for several weeks before being placed in the experimental systems. This carbon dioxide obligate species was selected to drive the pH fluctuations of the recirculation systems due to its efficiency at carbon dioxide removal (R. D. Doyle, pers. comm.).

Plants were placed in plastic pots with an inner diameter of 11 cm and a depth of 12 cm. They were potted in sand with 5 g of osmocote fertilizer (Osmocote-plus, Scotts-Sierra Horticultural Products Co., Marysville, Ohio) at a depth of approximately 6 cm. In all systems, the plants were placed at the end of the reservoir tank opposite the ball valve and inlet filter. High intensity plant lights (DLX ABS, 220-W, Hamilton Technology Corp., Gardena, California) were situated directly above the plants to promote high levels of photosynthetic activity. A black plastic light shield inside of the reservoir tank behind the plants prevented exposure of salamanders to the high intensity light.

Several methods were used to vary the degree of pH fluctuation between the experimental systems. Different amounts of *V. americana* were placed in each system. EX3 contained the most plant material (6 to 12 pots) and was expected to exhibit the highest amount of diel pH fluctuation. EX2 held three to five pots, while EX1 contained only one pot of *V. americana*. Plant number was adjusted depending on the pH level of the water determined by frequent water quality monitoring. To reduce the photosynthetic activity of plants within EX1 and EX2, only half of the high intensity lighting power was used. Approximately 11.6 L/min more well water (pH ≈ 7.2) was added to the EX1 system than the other systems to stabilize pH. Additionally, a carbon dioxide generator (Carbo-plus, Aquarium Products, Glen Burnie, Maryland) was installed near the recirculation inlet in the EX1 system to help maintain low levels of diel pH fluctuation.

The partially enclosed building that housed the culture systems received reflected sunlight and low levels of fluorescent lighting. Additional fluorescent lighting was installed above each experimental unit. All lighting systems in the building were controlled by digital timers adjusted weekly to follow sunrise and sunset times published for the San Marcos area by the Nautical Almanac Office. Each set of lights was offset 15 min from one another. Lights over the experimental systems were the last to turn on in the morning and the first to turn off at dusk. This was done to reduce the intensity of light level changes and presumably startle-response behavior by salamanders as lighting abruptly changed.

In each system, eight experimental aquaria contained salamanders directly involved in the study (Fig. 1b). Two auxiliary aquaria housed salamanders (four males in one, and four females the other) to be used as replacements for those that were lost in the experimental aquaria. Two other auxiliary aquaria were used in each system for monitoring water quality. No salamanders were kept in these aquaria to avoid disturbances caused by water quality testing.

Each experimental aquarium housed one male and one female. Salamanders were randomly selected, but those with few eggs or other anomalies (e.g., curvature of the spine, shrunken or swollen abdomen) were excluded from the selection pool. Paired salamanders were kept together for the duration of the experiment which was conducted over a 9-month period from 12 January to 12 October 2000.

Eggs produced by breeding pairs during the study were removed from aquaria 3 days after they were first observed. A plastic baster was used to gently dislocate the eggs from the moss or marbles. They were placed on a mesh substrate in a separate aquarium that received both well water and recirculated flow. The eggs and larvae were not exposed to any of the pH or upwelling treatments after their removal from the experimental aquaria.

Water Quality Testing

Water quality measurements were performed regularly in each experimental system (Table 1). Dissolved oxygen and pH were recorded three times a week to ensure these variables were fluctuating at different levels in EX1, EX2, and EX3. Temperature, saturation of gases, alkalinity, and total ammonia were measured to detect potentially harmful water conditions and to establish that these variables were similar in all systems throughout the experiment. The potentiometric titration method and the ammonia-selective electrode method (American Public Health Association, 1995) were used to measure alkalinity and ammonia, respectively. Approximately 2 months after the experiment began, a Hydrolab probe (DataSonde 4®, Hydrolab Corporation) and data display unit (Surveyor 4®, Hydrolab Corporation) were used to better depict water quality fluctuations on a diel basis. The Hydrolab unit was programmed to record pH, dissolved oxygen, and temperature every hour over a 24-h period. These measurements were taken once a week in each experimental system.

Collection of temperature data was supplemented by the use of remote temperature recorders (Stowaway, Onset Computer Corporation, Pocasset, Massachusetts). Enclosed in weatherproof cases, these devices were attached to sensors suspended in the center of each reservoir tank. Each was programmed to record water temperature inside the reservoir tanks at 30-min intervals. They were used to ensure all systems were fluctuating similarly and to document any abnormalities in temperature during the experiment.

Salamander Measurement Procedure

Salamanders were measured prior to placement in experimental aquaria and approximately every 30 days for the following 9 months. A small aquarium net was used to retrieve salamanders from the aquaria. Individuals found in the upwelling tubes were retrieved by removing the coupling, plastic, and mesh cloth at the base of the tube, allowing salamanders to be flushed into a large bucket of water. They were sedated in a 150 mg/L solution of tricane methanesulfonate (MS-222, Argent Chemical Laboratories, Redmond, Washington) for no more than 5 min until they appeared sedated enough to be measured. The sex of each individual was determined and weights (g) and lengths (mm) were recorded. Salamanders were promptly returned to their designated aquarium.

Feeding Procedure

Salamanders were fed commercially raised annelids (Blackworms, Aquatic Foods, Fresno, California) three times a week. Zooplankton harvested from the ponds on the SMNFH&TC station also was fed to salamanders when available. Samples of zooplankton were collected and examined under a dissecting microscope prior to being fed to the salamanders. Samples containing any cyclopoid copepods or *Hydra* sp. were not used, as it has been noted that these organisms can be extremely harmful to salamanders in culture systems (J. N. Fries, pers. comm.). The moss also housed a substantial number of amphipods which provided the salamanders with a constant food supply throughout the experiment. A small amount of tropical fish flakes (F0C, Aquatic Ecosystems Inc.) was administered to each aquarium biweekly to provide nutrition for annelids, zooplankton, and snails not immediately eaten by salamanders. To ensure each aquarium received equal amounts of food, blackworms and zooplankton were distributed using plastic, calibrated basters. Food was drawn into a baster and measured before being released into an aquarium. Likewise, tropical fish flakes were measured in the cap of a small vial prior to distribution.

Statistical Analyses

An analysis of variance (ANOVA) was conducted each month among the three experimental systems to determine if the lengths and weights of salamanders differed significantly ($P < 0.05$) as a result of diel pH fluctuations. The same statistical test was used to examine differences in the lengths and weights of the salamanders in the two upwelling treatments. If salamanders were replaced in the study, two ANOVAs were performed, one including and one excluding the measurement of the replacement salamander. If ANOVA results of both tests showed no significant difference, it was assumed that the replacement did not bias the experiment.

RESULTS

Spring Lake Data Collection

The average measurements as well as the maximum amount of diel fluctuation of pH, temperature, and dissolved oxygen recorded at both spring and non-spring sites in Spring Lake are presented in Table 4. Results of water quality sampling indicated that these waters may exhibit as much as a 0.98 change in pH over a 16-h period. Temperature generally was between 21.0 and 22.0°C but reached 23.1°C in the middle of the afternoon. The maximum amount of diel temperature change in Spring Lake was 1.7°C. Dissolved oxygen at the sites nearest to spring openings was between 4.05 and 4.44 mg/L, while dissolved oxygen at sites located farther from spring openings was between 3.14 and 5.23 mg/L. Samples taken at non-spring sites showed a higher degree of diel fluctuation with respect to dissolved oxygen (3.80 mg/L change) than sites located near spring openings (1.97 mg/L change).

Water Quality

The minimum, maximum, and mean values of pH, dissolved oxygen, temperature, alkalinity, total ammonia, and percent saturation of gases are presented in Table 5. Graphs of water quality data collected in the three experimental systems are presented in Figures 10-15. The average pH was greater in EX3 (7.55) than in EX2 (7.44) and EX1 (7.12). Average dissolved oxygen was greater in EX3 (7.30 mg/L) than in EX2 (7.15 mg/L) and EX1 (5.97 mg/L). There was very little variation between the systems in temperature during the study (Fig. 12). The mean temperature in EX1, EX2, and EX3 was 21.7, 21.6, and 21.7°C, respectively. Alkalinity was greater on average in EX1 (252 mg $CaCO_3$/L), than in EX2 (245 mg $CaCO_3$/L) and EX1 (240 mg $CaCO_3$/L). Total ammonia remained low in all systems throughout most of the experiment (Fig. 14). Mean total ammonia values for EX1, EX2, and EX3 were 0.06, 0.05, and 0.04, respectively. The highest ammonia levels recorded for each system occurred on the final day of data collection when electrical power failed at the SMNFH&TC. Mean total gas saturation (Fig. 15) was similar for EX1 (91.2%), EX2 (89.8%), and EX1 (92.1%).

The maximum diel fluctuations that occurred weekly over a 24-h period using the Hydrolab Datasonde®4 in pH, dissolved oxygen, and temperature are reported in Tables 6-8. The average change in pH was slightly greater in EX3 (0.10) than in EX2 (0.08) and EX1 (0.06). Mean change in dissolved oxygen was 0.81, 0.87, and 0.88 mg/L in EX1, EX2, and EX3, respectively. The average change in temperature over a 24-h period was greater in EX2 (2.25°C) than in EX1 (1.63°C) and EX3 (1.48°C).

Growth and Size

The monthly length and weight of each salamander involved in the experiment are presented in Appendix 1. Average lengths and weights of male and female salamanders recorded monthly throughout the 9-month period are presented in graphs (Fig. 2-9). Both male and female salamanders increased in length during the 9-month experiment, however, males grew an average of 3.1 mm more than females in the same amount of time. Both sexes showed an overall increase in weight during the study as well.

Table 3 gives the *P*-values from analyses of variance conducted to determine significant differences between monthly length and weight measurements of male and female *E. nana* in both the pH and upwelling treatments. No significant differences were found among monthly length and weight measurements of male salamanders in the three pH treatments (Table 9). Likewise, there were no significant differences during any month of the experiment in the lengths and weights of females in the three pH treatments. Male salamanders with access to upwelling tubes were significantly greater in weight than those without access during the last month of the study (Table 10; 1.45 g vs. 1.25 g; $P < 0.05$). There were no significant differences in lengths or weights of female salamanders in the two upwelling treatments.

Reproduction

A summary of reproductive events is given in Table 2. Seven clutches were produced during the 9-month experiment resulting in a total of 243 eggs deposited and 72 hatchlings. The average number of eggs per clutch was 34.7 with an average of 10.3 hatchlings per clutch. The average hatching success for each reproductive event was 22.9%. The number of eggs deposited by one female during a single reproductive event ranged from 2 to 73. All eggs were deposited between 3 March 2000 and 21 July 2000. Six of the seven clutches produced in this period resulted in the hatching of larvae. The time between oviposition and hatching ranged from 12 to 23 days, with an average of 18.5 days.

Four reproductive events occurred in the system with the lowest degree of diel pH fluctuation (EX1). The number of eggs produced in this system totaled 205, with an overall hatching success of 31.7%. The average number of eggs produced in each clutch deposited by breeding pairs in the EX1 system was 51.2. The average hatching success of each clutch produced by breeding pairs in EX1 was 30.3%. Only one breeding pair from the EX2 system reproduced during the experiment. Eighteen eggs were produced in this system with a hatching success of 33.3%. Two reproductive events resulted from paired salamanders in the system with the highest degree of diel pH fluctuation (EX3). A total of 20 eggs were produced in this system with an overall hatching success of 5.6% and an average hatching success of 2.8% per clutch.

Four reproductive events occurred in aquaria equipped with upwelling-flow tubes and three reproductive events occurred in aquaria without upwelling-flow tubes. Female salamanders in aquaria receiving upwelling flow deposited a total of 150 eggs and females without access to upwelling flow deposited a total of 93 eggs. Of the 150 eggs deposited by salamanders in aquaria supplied with upwelling-flow tubes, 52 were found on marbles within the tubes and 98 were located on moss inside the aquarium. The average hatching success of eggs produced from breeding pairs in aquaria with upwelling flow was 28.3%. Average hatching success of eggs produced from breeding pairs in aquaria without upwelling flow was 15.6%. Females with access to upwelling flow deposited an average of 37.5 eggs per clutch and an average of 31.0 eggs were deposited by females without access to upwelling flow.

One breeding pair reproduced three times during a 138-day period (less than 5 months). A total of 132 eggs and 55 hatchlings resulted from the reproductive events of this pair. An average of 44 eggs were deposited during each reproductive event and the average hatching success for each clutch was 35.9%. There was a 72-day period between the time in which the first and second clutches were deposited and a 66-day period between the second and third clutches. Newly developed eggs were observed in the abdomen of the female 24, 22, and 28 days after the first, second, and third reproductive events, respectively. She was fully gravid with large eggs (estimated to be approximately 2 mm in diameter) 45 days following the deposition of the first clutch and 44 days following the deposition of the second clutch. Fifty-nine days after the third reproductive event, the female was noticeably gravid with eggs of many different sizes.

Observations

Of those salamanders housed in aquaria equipped with upwelling-flow tubes, at least one salamander in each aquarium was found in the upwelling tube 94% of the time. Both salamanders in each aquarium were found in the tube at least 56% of the time. Male salamanders were found in an upwelling tube at least 71.5% of the time, while females were observed in the tube at least 68.5% of the time. No salamanders were observed in any of the upwelling tubes when the electrical power failed on 11 October 2000.

Mortality

Two mortalities occurred during the study. One female salamander was lost during an attempt to remove it for measurement from an upwelling tube. The salamander became wedged between two marbles, was eventually retrieved, but died soon after it was measured. Another female was taken from an auxiliary aquarium to replace the lost salamander. The length and weight measurements of this individual were recorded. The second salamander lost was a male, which was assumed to be dead after it was not found in its aquarium during the monthly measurement procedures. A male replacement salamander was taken from an auxiliary aquarium, measured, and placed into the aquarium (EX3-7) of the lost salamander.

Another male salamander was missing and was assumed to be dead on 14 April 2000. A replacement male salamander was taken from the appropriate auxiliary aquarium and was measured. It was then placed into the aquarium (EX2-3) of the lost male. Three months later, both males were found, alive, in the same aquarium. The measurements taken while the original salamander was presumed dead most closely corresponded to measurements of the replacement salamander recorded in April. Therefore, the original male was taken out of the system and measurements of the replacement male were used in the statistical analysis of growth as though an actual mortality had occurred. Although no eggs were produced in EX2-3, it is important to note that the sex ratio was not 1:1 in this aquarium during April, May, and June of the study.

