Anuran Metamorphosis: a Model for Gravitational Study on Motor Development

Jae Seung Shin, Jin Cheul Park, Masamichi Yamashita[1], and In-Ho Choi*

Department of Life Science, College of Liberal Arts and Sciences, Yonsei University, Wonju 222-710, Korea;
[1]*Institute of Space and Astronautical Science, Sagamihara 229-8510, Japan*

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Limbs and supporting structures of an organism experience a full weight of its own when it lands from water, because neutral buoyancy in the aquatic habitat will be no longer available in the terrestrial world. Metamorphosis of anuran amphibians presents a good research model to examine how this transition from non-loading to weight-loading affects development of motor capacity at the time of their first emergence on land. Our video analysis of the transitional anurans, *Rana catesbeiana*, at Gosner stage 46 (the stage of complete transformation) demonstrated that the take-off speed increased 1.23-fold after the first six hours of weight-loading on the wet ground. It did not increase further during the following three days of loading, and was close to the level of mature frogs with different body mass. During development of larvae in deep water with no chance of landing through metamorphosis, both tension and power of a hindlimb anti-gravity muscle increased 5-fold between stages 37 and 46. However, the muscle contractility increased more rapidly when the larvae could access the wet ground by their natural landing behavior after stages 41-42. Muscle power, one of major factors affecting locomotory speed, was 1.29-fold greater in the loaded than in the non-loaded larvae at the transitional stage. Thus, weight-loading had a potentially significant effect on the elevation of motor capacity, with a similar extent of increment in locomotory speed and muscle power during the last stages of metamorphosis. Such a motor adjustment of the froglets in a relatively short transitional period would be important for effective ecological interactions and survival in their inexperienced terrestrial life.

A variation in external condition influences many aspects of physiology in life forms (Schmidt-Nielsen, 1990). Because of its pervasive effect, even a slight extrinsic alteration may cause a problem in survival or reproduction of an organism. Individuals should exhibit a proper solution or solutions to the problem, the ability which may essentially affect its fitness (Fig. 1A).

Gravity is a physical interaction produced by every mass of matter that pulls other mass of objects including life forms. It defines a coordinate for orientation and posture in animals (Young et al., 1984) and directions of growth in plants (Sievers and Hensel 1990). It affects reproductive, developmental and immune responses of most organisms through either direct or indirect paths (Malacinski, 1990; Tipton et al., 1996). Functional capacity of an anti-gravity muscle (e.g., the soleus muscle), for instance, is important for postural maintenance under the gravitational load. If the muscle

is exposed to microgravity, as in the case of spaceflight, it faces transition of fiber types and atrophy with significant reduction in fiber size, metabolic potential, and force generation capacity (Ohira et al., 1992; Baldwin, 1996; Widrick et al., 1999). Postflight animals tend to exhibit abnormally low body posture and slow, unsteady movements on the day of landing, reflecting muscle weakness and poor coordination against the load of gravity (Riley et al., 1996; Yamashita et al., 1997). Recovery of functional properties from spaceflight takes hours to weeks, depending upon type of physiological systems, age, sex, size, and species of subjects (Baldwin, 1996).

Gravity changes little over geographically similar regions and through the whole history of life on the earth. Yet, load impinged on supporting structures (e.g., limbs) of an organism can be altered if an organism changes posture or locomotory condition in relation to gravity. The cases of long-term bed rest, limb suspension, or aquatic dwellers with neutral buoyancy are good examples (LeBlanc et al., 1988; Stump et al., 1997). Subjects including humans show signifi-

*To whom correspondence should be addressed.
Tel: 82-33-760-2244, Fax: 82-33-760-2183
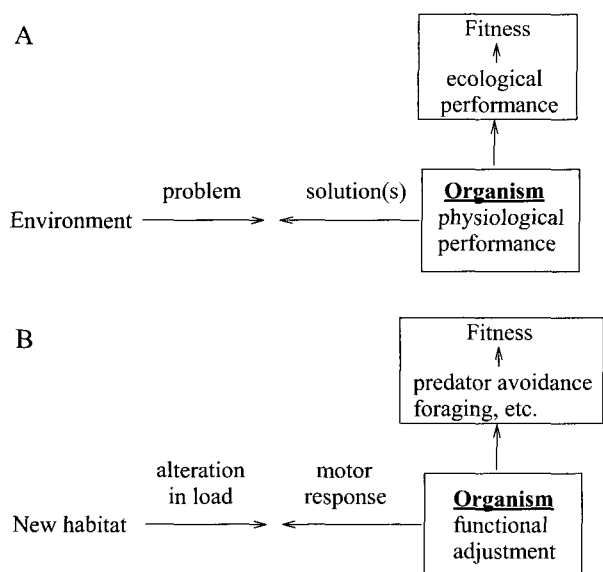E-mail: ichoi@dragon.yonsei.ac.kr