DISCUSSION

Spring Lake Data Collection

Data collected at Spring Lake may be unreliable because the equipment used for measuring pH failed several times. The unusually high level of diel fluctuation noted in the lake was unexpected because of the near constant water conditions issuing from the springs. The relatively constant temperatures and lower diel dissolved oxygen fluctuations at sites located near spring openings may be attributed to the constant flow of large amounts of water emerging from the aquifer which also has these characteristics (A. W. Groeger, pers. comm.).

Water Quality

Measurements taken with a hand held pH meter showed the system with the greatest amount of plant material (EX3) had the highest mean pH and had the greatest amount of diel pH fluctuation during the study. This indicated that the removal of carbon dioxide by plants for photosynthesis caused an increase in pH during daylight hours as expected. The additional well water supplied to EX1 may have contributed to the low pH levels due to a high level of bacterial respiration that takes place in the Edwards Aquifer. Increased respiration adds carbon dioxide to an aquatic system, resulting in depressed pH levels.

The similarity of dissolved oxygen in EX2 and EX3 was not expected since the latter had more photosynthetic activity as evidenced by the high diel pH fluctuations. It is possible that this similarity was due to the greater amount of plant substrate that was present in the reservoir tank of EX3. This substrate may have provided a site for bacterial respiration to occur. Increased bacterial respiration would have depressed dissolved oxygen levels in the system faster than they could have been replenished by the photosynthetic activity of the plants. Additional well water in EX1 helped to maintain low levels of dissolved oxygen in this system. The chiller unit on EX1 had difficulties maintaining temperatures between 21 and 22°C during the warm summer of 2000. The additional well water supplied to this reservoir tank was pumped from a well over 1.6 km away and may have been responsible for causing increased temperatures in this system. In June 2000, an additional 1-hp chiller unit was installed on the EX1 system to maintain lower water temperatures. Temperatures as low as 17.7°C were found only during the last week of the study when power failed on the SMNFH&TC station. The higher degree of diel change in temperature noted for EX2 when compared to the other two systems may be attributed to differences in heater/chiller unit capabilities. The lower mean alkalinity values in EX3 may be attributed to a greater amount of calcium carbonate that was precipitated out of the system during the removal of carbon dioxide by the plants for photosynthesis (Wetzel, 1975).

Total ammonia was lowest during the first few weeks of the experiment after the recirculation systems had been washed. However, ammonia levels reached potentially stressful levels during the last week of the study when the electricity failed. During the power outage, flow of oxygenated water through the biofiltration media stopped. Therefore, aerobic bacteria could not break down the nitrogenous wastes which caused a build-up of ammonia in the systems.

The ball valve used for controlling total dissolved gases worked fairly well, as none of the systems reached 100% saturation, but required occasional adjustment. During the last few months of the experiment, calcium carbonate deposits made the valves nonfunctional, however, percent saturation remained similar for each recirculation system throughout the experiment.

Growth and Size

Males in aquaria supplied with an upwelling-flow tube were more similar in weight than those housed in aquaria without upwelling-flow tubes except during the last month of the study. It is possible that the upwelling tubes benefited salamanders in the study by providing them with an environment similar to their habitat and that effects of upwelling flow may not become evident until salamanders are exposed to this condition for many months. Because they must allocate energy towards egg production, the effects of upwelling flow may not be detectable in females.

Male salamanders grew more in length than females during the experiment. This may have occurred because males used in the study had been housed at SMNFH&TC for approximately 10 months longer than females. The males had been acclimated to aquarium conditions longer, therefore enabling them to grow to a larger size. Males also may have had more energy to devote to growth because they did not have to donate energy to egg production as did the females.

Reproduction

Egg production from salamanders in this study was not sufficient to determine if any of the treatments affected reproduction. The number of eggs (205) deposited in the system with the lowest degree of diel pH fluctuations (EX1) was five times greater than the number of eggs deposited in other two systems combined (38). A greater number of eggs also were deposited by females in the aquaria equipped with upwelling-flow tubes (150) than those in aquaria without upwelling-flow tubes (93). The high number of eggs produced within the EX1 and upwelling treatments is largely due to reproduction of a single breeding pair which was responsible for depositing 132 eggs during three separate reproductive events. Because this breeding pair reproduced more than once during the 9-month study and others did not, mate selection may play an important role in the reproduction of this species. Olfactory cues, which stimulate reproduction during the early stages of courtship, may be involved in mate selection (Duellman and Trueb, 1986). Courtship pheromones affect reproductive success in both terrestrial and aquatic salamanders because they increase the probability of female receptivity (Houck, 1986). Glands located on the chins of male plethodontid salamanders produce courtship pheromones which are delivered to females through rubbing of the premaxillary teeth (Houck and Reagan, 1990). These mental hedonic glands (Sever, 1985) and premaxillary teeth (Bishop, 1941) have been identified in male *E. nana*. Houck (1986) stated that the presence of these structures is evidence for the use of courtship pheromones in the sexual behavior of salamanders, however, differences in male mating success may be due to differences in pheromone production and delivery rate (Houck and Reagan, 1990). These factors may influence reproduction in *E. nana*, but more study of their courtship behavior is needed to understand variation in male mating success.

Average hatching success for eggs produced during the experiment was approximately 23%. Eggs that were deposited and did not develop into embryos eventually became covered by fungus. It is not known whether the fungus developed on the eggs because the eggs were not viable or if the fungus caused the cessation of embryo development. The low hatching success suggests that some eggs in clutches may not be fertilized before they are deposited. If this is the case, female salamanders would waste the energy needed for the development and oviposition of non-viable eggs. Hatching success of eggs produced by *E. nana* at the SMNFH&TC has ranged from 0 to 98.3% (J. N. Fries, pers. comm.). Therefore, if females can produce clutches of both fertilized and unfertilized eggs, the number of unfertilized eggs is probably highly variable between clutches.

One female salamander in the experiment deposited only two eggs in one clutch. Approximately 2 days after the first observation of these eggs, they were not found again in the aquarium. It is possible that the snails, worms, or salamanders ingested these eggs. The maximum number of eggs deposited in this study by a single female salamander during one reproductive event was 73. Oviposited eggs were estimated to be approximately 2 mm in diameter. Tupa and Davis (1976) hypothesized that the abdominal region of female *E. nana* could accommodate no more than 20 eggs of this size. Wood and Duellman (1951) found that female *Eurycea bislineata rivicola*, which is on average 20 to 60 mm greater in length than *E. nana* (Petranka, 1998) could carry as many as 95 large eggs (1.5 to 2.5 mm in diameter) in their ovaries. No eggs of *Eurycea nana* have been found in its habitat, therefore it cannot be determined if clutch sizes of 73 occur in the wild. Salamanders housed at the SMNFH&TC were fed at least every other day, provided with adequate cover, and were isolated from potential predators. This environment may have allowed female salamanders to devote more energy toward egg production in captivity than they can in the wild.

Observations

Salamanders with access to an upwelling tube tended to be observed in the tube more often than in the aquarium. The salamanders may have occupied the tube because of the dark environment, the upwelling action of the recirculated water, or both. The electrical failure during the last week of the study resulted in a loss of upwelling flow from the tubes to the aquaria. No salamanders were found in any of the tubes during this time. This suggests that the salamanders did not occupy the tubes solely because of the dark environment, but that upwelling flow also was a factor in the presence of salamanders within the tubes. The salamanders also may have been avoiding the stressful water quality conditions (e.g., increase in ammonia, decrease in pH, decrease in dissolved oxygen) created by the lack of flow within the tubes.

Summary

Egg production by *E. nana* was insufficient to determine diel pH fluctuation or upwelling flow affected reproduction, however, it was found that a single captive female can deposit 73 eggs in during one reproductive event. It also was demonstrated that breeding pairs can reproduce at least three times during 1 year. For this reason, further research of mate selection and breeding behavior may be beneficial to identify factors that stimulate reproduction in this species.

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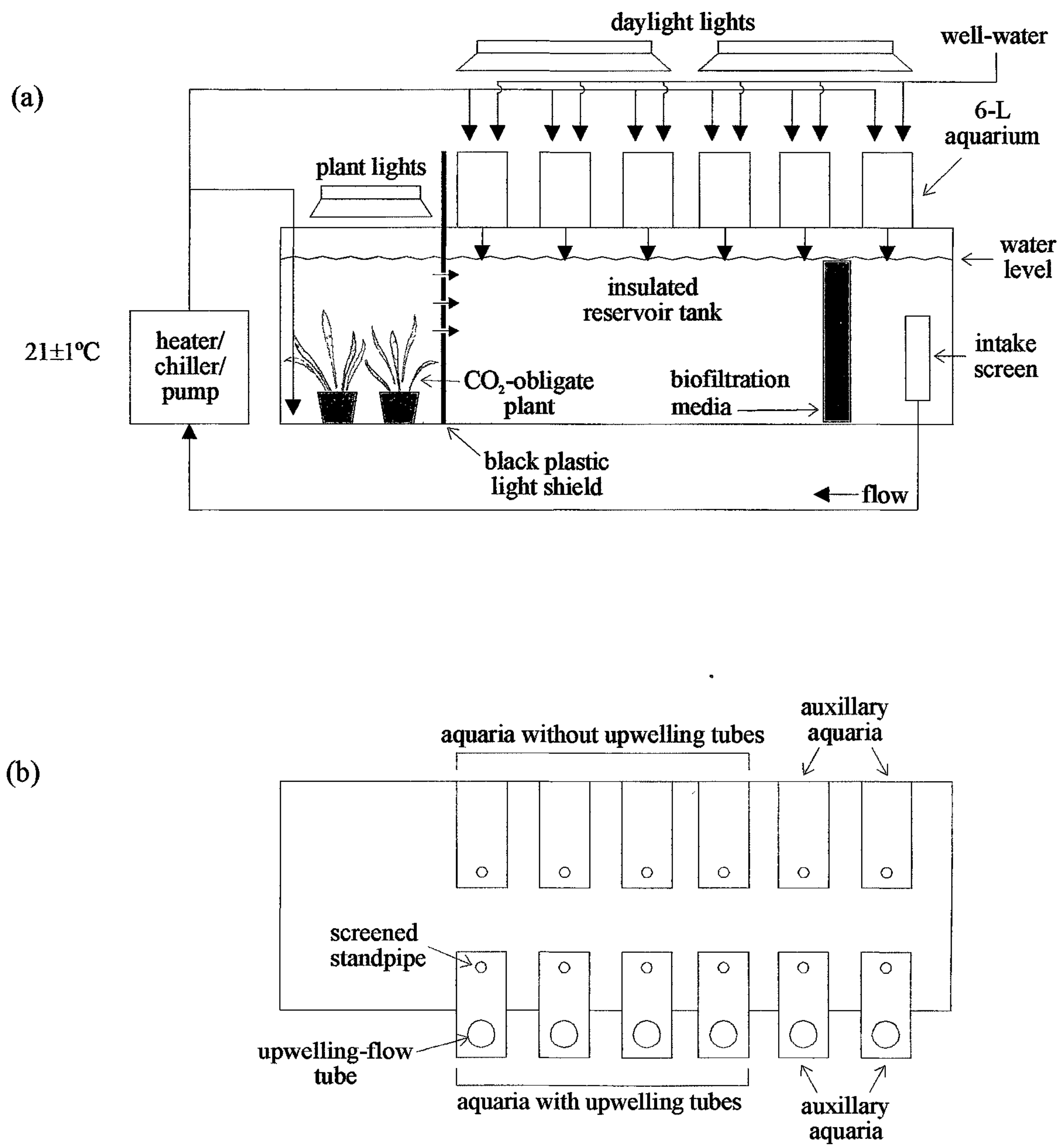

Figure 1. Schematic of one of three water recirculation systems with (a) a side view of the interactions of the different components of a system and (b) a top view of the arrangement of aquaria used in the 9-month experiment to examine the effects of diel water quality fluctuations and upwelling flow in the San Marcos salamander. Overflow pipe not shown. Auxiliary aquaria used for taking water quality measurements and holding replacement salamanders. Not to scale.

Table 1. Summary of sampling frequencies and equipment used to monitor water quality conditions in each of the three experimental systems (EX1, EX2, and EX3) housed at the San Marços National Fish Hatchery and Technology Center from 12 January to 12 October 2000.

Variable	Units	Sampling Frequency	Instrument
pH	--	3 times per week	Y60 model, YSI[1]
Temperature	°C	3 times per week	Y60 model, YSI[1]
Dissolved oxygen	mg/L	3 times per week	Y95 model, YSI[1]
Total gas	% saturation	weekly	model DS-IB, SA[2]
Total ammonia	mg NH_3/L	weekly	model 250, DI[3]
Alkalinity	mg $CaCO_3$/L	weekly	Basic model, DI[3]

[1] Yellow Springs Instruments Co., Inc., Yellow Springs, Ohio

[2] Sweeny Aquametrics, Stony Creek, Connecticut

[3] Denver Instruments Co., Ltd., Arvada, Colorado

Table 2. Summary of the seven reproductive events resulting from pairings of *Eurycea nana* in three experimental culture systems (EX1, EX2, and EX3) housed at the San Marcos National Fish Hatchery and Technology Center from 12 January to 12 October 2000.

Date	Aquarium Number	# Eggs	# Hatched in Clutch	% Hatching Success	Treatments
3 March	EX1-6	46	22	47.8	upwelling/low pH fluctuation
3 April	EX1-10	73	10	13.6	non-upwelling/low pH fluctuation
24 April	EX2-9	18	6	33.3	non-upwelling/medium pH fluctuation
24 April	EX3-4	18	1	5.6	upwelling/high pH fluctuation
15 May	EX3-10	2	0	0	non-upwelling/high pH fluctuation
15 May	EX1-6	59	31	52.5	upwelling/low pH fluctuation
21 July	EX1-6	27	2	7.4	upwelling/low pH fluctuation
	Total	243	72		
	Average	34.7	10.3	22.9	

Table 3. Probabilities of Type I errors (P) occurring from analyses of variance of monthly length and weight measurements of male and female *Eurycea nana* in three experimental systems (EX1, EX2, and EX3), each with a different level of diel pH fluctuation ($n = 8$), and with the presence or absence of upwelling flow tubes ($n = 12$). Measurements were recorded from 12 January to 12 October 2000 at the San Marcos National Fish Hatchery and Technology Center.

	pH fluctuation				Upwelling treatment			
	Male		Female		Male		Female	
Day	L	W	L	W	L	W	L	W
1	0.53	0.71	0.89	0.17	0.55	0.73	0.57	0.26
36	0.45	0.51	0.78	0.77	0.53	0.98	0.55	0.15
64	0.98	0.96	0.61	0.89	1.00*	0.66	0.56	0.34
94	0.79	0.66	0.70	0.52	0.73	0.76	0.60	0.67
122	0.83	0.90	0.70	0.50	0.64	0.57	0.52	0.90
153	0.90	0.55	0.64	0.51	0.56	0.39	0.44	0.65
181	0.94	0.41	0.72	0.62	0.45	0.32	0.67	0.99
218	0.92	0.42	0.87	0.49	0.41	0.30	0.75	0.61
248	0.94	0.46	0.90	0.71	0.39	0.07	0.69	0.70
276	0.97	0.75	0.92	0.92	0.23	0.01	0.87	0.69

*No difference resulted from an analysis of variance comparing average lengths of males housed in aquaria equipped with upwelling flow-tubes and those males without access to upwelling flow on the 64th day of the study.

Table 4. Average diel measurements of water quality variables taken at Spring Lake during summer 1999. Maximum Δ represents the largest amount of fluctuation recorded over a 16-h period at a single location. The range of values for each variable is given in parentheses. Data were recorded at 0700, 1500, and 2300 hours.

	Spring sites				Non-spring sites			
Variable	0700	1500	2300	Max. Δ	0700	1500	2300	Max. Δ
pH	6.98 (6.84-7.12)	6.90 (6.31-7.25)	7.03 (6.79-7.39)	0.98	6.91 (6.53-7.11)	6.74 (6.07-7.00)	6.94 (6.60-7.39)	0.97
Temperature (°C)	21.31 (21.2-21.5)	21.38 (21.2-21.6)	21.30 (21.1-21.6)	0.40	21.38 (21.1-21.6)	21.99 (21.5-23.1)	21.43 (21.3-21.8)	1.70
Dissolved Oxygen (mg/L)	4.07 (3.30-5.44)	4.44 (3.53-5.58)	4.05 (3.51-4.87)	1.97	3.14 (2.20-4.73)	5.23 (3.61-6.89)	3.48 (2.27-4.42)	3.80

Table 5. Minimum, maximum, and mean values of six water quality variables measured over a 9-month period from 12 January to 12 October 2000 in three experimental systems (EX1, EX2, and EX3) housed at the San Marcos National Fish Hatchery and Technology Center.

		EX1			EX2			EX3		
Variable	*n*	Max.	Min.	$\bar{x}$	Max.	Min.	$\bar{x}$	Max.	Min.	$\bar{x}$
pH	130	7.41	6.85	7.12	7.81	7.13	7.44	7.92	7.23	7.55
Dissolved oxygen (mg/L)	124	7.50	4.36	5.97	8.50	5.95	7.15	8.81	5.43	7.30
Temperature (°C)	130	24.0[1]	19.3[2]	21.7	23.8[1]	19.0[2]	21.6	24.1[1]	17.7[2]	21.7
Alkalinity ($mgCaCO_3/L$)	38	268	209	252	258	215	245	252	206	240
Ammonia (total)	39	1.10[2]	$1.24X10^{-5}$	0.06	1.10[2]	$1.63X10^{-5}$	0.05	0.67[2]	$1.17 X10^{-5}$	0.04
Total gas (%)	46	99.4	76.3	91.2	99.7	72.3	89.8	99.9	75.7	92.1

[1]Measurements resulting from elevated ambient temperatures during summer months.
[2]Measurements resulting from power failure on the SMNFH&TC station from 10-11 October 2000.

Table 6. Maximum amounts of diel pH fluctuations determined from measurements recorded every hour over a 24-h period. Diel sampling of pH occurred separately for each experimental system (EX1, EX2, and EX3) once a week at the San Marcos National Fish Hatchery and Technology Center. Data were collected using a Hydrolab DataSonde® 4 instrument from March to October 2000.

	EX1	EX2	EX3
Dates	Δ pH	Δ pH	Δ pH
20-24 March	0.06	0.09	0.16
27-31 March	0.06	0.07	0.09
3-7 April	0.06	0.06	0.11
10-14 April	0.05	0.07	0.06
17-21 April	0.05	0.07	0.07
24-28 April	0.06	0.08	0.10
1-5 May	0.04	0.06	0.11
8-12 May	0.10	0.05	0.08
15-19 May	0.05	0.08	0.08
22-26 May	0.05	0.05	0.09
29 May-2 June	0.10[1]	0.08	0.06
5-9 June	0.04	0.06	0.09
12-16 June	0.04	0.06	0.05
19-23 June	0.08	0.09	0.07
26-30 June	0.07	0.07	0.08
3-7 July	0.05	0.09	0.06
10-14 July	0.05	0.13	0.13
17-21 July	*	*	*
24-28 July	0.07	0.10	0.11
31 July-4 August	0.09	0.09	0.06
7-11 August	0.19[1]	0.10	0.14
14-18 August	0.06	0.07	0.08
21-25 August	0.04	0.09	0.06
28 August-1 September	0.07	0.10	0.06
4-8 September	0.05	0.08	0.10
11-15 September	0.04	0.06	0.11
18-22 September	0.06	0.12	0.13
25-29 September	0.04	0.08	0.08
2-6 October	0.03	0.08	0.09
9-13 October	0.07	0.05	0.42[2]
Average Δ	0.06	0.08	0.10

*No data available due to operator error
[1] CO_2 generator was not working in EX1
[2] Power failure occurred at the SMNFH&TC

Table 7. Maximum amounts of diel dissolved oxygen (mg/L) fluctuations determined from measurements recorded every hour over a 24-h period. Diel sampling of DO occurred separately for each experimental system (EX1, EX2, and EX3) once a week at the San Marcos National Fish Hatchery and Technology Center. Data were collected using a Hydrolab DataSonde® 4 instrument from March to October 2000.

	EX1	EX2	EX3
Dates	Δ DO	Δ DO	Δ DO
20-24 March	1.67	0.64	0.74
27-31 March	0.61	1.96	1.28
3-7 April	0.84	0.62	0.98
10-14 April	0.82	0.68	0.65
17-21 April	0.61	0.69	0.76
24-28 April	0.91	0.68	0.79
1-5 May	1.49	0.69	1.17
8-12 May	0.89	0.73	0.90
15-19 May	0.60	0.65	0.77
22-26 May	0.72	0.75	0.74
29 May-2 June	1.43[1]	0.72	0.59
5-9 June	0.74	0.76	0.77
12-16 June	0.62	0.72	0.91
19-23 June	0.69	0.86	0.94
26-30 June	0.86	0.79	0.64
3-7 July	0.73	0.93	0.53
10-14 July	0.66	1.17	0.78
17-21 July	*	*	*
24-28 July	0.59	0.98	0.11
31 July-4 August	0.59	0.97	0.60
7-11 August	0.57[1]	0.93	1.87
14-18 August	0.58	0.86	0.62
21-25 August	0.43	0.79	1.73
28 August-1 September	0.87	0.93	*
4-8 September	0.75	0.95	1.01
11-15 September	0.98	0.83	0.99
18-22 September	0.71	1.02	0.76
25-29 September	0.93	0.66	0.69
2-6 October	0.73	0.83	1.08
9-13 October	0.76	1.45	1.37
Average Δ	0.81	0.87	0.88

*No data available due to operator error

[1] CO_2 generator was not working in EX1

Table 8. Maximum amounts of diel temperature fluctuations determined from measurements recorded every hour over a 24-h period. Diel sampling of temperature occurred separately for each experimental system (EX1, EX2, and EX3) at the San Marcos National Fish Hatchery and Technology Center once a week. Data were collected using a Hydrolab DataSonde® 4 instrument from March to October 2000.

	EX1	EX2	EX3
Dates	Δ Temp	Δ Temp	Δ Temp
20-24 March	0.68	1.32	0.74
27-31 March	0.70	2.05	0.68
3-7 April	1.59	2.36	0.56
10-14 April	0.50	1.56	0.67
17-21 April	1.17	1.17	0.74
24-28 April	0.45	1.17	0.70
1-5 May	1.72	1.16	0.55
8-12 May	3.49	1.24	0.70
15-19 May	3.12	1.48	0.70
22-26 May	1.77	1.45	0.85
29 May-2 June	2.62	1.29	1.46
5-9 June	0.81	1.48	0.85
12-16 June	1.10	1.41	1.12
19-23 June	1.26	1.80	1.37
26-30 June	0.91	2.32	1.38
3-7 July	1.32	2.87	1.77
10-14 July	1.95	4.12	1.85
17-21 July	*	*	*
24-28 July	1.50	3.80	4.32
31 July-4 August	0.88	3.38	1.11
7-11 August	1.63	3.26	2.53
14-18 August	4.25	2.30	2.25
21-25 August	3.69	3.20	2.39
28 August-1 September	2.32	4.58	2.60
4-8 September	0.94	3.58	4.20
11-15 September	1.29	2.11	0.95
18-22 September	1.89	3.38	0.82
25-29 September	0.74	1.15	1.13
2-6 October	1.48	1.24	0.66
9-13 October	1.63	3.03	3.38[1]
Average Δ	1.63	2.25	1.48

*No data available due to operator error

[1]Power failure occurred on the SMNFH&TC station

Table 9. Average monthly length (mm) and weight (g) measurements of male ($n = 24$) and female ($n = 24$) *Eurycea nana* in the three recirculation systems housed at the San Marcos National Fish Hatchery and Technology Center. There were no significant differences ($P > 0.05$) between salamanders exposed to the low (EX1), medium (EX2), or high (EX3) amounts of diel pH fluctuations in length or weight within a gender. Data were recorded over a 9-month period from 12 January to 12 October 2000.

	EX1				EX2				EX3			
Day	$\bar{x}$ ♂ L	$\bar{x}$ ♀ L	$\bar{x}$ ♂ W	$\bar{x}$ ♀ W	$\bar{x}$ ♂ L	$\bar{x}$ ♀ L	$\bar{x}$ ♂ W	$\bar{x}$ ♀ W	$\bar{x}$ ♂ L	$\bar{x}$ ♀ L	$\bar{x}$ ♂ W	$\bar{x}$ ♀ W
1	73.0	72.4	1.03	1.22	73.5	71.3	0.99	1.07	71.1	72.1	0.96	1.07
36	73.5	71.6	1.12	1.17	74.4	70.6	1.12	1.09	71.8	72.5	1.05	1.14
64	74.3	71.9	1.19	1.18	74.6	69.8	1.17	1.14	74.4	72.1	1.17	1.18
94	75.5	72.3	1.22	1.26	75.9	70.3	1.17	1.15	74.5	72.3	1.17	1.14
122	75.5	72.6	1.25	1.24	76.5	70.6	1.24	1.19	75.3	72.8	1.24	1.13
153	76.4	73.5	1.26	1.23	76.9	71.3	1.17	1.12	75.9	73.3	1.17	1.11
181	76.4	73.9	1.23	1.24	77.0	72.1	1.16	1.12	76.3	73.8	1.16	1.18
218	77.1	74.3	1.26	1.31	77.4	73.0	1.21	1.27	76.5	74.0	1.21	1.17
248	77.3	74.6	1.29	1.38	77.9	73.8	1.29	1.31	77.1	74.9	1.29	1.26
276	78.3	74.4	1.34	1.41	78.6	74.4	1.32	1.38	78.4	75.3	1.34	1.35

Table 10. Average monthly length (mm) and weight (g) measurements of male ($n = 24$) and female ($n = 24$) *Eurycea nana* housed in aquaria equipped with upwelling-flow tubes and in aquaria without upwelling flow at the San Marcos National Fish Hatchery and Technology Center. Data were recorded over a 9-month period from 12 January to 12 October 2000.

	Non-upwelling				Upwelling			
Day	$\bar{x}$ ♂ L	$\bar{x}$ ♀ L	$\bar{x}$ ♂ W	$\bar{x}$ ♀ W	$\bar{x}$ ♂ L	$\bar{x}$ ♀ L	$\bar{x}$ ♂ W	$\bar{x}$ ♀ W
1	72.0	72.5	0.98	1.16	73.1	71.3	1.00	1.09
36	72.7	72.3	1.07	1.19	73.8	70.9	1.09	1.07
64	74.4	71.9	1.19	1.21	74.4	70.6	1.16	1.10
94	75.0	72.2	1.20	1.16	75.6	71.0	1.22	1.20
122	75.3	72.8	1.24	1.19	76.2	71.3	1.27	1.18
153	75.8	73.5	1.19	1.17	76.9	71.8	1.25	1.13
181	75.8	73.7	1.18	1.18	77.3	72.8	1.25	1.18
218	76.3	74.1	1.22	1.23	77.8	73.4	1.29	1.28
248	76.6	74.8	1.25	1.29	78.3	74.0	1.38	1.34
276	78.3	74.8	1.25*	1.36	79.5	74.5	1.45*	1.36

*Males housed in aquaria equipped with upwelling-flow tubes were significantly greater in weight ($P < 0.05$) than those without access to upwelling flow during the last month of the study.