A

B

Fig. 1. Problem of the environment versus solution presented by an organism (A). An organism who reacts properly to an altered load in a new environment may attain better ecological performance and thus higher chance to increase its fitness (B).

cant atrophic responses in anti-gravity or locomotory muscles, that is found to be comparable to those exposed to spaceflight. For instance, the soleus muscle of rats exhibited 25% and 34% reduction in cross- sectional area after 14 d of spaceflight and of hindlimb suspension, respectively (Ohira et al., 1992).

Load of gravity on the supporting structures can be also changed significantly when the organism moves to different habitats that contain media of different density. For an animal, transition of living environment from water to land results in a full load of its own because buoyancy in the aquatic habitat is reduced to three orders less in air. The opposite is also true in immigrants from land to water. They experience an almost weightless condition at neutral buoyancy, supposing that density of live tissues is nearly equal to that of water.

Transition of media differing in density is quite common in animals. Most anurans undergo such transition at the end of metamorphosis. Many species of reptiles, birds, and mammals experience a similar transition at the time of hatching or birth when the young depart from the maternal amniotic environment. In the beginning of a new life in a different medium, the altered load is problematic for an animal, and should be solved as soon as possible for effective ecological interactions (predator avoidance, feeding, etc.) (Fig. 1B). The moment of transition could be critical to an organism over its life history, if the animal might not be ready for full capacity of performance (Wassersug and Sperry, 1977). Thus, when extrinsic alteration inevitably comes at a certain stage of growth, the animal should internally activate compensatory mechanisms to overcome its adverse effect on fitness at the time of transition.

An Anuran Model for Gravitational Study

Metamorphosis of anuran presents a good research model to investigate motor adjustment of animals during transition from a non-loading to a weight-loading status. Anuran is the first vertebrate that comes on land, and repeats this epoch every time in its life history at metamorphosis. Anuran tadpoles undergo complete restructuring of body plan to fit for their post-metamorphic terrestrial life (Burggren and Just, 1992; Shi, 2000). One of the prominent modifications over this period occurs in their locomotory system and its style. Larval undulatory swimming with the tail fin changes to pedal saltatory locomotion in frog. However, transition of the locomotory design is not enough for successful landing. The animals must have supporting and locomotory elements adjusted to bear their own weight during or before the time of early landing attempts, as they would lose the aquatic buoyancy after the transition. In this paper, we present preliminary results using of an anuran system on how locomotory capacity changes as the animals undergoing metamorphosis move from water to land in the first time of their lives.

According to Gosner's staging (1960), anurans exhibit emergence of hindlimb at stage 26, of forelimb at stage 42, and tail resorption, intestinal change and lung development at stages through 42 to 46. Transformation is completed at stage 46, and the animals, now froglets, have a short body trunk and long, slender limbs that make their terrestrial locomotion effective (Choi and Park, 1996). Over the climax period of metamorphosis (stages 42-46), tadpoles experience a remarkable change in their locomotory ability and style. According to Huey (1980), swimming speed of *Bufo boreas* tadpoles peaked at stages 41-42, and decreased rapidly thereafter until the end of transformation. This change in swimming performance was in parallel with the change in tail length. Around the time of complete transformation, anurans would be most vulnerable to predation since they are neither good swimmers nor fast jumpers (Huey, 1980). In fact, tadpoles at late metamorphic stages were the major prey item for snake predators (Wassersug and Sperry, 1977). Unless they have other defense mechanisms (e.g., toxin secretion, Daly, 1995), the tadpoles (or froglets) must be able to take off at their full capacity on land. It is thus reasonable to assert that the whole-animal as well as the hindlimb muscles should adjust to their own weight (and to other new factors such as the relatively dry terrain surface) as quickly as possible during the first landing trials for successful terrestrial life.