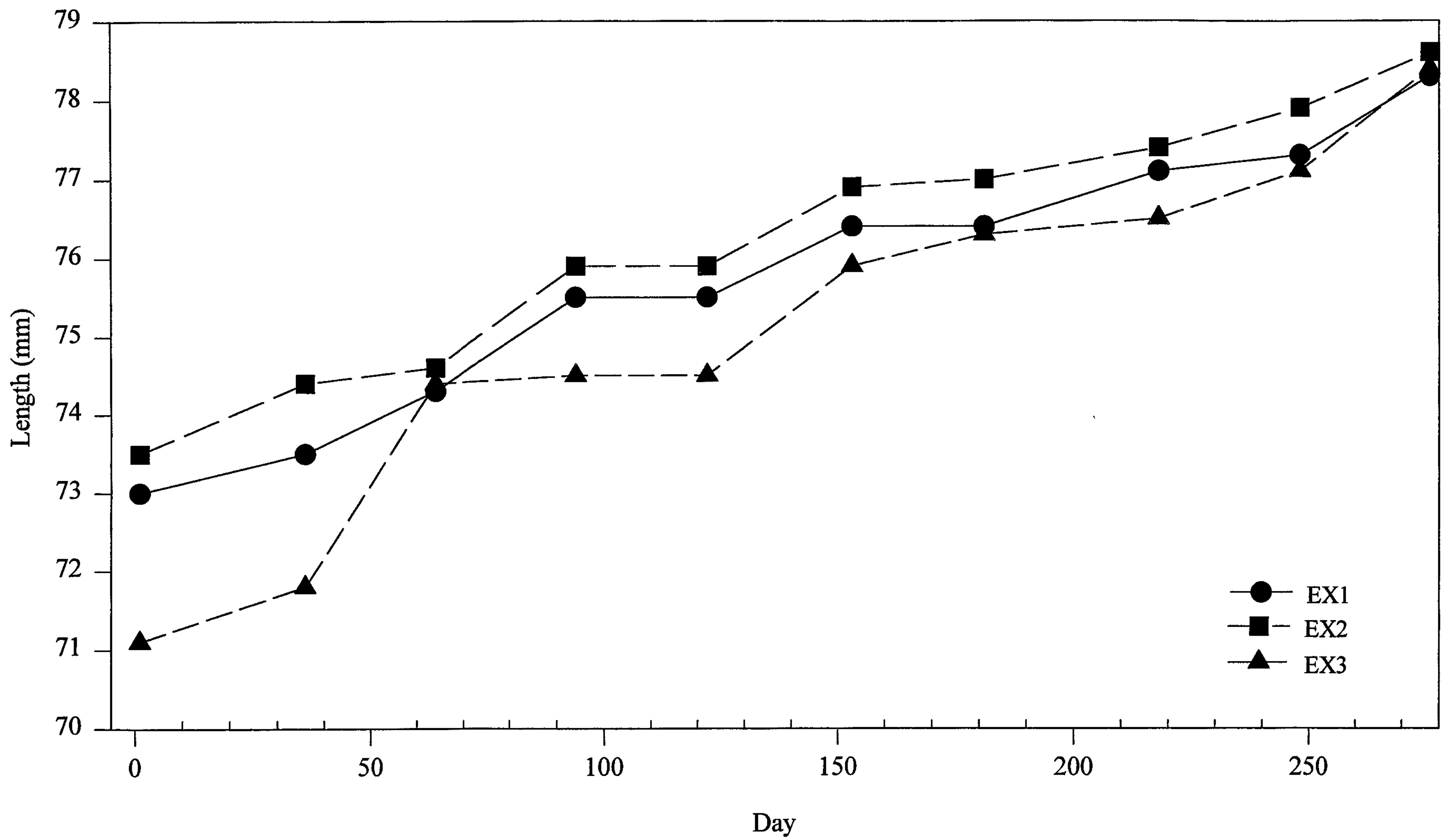

Figure 2. Average lengths of male *Eurycea nana* recorded monthly over a 9-month period in three recirculation systems (EX1, EX2, and EX3) housed at the San Marcos National Fish Hatchery and Technology Center.

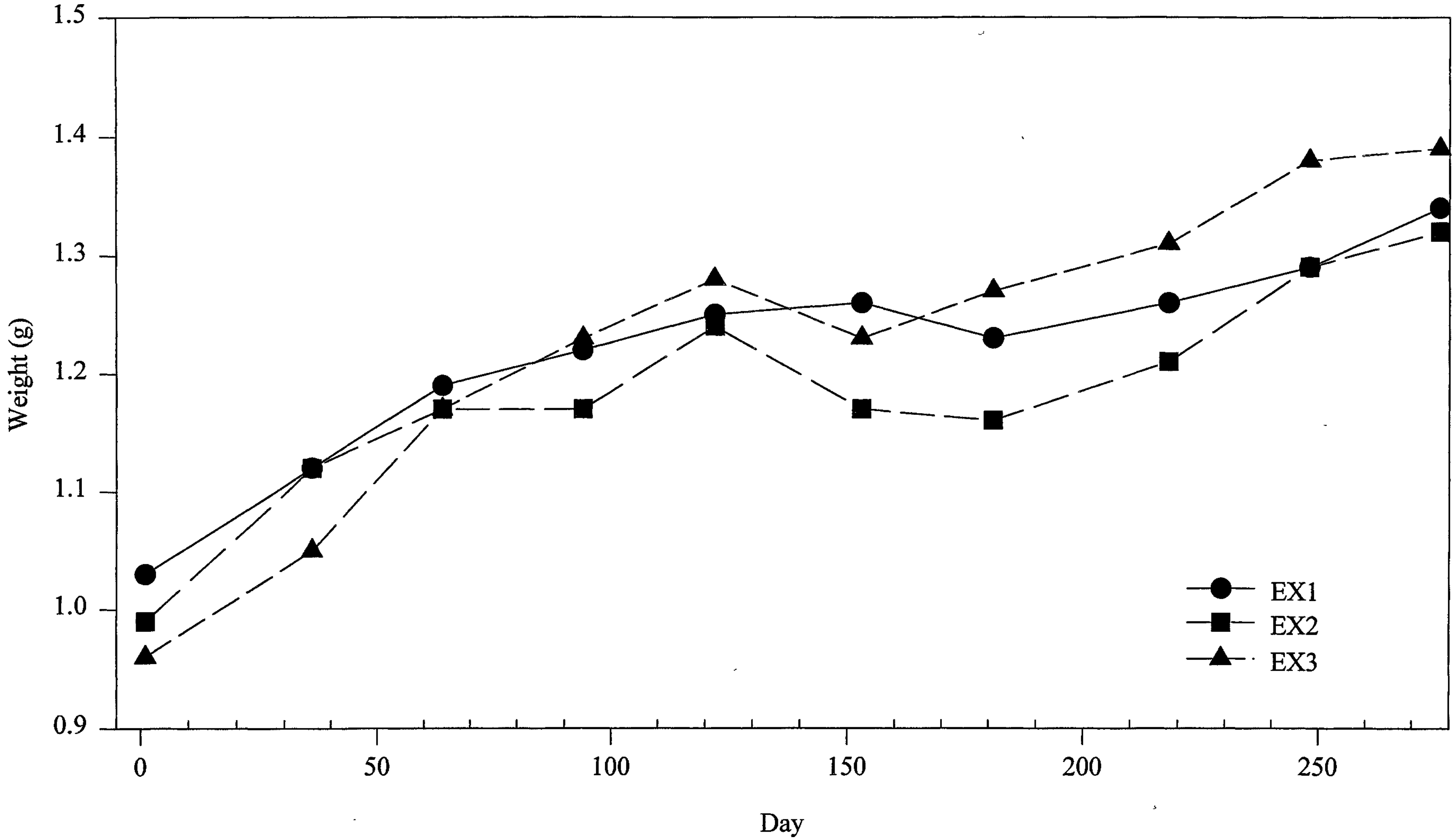

Figure 3. Average weights of male *Eurycea nana* recorded monthly over a 9-month period in three recirculation systems (EX1, EX2, and EX3) housed at the San Marcos National Fish Hatchery and Technology Center.

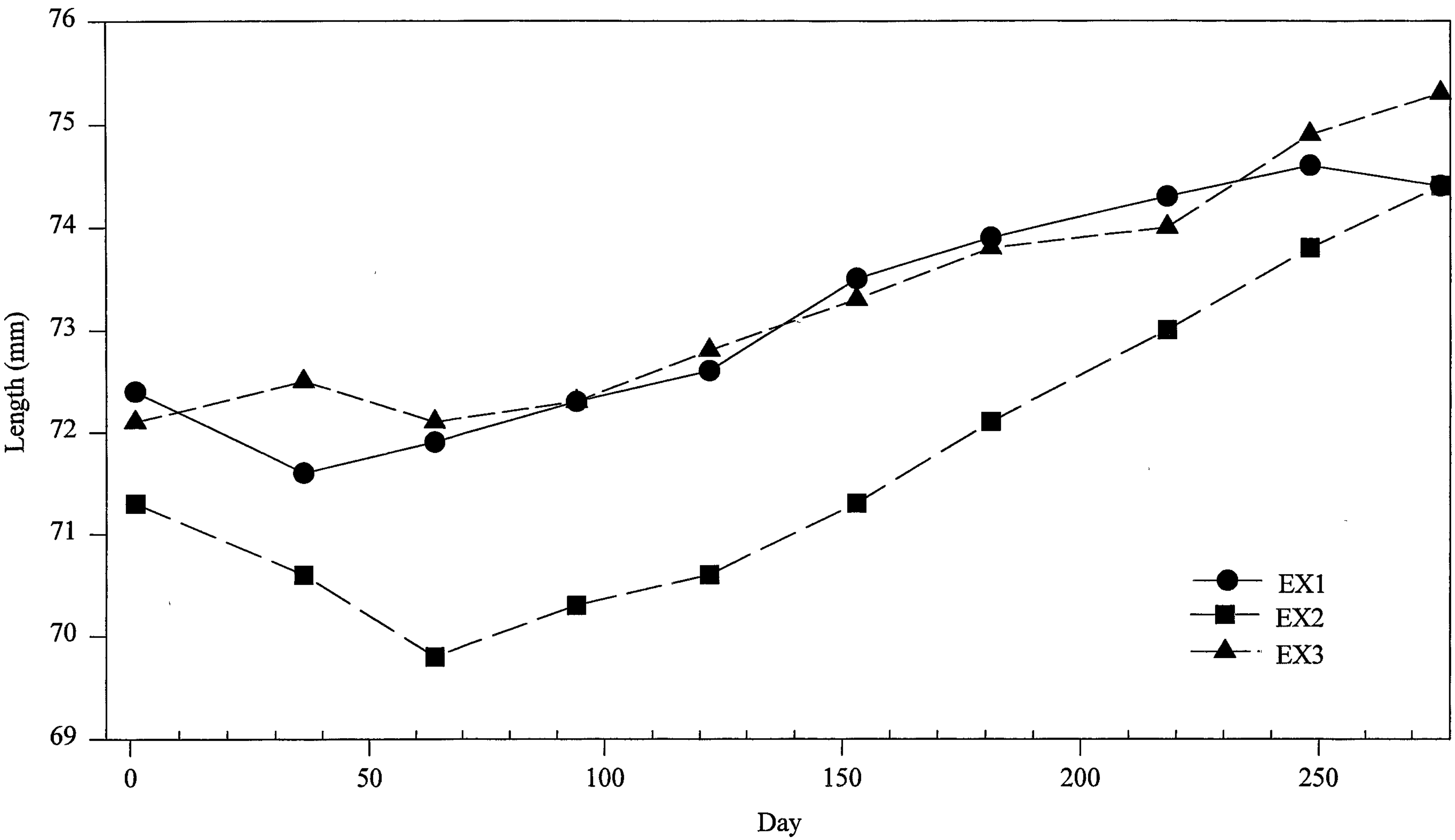

Figure 4. Average lengths of female *Eurycea nana* recorded monthly over a 9-month period in three recirculation systems (EX1, EX2, and EX3) housed at the San Marcos National Fish Hatchery and Technology Center.

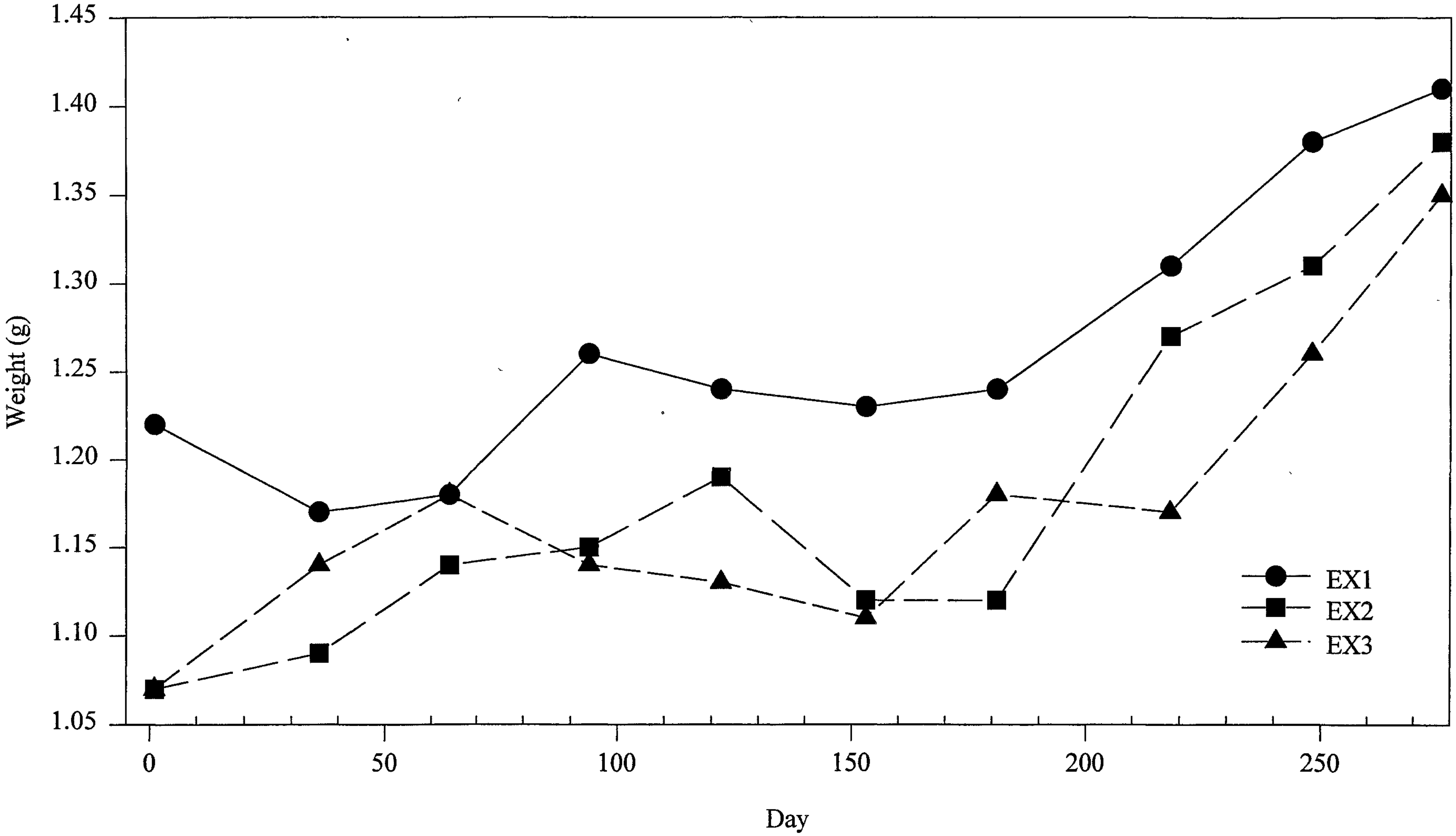

Figure 5. Average weights of female *Eurycea nana* recorded monthly over a 9-month period in three recirculation systems (EX1, EX2, and EX3) housed at the San Marcos National Fish Hatchery and Technology Center.

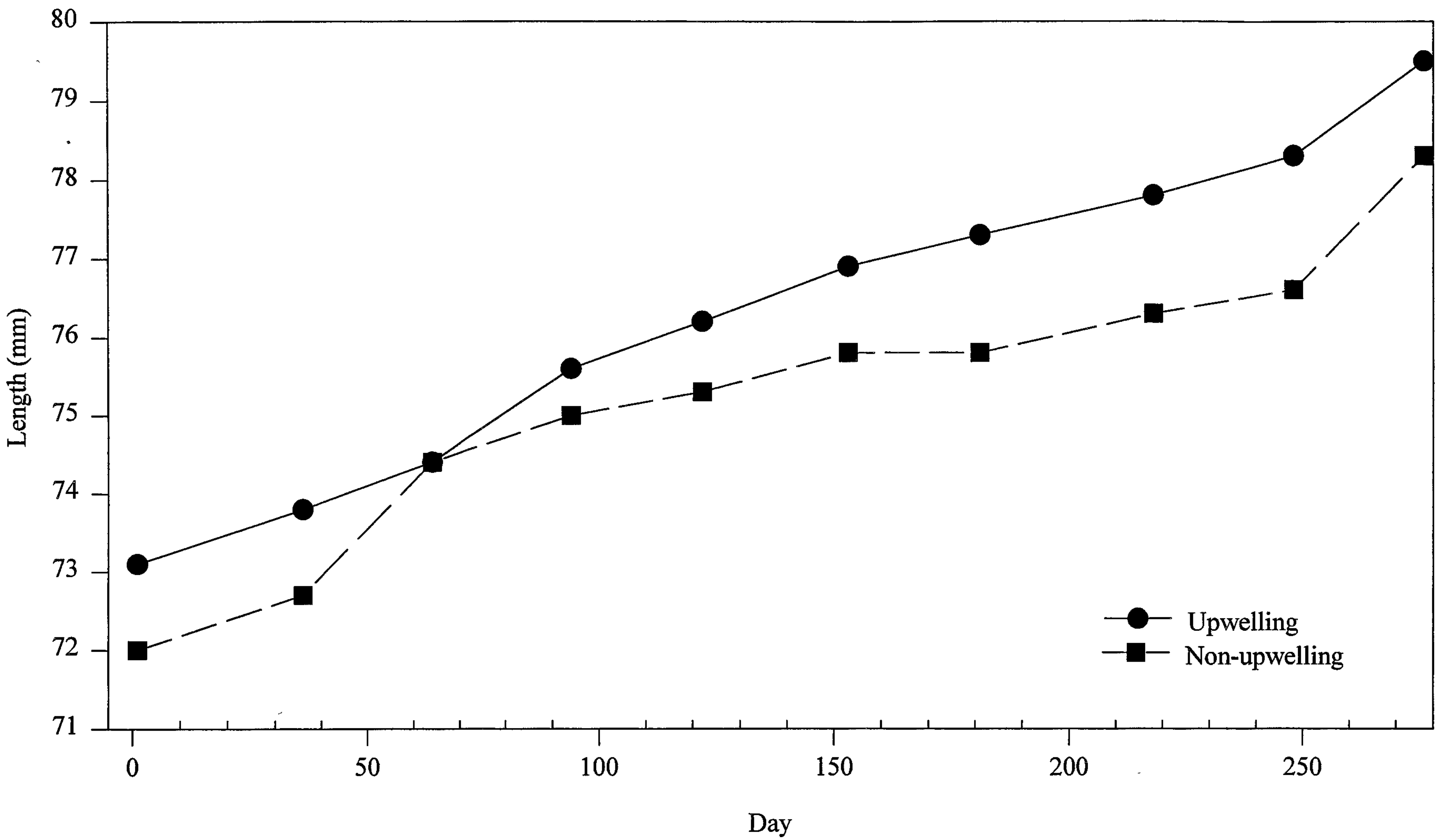

Figure 6. The average lengths for male *Eurycea nana* recorded monthly over a 9-month period in aquaria equipped with upwelling flow and aquaria without upwelling flow at the San Marcos National Fish Hatchery and Technology Center.

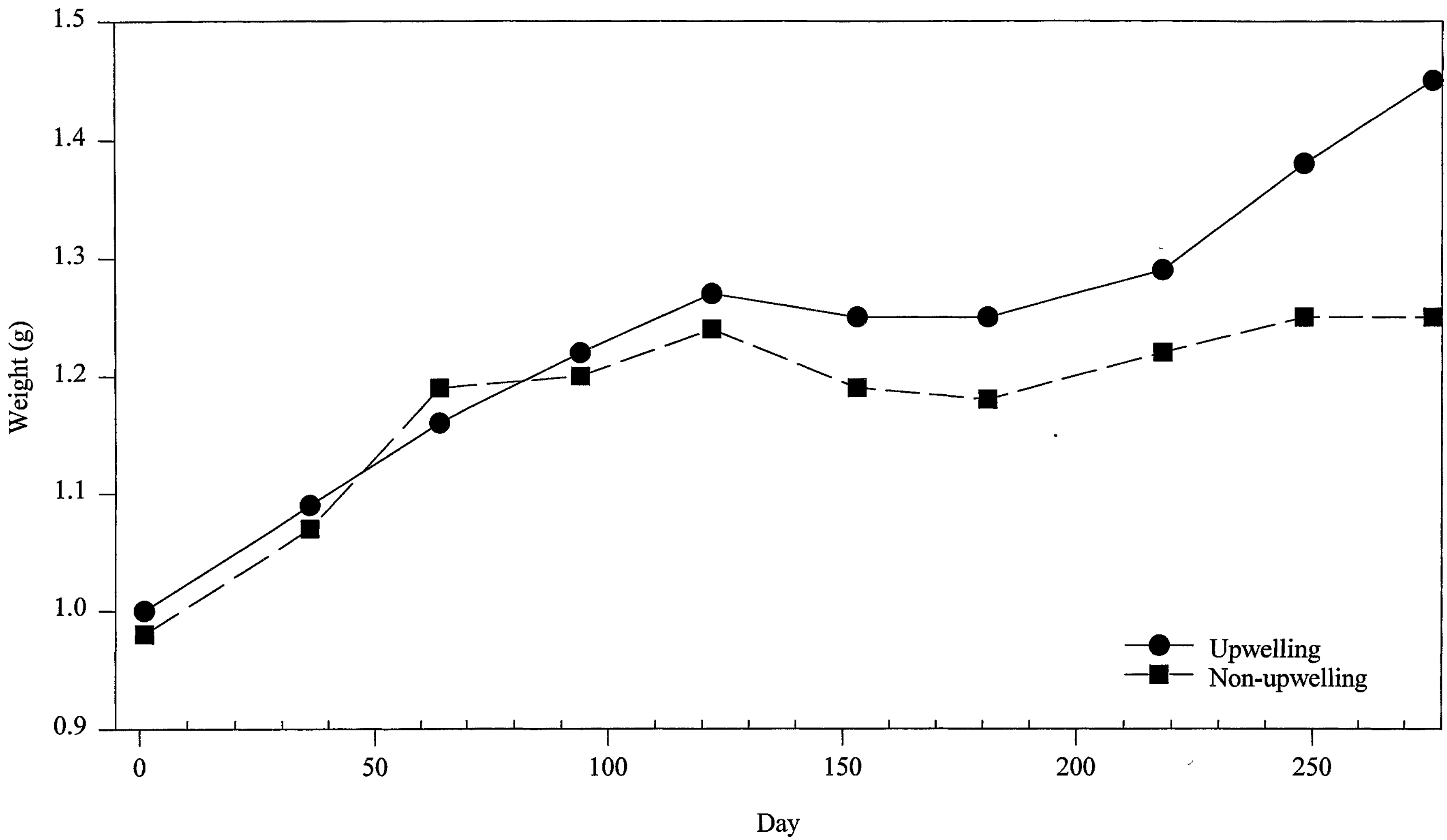

Figure 7. The average weights for male *Eurycea nana* recorded monthly over a 9-month period in aquaria equipped with upwelling flow and aquaria without upwelling flow at the San Marcos National Fish Hatchery and Technology Center.

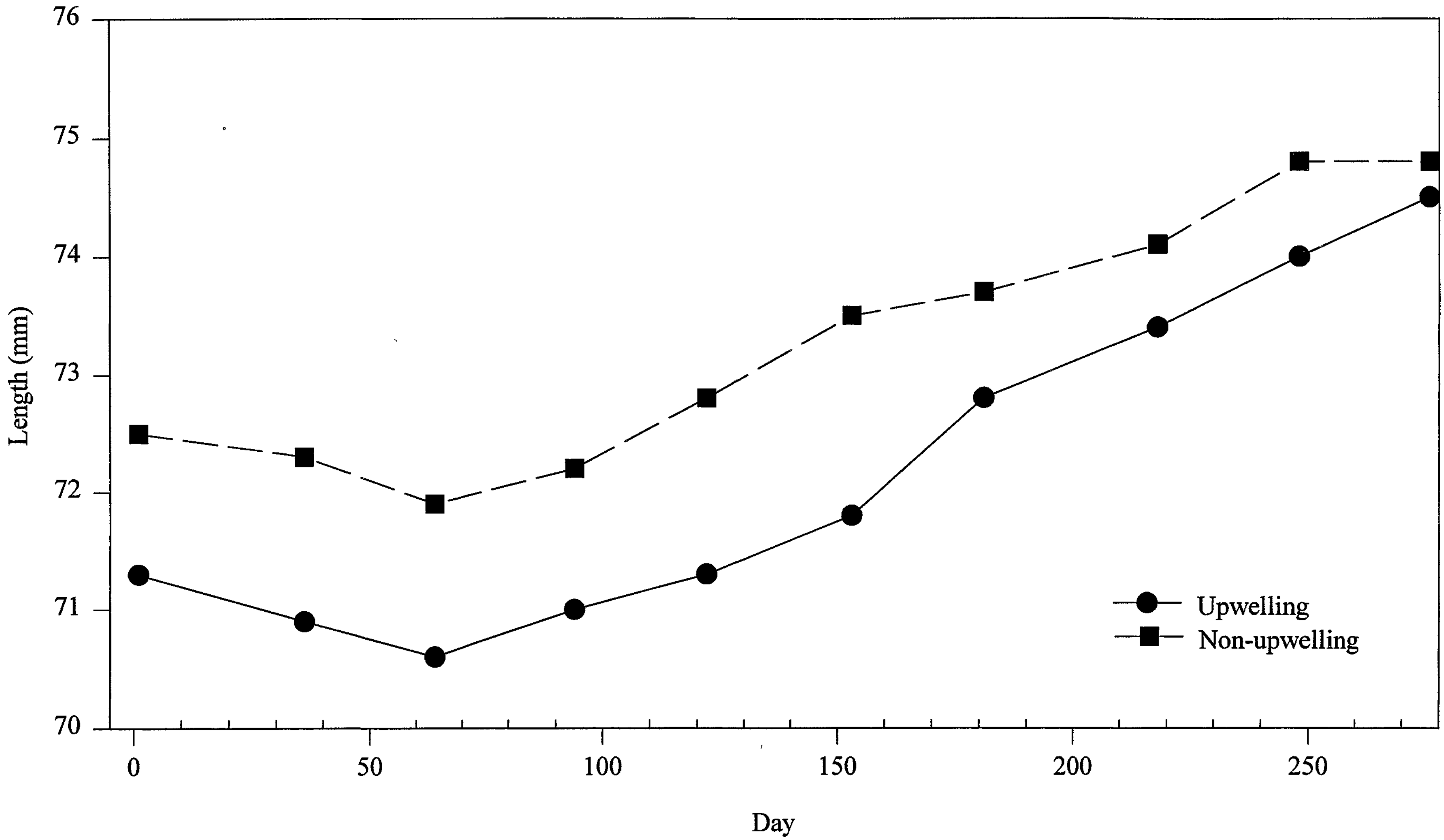

Figure 8. The average lengths for female *Eurycea nana* recorded monthly over a 9-month period in aquaria equipped with upwelling flow and aquaria without upwelling flow at the San Marcos National Fish Hatchery and Technology Center.

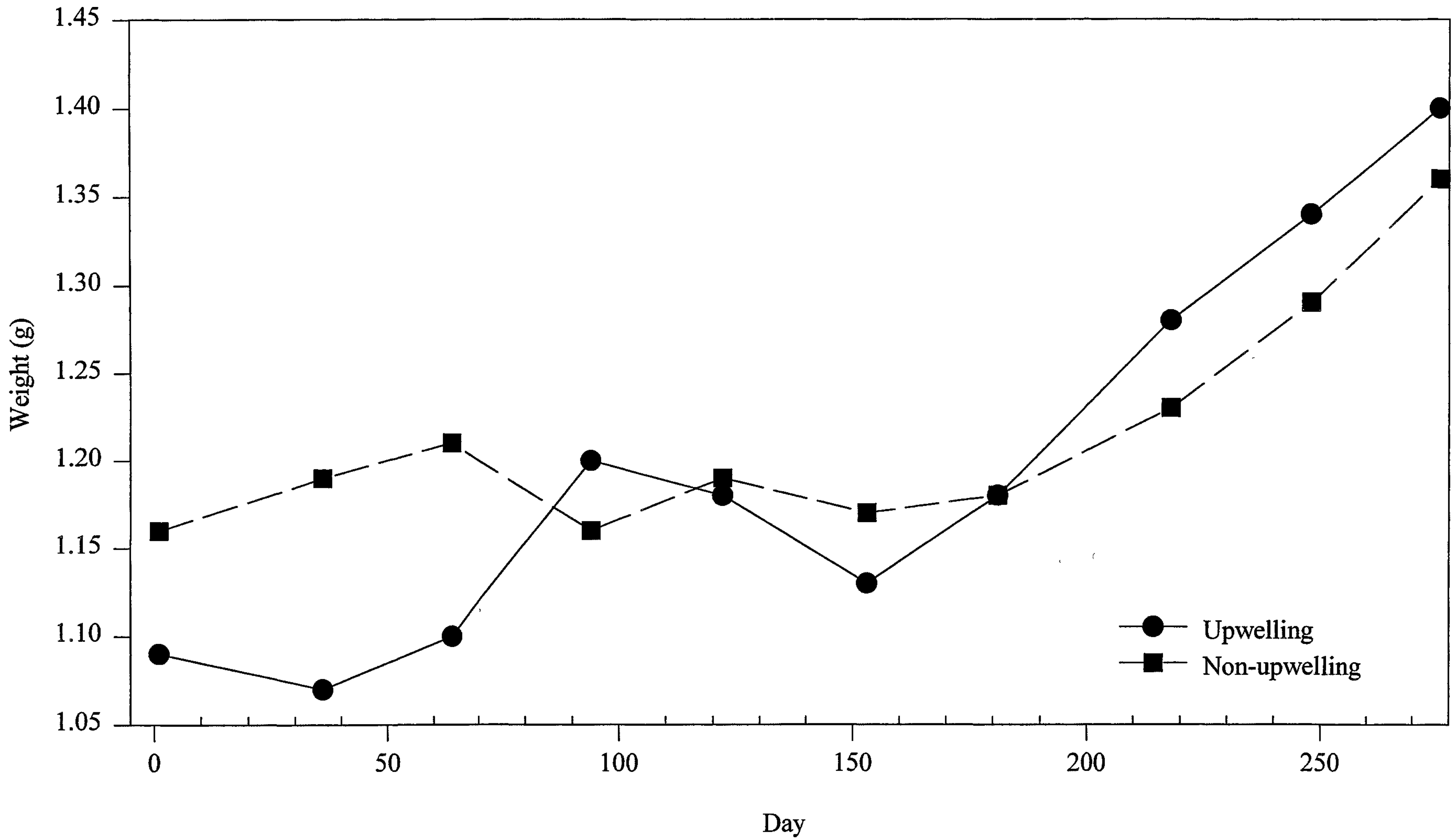

Figure 9. The average weights of female *Eurycea nana* recorded monthly over a 9-month period in aquaria equipped with upwelling flow and aquaria without upwelling flow at the San Marcos National Fish Hatchery and Technology Center.

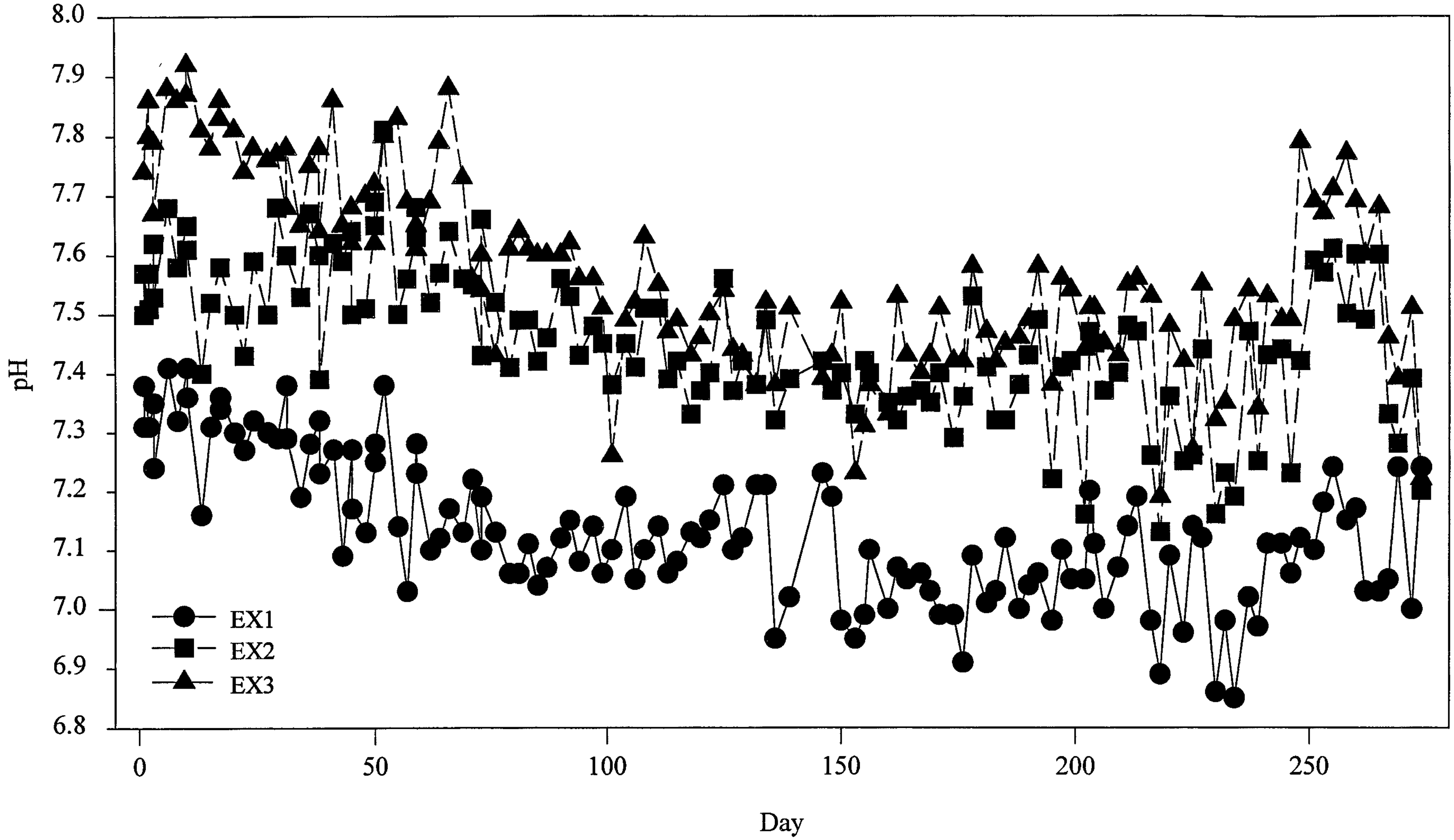

Figure 10. Values of pH recorded three times weekly over a 9-month period in three experimental systems (EX1, EX2, and EX3) housed at the San Marcos National Fish Hatchery and Technology Center. Data recorded from 12 January to 12 October 2000.

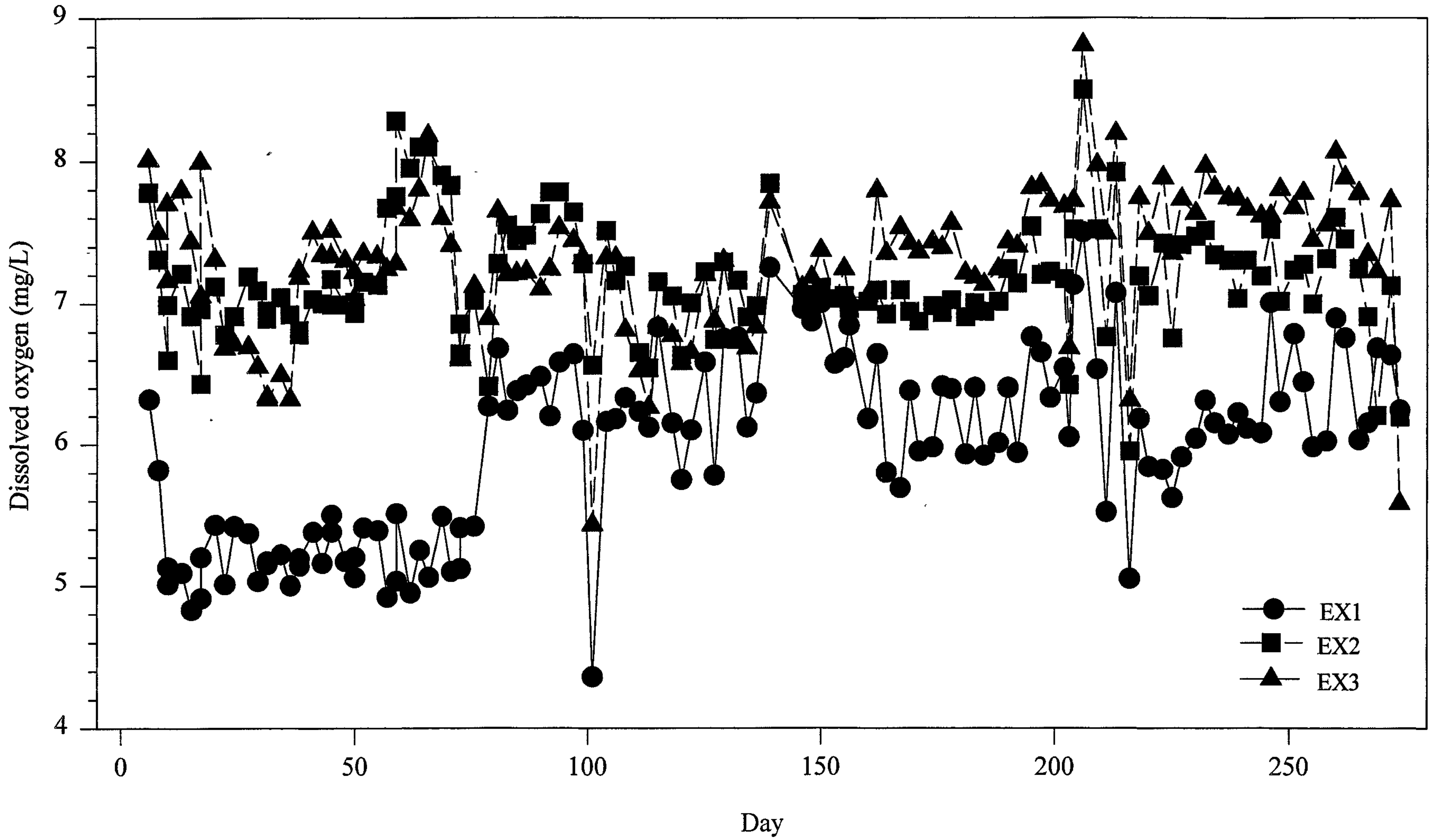

Figure 11. Dissolved oxygen values recorded three times weekly over a 9-month period in the three experimental systems (EX1, EX2, and EX3) at the San Marcos National Fish Hatchery and Technology Center. Data recorded from 12 January to 12 October 2000.

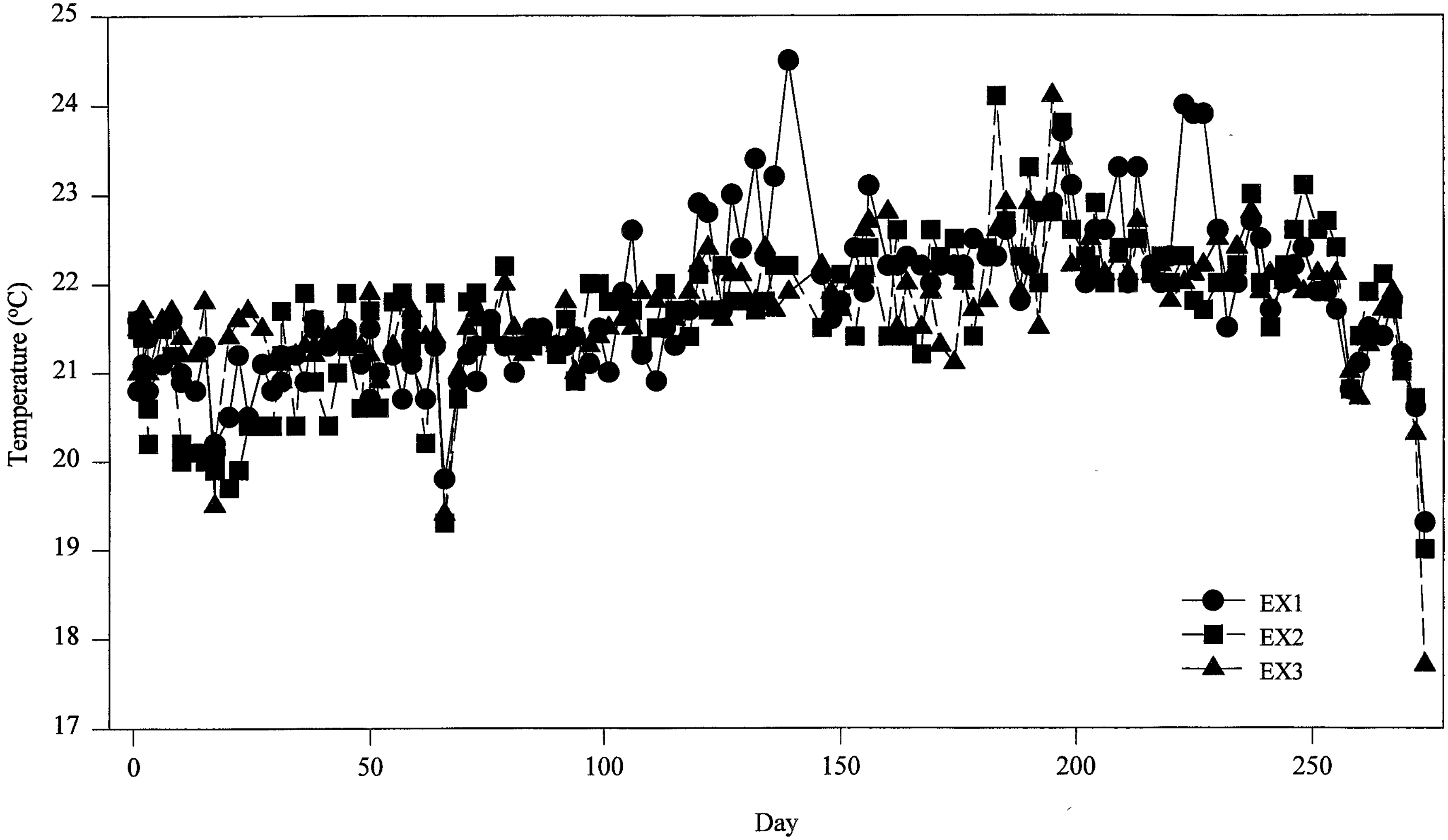

Figure 12. Temperature values recorded three times weekly over a 9-month period in the three recirculation systems at the San Marcos National Fish Hatchery and Technology Center. Data were collected from 12 January to 12 October 2000.

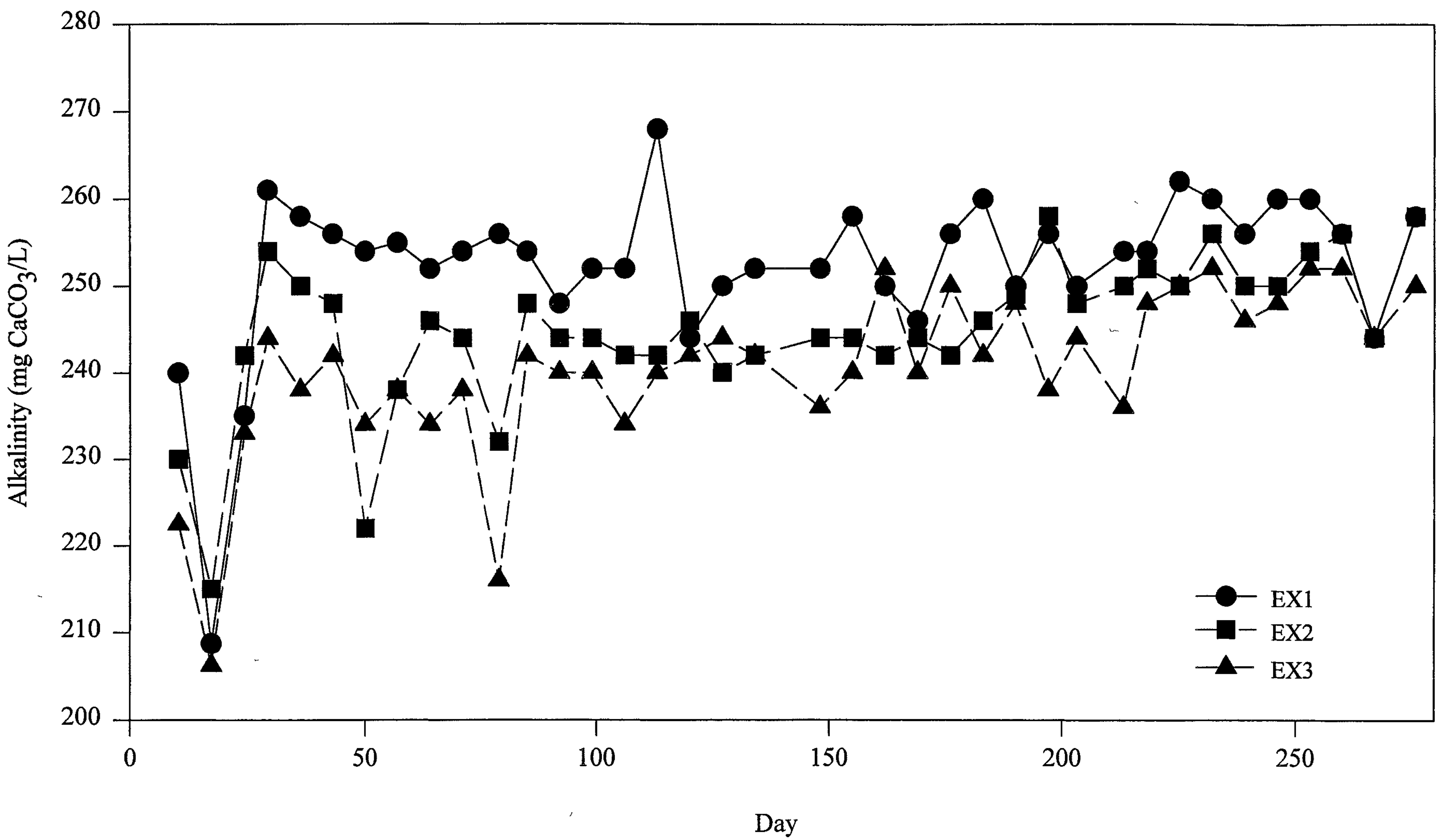

Figure 13. Alkalinity values recorded weekly over a 9-month period in three recirulation systems (EX1, EX2, and EX3) housed at the San Marcos National Fish Hatchery and Technology Center. Data were recorded from 12 January to 12 October 2000.

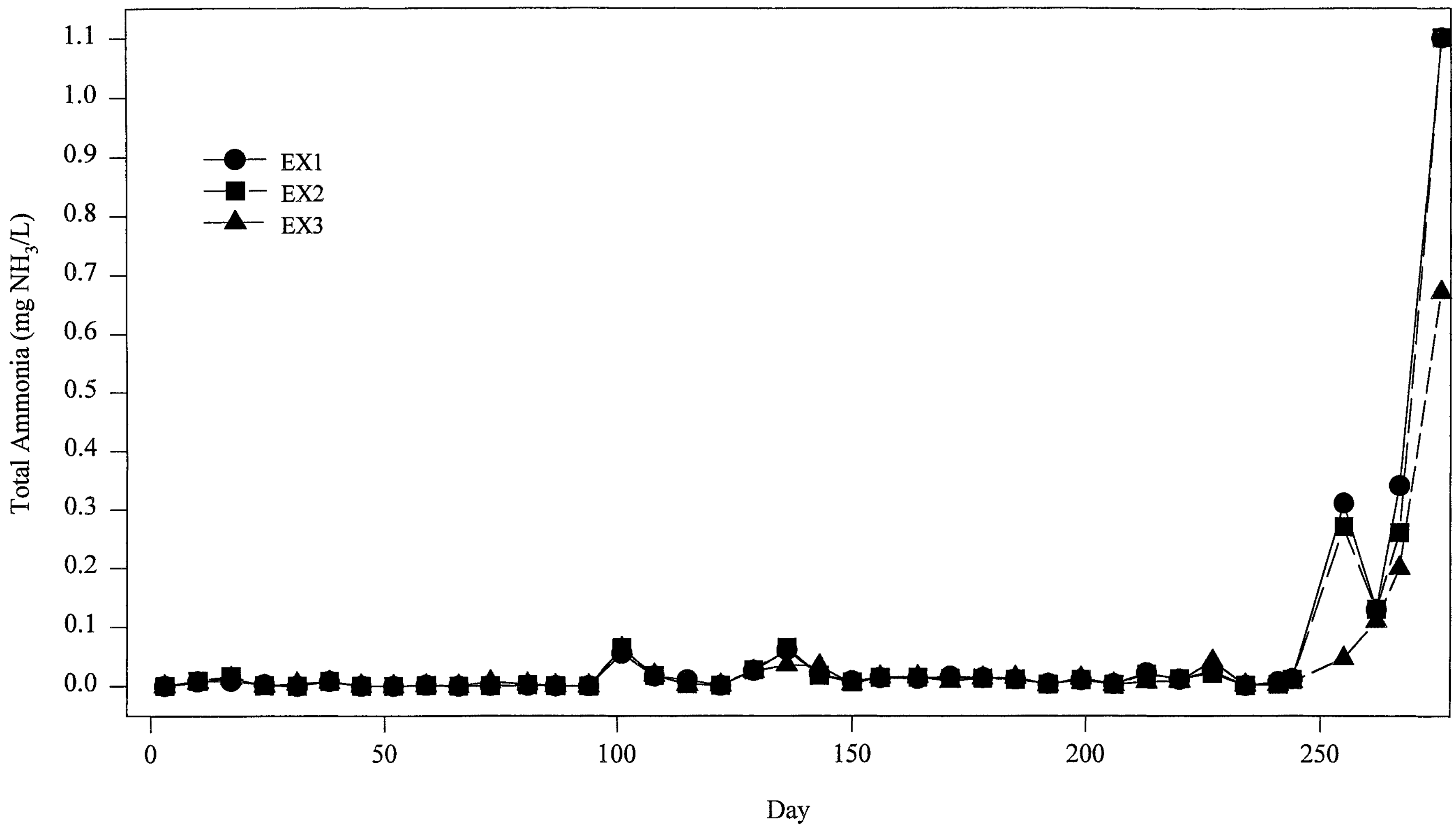

Figure 14. Total ammonia values recorded weekly over a 9-month period in three recirculation systems (EX1, EX2, and EX3) housed at the San Marcos National Fish Hatchery and Technology Center. Data were collected from 12 January to 12 October 2000.

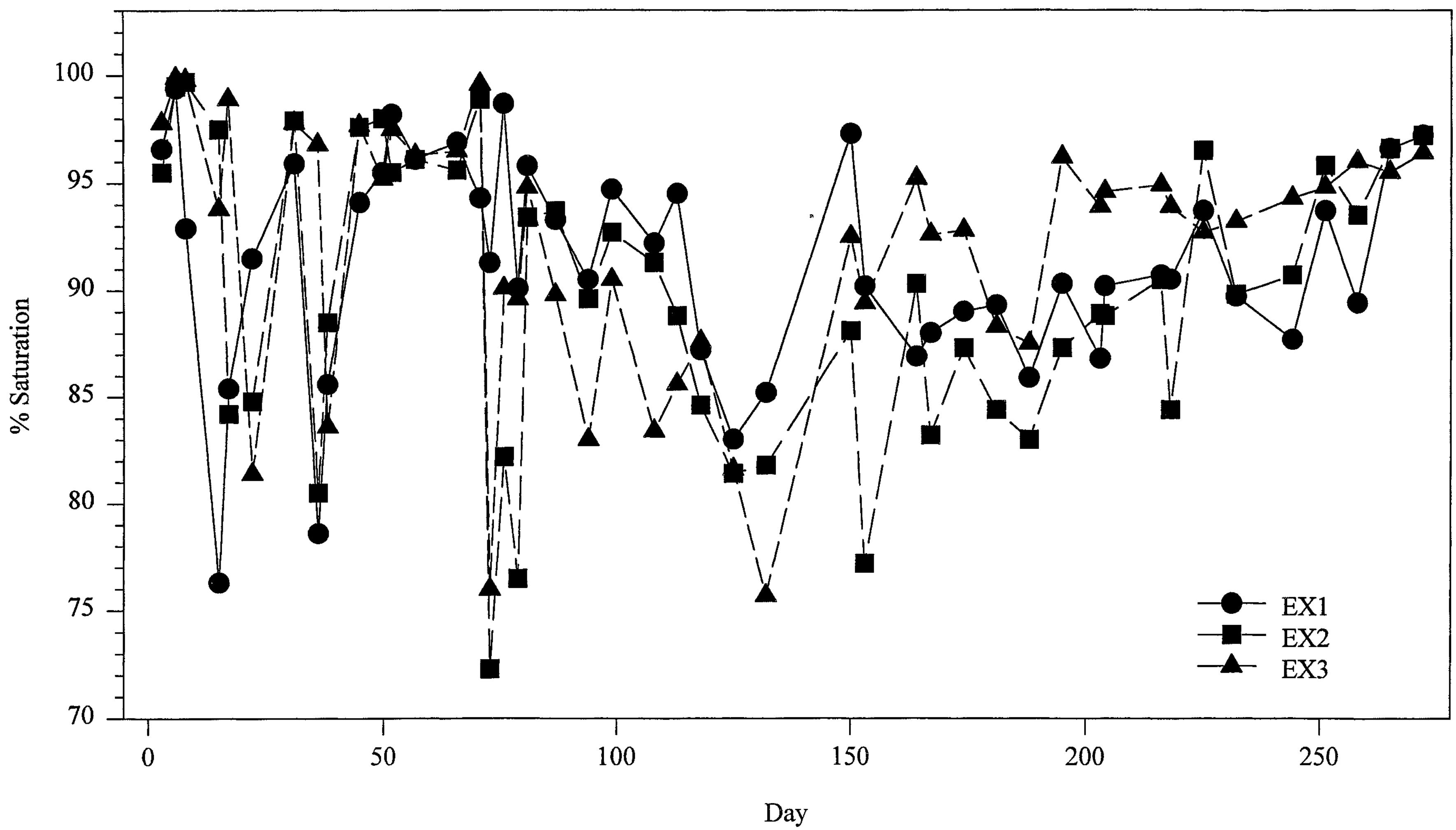

Figure 15. Percent saturation of gases recorded weekly over a 9-month period in three recirculation systems (EX1, EX2, and EX3) housed at the San Marcos National Fish Hatchery and Technology Center. Data were recorded from 12 January to 12 October 2000.

APPENDIX 1

Length and weight measurements of *Eurycea nana* in experimental aquaria recorded approximately every 30 days from 12 January to 16 October 2000 at the San Marcos National Fish Hatchery and Technology Center

Appendix 1. Length and weight measurements of *Eurycea nana* in experimental aquaria recorded approximately every 30 days from 12 January to 16 October 2000 at the San Marcos National Fish Hatchery and Technology Center.

		Male		Female	
Date	Aquarium Number	Length (mm)	Weight (g)	Length (mm)	Weight (g)
12 January	EX1-3	70	1.03	72	1.14
12 January	EX1-4	77	1.31	72	1.38
12 January	EX1-5	71	0.91	68	1.22
12 January	EX1-6	77	1.05	72	0.97
12 January	EX1-7	73	0.96	68	1.14
12 January	EX1-8	68	0.89	71	1.32
12 January	EX1-9	78	1.10	76	1.16
12 January	EX1-10	70	1.00	80	1.44
12 January	EX2-3	75	1.08	78	1.25
12 January	EX2-4	73	0.96	70	0.91
12 January	EX2-5	69	0.83	70	1.05
12 January	EX2-6	73	0.83	70	0.94
12 January	EX2-7	67	0.73	76	1.14
12 January	EX2-8	73	0.96	78	1.25
12 January	EX2-9	74	1.22	63	0.85
12 January	EX2-10	84	1.27	65	1.20
12 January	EX3-3	74	1.18	74	1.02
12 January	EX3-4	72	0.85	74	1.18
12 January	EX3-5	70	0.96	70	1.10
12 January	EX3-6	76	1.06	66	0.79
12 January	EX3-7	65	0.63	72	0.97
12 January	EX3-8	75	0.87	67	0.87
12 January	EX3-9	68	1.01	76	1.35
12 January	EX3-10	69	1.12	78	1.26
16 February	EX1-3	73	1.30	71	1.05
16 February	EX1-4	77	1.27	71	1.31
16 February	EX1-5	70	1.04	68	1.14
16 February	EX1-6	78	1.12	71	1.05
16 February	EX1-7	73	1.13	67	1.04
16 February	EX1-8	69	0.99	71	1.29
16 February	EX1-9	78	1.14	75	1.08
16 February	EX1-10	70	0.99	79	1.36
16 February	EX2-3	76	1.05	77	1.30
16 February	EX2-4	74	1.11	68	0.85

Appendix 1. Continued.

Date	Aquarium Number	Male Length (mm)	Male Weight (g)	Female Length (mm)	Female Weight (g)
16 February	EX2-5	70	1.00	70	0.93
16 February	EX2-6	74	0.98	69	0.89
16 February	EX2-7	69	1.00	77	1.37
16 February	EX2-8	74	1.06	78	1.36
16 February	EX2-9	74	1.31	61	0.81
16 February	EX2-10	84	1.42	65	1.23
16 February	EX3-3	74	1.16	74	1.24
16 February	EX3-4	73	0.98	73	1.09
16 February	EX3-5	70	1.03	73	1.22
16 February	EX3-6	76	1.09	66	0.78
16 February	EX3-7	65	0.73	71	1.03
16 February	EX3-8	76	1.02	66	1.00
16 February	EX3-9	70	1.08	77	1.47
16 February	EX3-10	70	1.28	80	1.29
15 March	EX1-3	74	1.49	72	1.10
15 March	EX1-4	78	1.45	72	1.40
15 March	EX1-5	71	1.04	67	1.25
15 March	EX1-6	78	1.08	72	0.91
15 March	EX1-7	74	1.16	67	1.00
15 March	EX1-8	70	1.06	71	1.27
15 March	EX1-9	78	1.20	75	1.23
15 March	EX1-10	71	1.05	79	1.30
15 March	EX2-3	76	1.11	77	1.41
15 March	EX2-4	75	1.26	63	0.89
15 March	EX2-5	70	0.96	70	1.00
15 March	EX2-6	74	1.02	70	0.94
15 March	EX2-7	70	1.04	75	1.31
15 March	EX2-8	74	1.11	78	1.38
15 March	EX2-9	74	1.26	60	0.84
15 March	EX2-10	84	1.61	65	1.34
15 March	EX3-3	75	1.24	74	1.27
15 March	EX3-4	74	1.07	71	1.10
15 March	EX3-5	71	1.03	72*	1.44*
15 March	EX3-5	--	--	74[1]	1.15[1]
15 March	EX3-6	77	1.20	65	0.80
15 March	EX3-7	80[2]	1.21[2]	71	1.11
15 March	EX3-8	77	1.05	67	1.00

Appendix 1. Continued.

Date	Aquarium Number	Male Length (mm)	Male Weight (g)	Female Length (mm)	Female Weight (g)
15 March	EX3-9	71	1.26	77	1.49
15 March	EX3-10	70	1.31	78	1.27
14 April	EX1-3	75	1.36	73	1.33
14 April	EX1-4	79	1.44	72	1.66
14 April	EX1-5	73	1.08	68	1.26
14 April	EX1-6	79	1.28	72	1.27
14 April	EX1-7	75	1.14	67	1.00
14 April	EX1-8	72	1.07	71	1.24
14 April	EX1-9	79	1.24	75	1.23
14 April	EX1-10	72	1.15	80	1.08
14 April	EX2-3	79[3]	1.18[3]	78	1.51
14 April	EX2-4	76	1.28	65	0.97
14 April	EX2-5	71	1.02	70	1.11
14 April	EX2-6	76	1.03	70	1.06
14 April	EX2-7	70	0.97	75	1.24
14 April	EX2-8	75	1.18	78	1.42
14 April	EX2-9	75	1.23	61	0.75
14 April	EX2-10	85	1.49	65	1.16
14 April	EX3-3	76	1.33	74	1.32
14 April	EX3-4	75	1.22	71	0.99
14 April	EX3-5	71	1.10	74[1]	1.16[1]
14 April	EX3-6	77	1.28	65	0.80
14 April	EX3-7	80[2]	1.30[2]	72	1.03
14 April	EX3-8	77	1.12	67	0.98
14 April	EX3-9	70	1.17	77	1.54
14 April	EX3-10	70	1.33	78	1.29
12 May	EX1-3	76	1.28	73	1.15
12 May	EX1-4	79	1.44	73	1.61
12 May	EX1-5	72	1.21	67	1.16
12 May	EX1-6	80	1.30	71	1.10
12 May	EX1-7	76	1.23	68	1.16
12 May	EX1-8	70	1.02	73	1.22
12 May	EX1-9	79	1.30	75	1.19
12 May	EX1-10	72	1.19	81	1.36
12 May	EX2-3	80[3]	1.26[3]	79	1.46
12 May	EX2-4	77	1.35	65	1.12
12 May	EX2-5	72	1.22	71	1.19

Appendix 1. Continued.

Date	Aquarium Number	Male Length (mm)	Male Weight (g)	Female Length (mm)	Female Weight (g)
12 May	EX2-6	76	1.09	70	1.00
12 May	EX2-7	70	0.96	76	1.19
12 May	EX2-8	77	1.16	78	1.50
12 May	EX2-9	76	1.37	62	0.87
12 May	EX2-10	84	1.51	64	1.20
12 May	EX3-3	76	1.31	74	1.24
12 May	EX3-4	76	1.24	71	1.00
12 May	EX3-5	72	1.18	75[1]	1.21[1]
12 May	EX3-6	78	1.39	66	0.95
12 May	EX3-7	82[2]	1.52[2]	72	0.97
12 May	EX3-8	77	1.09	67	1.01
12 May	EX3-9	71	1.08	77	1.36
12 May	EX3-10	70	1.39	80	1.28
12 June	EX1-3	76	1.36	73	1.13
12 June	EX1-4	80	1.55	74	1.56
12 June	EX1-5	75	1.15	68	1.08
12 June	EX1-6	80	1.36	72	1.03
12 June	EX1-7	76	1.22	71	1.20
12 June	EX1-8	70	0.97	72	1.31
12 June	EX1-9	80	1.27	76	1.11
12 June	EX1-10	74	1.20	82	1.39
12 June	EX2-3	80[3]	1.29[3]	80	1.46
12 June	EX2-4	78	1.36	66	1.01
12 June	EX2-5	72	1.04	71	1.03
12 June	EX2-6	77	0.97	70	0.95
12 June	EX2-7	71	1.01	76	1.13
12 June	EX2-8	77	1.08	78	1.49
12 June	EX2-9	75	1.25	64	0.72
12 June	EX2-10	85	1.39	65	1.18
12 June	EX3-3	78	1.26	75	1.23
12 June	EX3-4	76	1.15	71	1.03
12 June	EX3-5	72	1.13	75[1]	1.19[1]
12 June	EX3-6	79	1.38	67	0.88
12 June	EX3-7	83[2]	1.33[2]	72	0.97
12 June	EX3-8	78	1.11	69	1.02
12 June	EX3-9	71	1.07	77	1.36
12 June	EX3-10	70	1.42	80	1.19

Appendix 1. Continued.

Date	Aquarium Number	Male Length (mm)	Male Weight (g)	Female Length (mm)	Female Weight (g)
10 July	EX1-3	77	1.37	74	1.26
10 July	EX1-4	80	1.51	75	1.67
10 July	EX1-5	74	1.21	70	1.13
10 July	EX1-6	80	1.27	72	1.10
10 July	EX1-7	76	1.24	71	1.29
10 July	EX1-8	70	0.95	72	1.20
10 July	EX1-9	80	1.21	76	1.06
10 July	EX1-10	74	1.06	81	1.18
10 July	EX2-3	81[3]	1.28[3]	80	1.47
10 July	EX2-4	79	1.35	69	0.85
10 July	EX2-5	72	1.00	71	1.04
10 July	EX2-6	77	0.99	72	1.01
10 July	EX2-7	71	0.93	75	1.06
10 July	EX2-8	76	1.06	79	1.53
10 July	EX2-9	75	1.22	66	0.95
10 July	EX2-10	85	1.42	65	1.05
10 July	EX3-3	77	1.32	74	1.35
10 July	EX3-4	77	1.18	73	1.11
10 July	EX3-5	73	1.12	77[1]	1.30[1]
10 July	EX3-6	80	1.40	67	0.86
10 July	EX3-7	83[2]	1.43[2]	73	0.92
10 July	EX3-8	78	1.16	69	1.11
10 July	EX3-9	72	1.11	78	1.50
10 July	EX3-10	70	1.40	79	1.28
16 August	EX1-3	79	1.39	76	1.43
16 August	EX1-4	80	1.36	76	1.66
16 August	EX1-5	74	1.23	68	1.19
16 August	EX1-6	81	1.40	73	1.12
16 August	EX1-7	77	1.22	72	1.26
16 August	EX1-8	71	1.05	73	1.39
16 August	EX1-9	80	1.34	75	1.12
16 August	EX1-10	75	1.06	81	1.33
16 August	EX2-3	82[3]	1.42[3]	81	1.69
16 August	EX2-4	78	1.40	66	1.05
16 August	EX2-5	73	0.96	73	1.21
16 August	EX2-6	77	1.08	74	1.20
16 August	EX2-7	71	1.07	77	1.21

Appendix 1. Continued.

Date	Aquarium Number	Male Length (mm)	Male Weight (g)	Female Length (mm)	Female Weight (g)
16 August	EX2-8	77	1.08	80	1.80
16 August	EX2-9	76	1.16	68	1.06
16 August	EX2-10	85	1.48	65	0.96
16 August	EX3-3	77	1.33	75	1.27
16 August	EX3-4	77	1.15	73	1.20
16 August	EX3-5	75	1.31	77[1]	1.30[1]
16 August	EX3-6	80	1.46	69	1.01
16 August	EX3-7	83[2]	1.41[2]	72	0.86
16 August	EX3-8	79	1.24	69	1.08
16 August	EX3-9	71	1.21	77	1.27
16 August	EX3-10	70	1.37	80	1.36
15 September	EX1-3	78	1.40	77	1.61
15 September	EX1-4	80	1.43	76	1.76
15 September	EX1-5	75	1.26	68	1.17
15 September	EX1-6	82	1.49	73	1.41
15 September	EX1-7	78	1.35	72	1.37
15 September	EX1-8	70	0.97	73	1.38
15 September	EX1-9	80	1.25	77	0.99
15 September	EX1-10	75	1.15	81	1.32
15 September	EX2-3	83[3]	1.54[3]	81	1.71
15 September	EX2-4	80	1.45	66	0.89
15 September	EX2-5	73	1.20	74	1.23
15 September	EX2-6	78	1.14	75	1.21
15 September	EX2-7	71	1.05	77	1.23
15 September	EX2-8	77	1.03	81	1.83
15 September	EX2-9	76	1.40	71	1.31
15 September	EX2-10	85	1.47	65	1.05
15 September	EX3-3	78	1.44	75	1.36
15 September	EX3-4	78	1.33	75	1.28
15 September	EX3-5	74	1.34	78[1]	1.36[1]
15 September	EX3-6	80	1.58	70	1.06
15 September	EX3-7	84[2]	1.56[2]	73	0.90
15 September	EX3-8	80	1.29	70	1.12
15 September	EX3-9	73	1.13	78	1.54
15 September	EX3-10	70	1.36	80	1.49
13 October	EX1-3	80	1.51	79	1.75

Appendix 1. Continued.

Date	Aquarium Number	Male Length (mm)	Male Weight (g)	Female Length (mm)	Female Weight (g)
13 October	EX1-4	81	1.51	76	1.65
13 October	EX1-5	76	1.33	68	1.25
13 October	EX1-6	83	1.62	72	1.39
13 October	EX1-7	79	1.43	70	1.31
13 October	EX1-8	71	0.92	73	1.34
13 October	EX1-9	81	1.21	76	1.19
13 October	EX1-10	75	1.15	81	1.37
13 October	EX2-3	84[3]	1.63[3]	81	1.81
13 October	EX2-4	81	1.56	67	1.01
13 October	EX2-5	74	1.16	75	1.27
13 October	EX2-6	79	1.19	77	1.30
13 October	EX2-7	73	0.99	77	1.29
13 October	EX2-8	77	1.17	81	1.97
13 October	EX2-9	76	1.36	72	1.28
13 October	EX2-10	85	1.48	65	1.12
13 October	EX3-3	79	1.54	76	1.43
13 October	EX3-4	79	1.38	75	1.44
13 October	EX3-5	75	1.29	77[1]	1.38[1]
13 October	EX3-6	83	1.64	71	1.14
13 October	EX3-7	85[2]	1.43[2]	73	1.05
13 October	EX3-8	80	1.26	71	1.18
13 October	EX3-9	74	1.22	78	1.57
13 October	EX3-10	70	1.32	81	1.62

*Measurements of the female salamander in EX3-5 that was killed during removal from the upwelling tube.

[1]Measurements of the female salamander used replace female lost in EX3-5.

[2]Measurements of the male salamander used to replace the male lost to mortality in EX3-7.

[3]Measurements of the male salamander used to replace the male lost to mortality in EX2-3.

VITA

Paige Anne Najvar was born in Corpus Christi, Texas on June 9, 1975. She is the daughter of Dr. Brian Richard Chapman and Ms. Donna Marie Chapman. After graduating from Tuloso-Midway Rand Morgan High School in May 1993, she enrolled at Del Mar College in Corpus Christi, Texas. She transferred to the University of Texas in Austin, Texas, in August 1995. She earned the degree of Bachelor of Arts in Biology from the University of Texas in December 1997 and entered the Graduate College of Southwest Texas State University in San Marcos, Texas, in January 1998. She married David Gregory Najvar on January 9, 1999. During her enrollment as a graduate student she worked as an instructional assistant, teaching Zoology, Mammalogy, and Modern Biology. Also during this time, she worked as a wildife biologist for the United States Fish and Wildlife Service at the San Marcos National Fish Hatchery and Technology Center in San Marcos, Texas.

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