In this study, we examined motor development of bullfrogs (*Rana catesbeiana*) at several stages before and after complete transformation. We chose take-off speed and angle of the whole-animal, and tension and power of a hindlimb muscle to gauge motor ability of

the animals during transformation process. We expected that the ability of the anurans to perform these variables depends not only upon functional development of the system but also on the degree of their internal adjustment to the load change.

Materials and Methods

Subjects

We purchased bullfrog tadpoles (*R. catesbeiana*) at stages < 41 from a local farm in southwest Korean Peninsula during the summers of 1999 and 2000. At these stages, forelimbs have not yet been erupted. Tadpoles were sorted according to the Gosner's method, housed in two acrylic aquaria ($l \times w \times h$=1 m × 0.5 m × 0.5 m), and fed with TetraBits fish feeders (Tetra, Germany) or meal worms everyday. The water level was maintained at about 0.12 m in depth. The aquaria were kept at 23±1℃ and 13 L: 11 D cycle, and were refreshed twice a week.

Take-off performance

We used two groups of transitional anurans (stage 46) to compare the effect of landing on take-off capacity. One was a 'non-loaded' group of which individuals were kept in water until experiments. The other was a 'loaded' group that was composed of individuals kept for 6 h on the wet ground (with a water level of about 3 mm) of labeled individual cages (0.4 m × 0.2 m × 0.13 m). By this way, individuals of the latter group had the head and trunk exposed to the air and the limbs and abdomen loaded by their own weight. We did not examine the take-off ability of animals at stages < 46 because the tail influenced their actual take-off capacity (Wassersug and Sperry, 1977). To investigate development of take-off performance, we examined two additional groups loaded for 1 d and 3 d, respectively, in the wet-surfaced cages after transformation (stage 46). The time point of complete transformation was judged by a dark spot which appeared on the vent at the last moment of the entire tail loss.

The experiments were conducted on a jump track (1.5 m × 0.2 m × 0.8 m) as described in Choi and Park (1996). Before the jump trials, we attempted to empty the urinary bladder of the animals by gently pressing their lower abdominal surface. We put individuals at one end of the track, and threatened them to jump by moving a dark pad down rapidly from behind. Jumping motions were recorded with a JVC GR-DVL9500 digital video camera at a high-speed mode (60 frames s⁻¹) with a shutter speed of 1/500 s. Each recording covered a lateral view of about 0.7 jumps. Five to seven trials were made for each frog, with a rest period of about 30 min between the trials. During the experiment, the cage and the track temperatures were kept at 20°-23℃ and were continuously monitored with a Cole-Parmer 91100-20 digital thermometer.

The recorded video images were analyzed frame by frame using the 'pause/advance' function of the JVC video system. The freeze-frame image was captured by the JVC JLIP Video Capture software, and was transferred to a graphic software, Adobe Photoshop (version 5.0) in an IBM 586 compatible PC. Each image (recorded at 60 frames s⁻¹) could be split into even and odd fields (thus, 120 fields s⁻¹) by a 'video filter' mode of the software. A tip of the subject's snout in each frame was traced on (x,y) coordinates of a cursor-based 17" PC monitor. Those jumping trials during which animals touched the side walls of the track were not included in our analyses.

From the video analyses, take-off speed was determined from two consecutive fields of the recording and was calculated using the distance the subject moved between the two frames multiplied by the recording rate (120 Hz). The highest take-off speed from five to seven trials of each frog was taken as the maximum take-off speed of that animal. The take-off angle was calculated from the tangent of the vertical to the horizontal distances that the animal moved between the two fields at the highest speed.

Muscle contraction

Preparation: Tadpoles of stages 37, 40, 43, and 46 were evaluated for muscle contractile development. From the jumping experiments, we found that the earliest natural landing behavior of this species started at stages 41 - 43. Furthermore, our preliminary results demonstrated that muscle contractility seemed quite established at stage 46, with no significant change in tension and shortening velocity after this stage. We thus decided to examine muscles of tadpoles at stages before the forelimb eruption (stage 37) and at stages exhibiting the first natural landing behavior (stages 40 and 43). In this muscle study, we divided tadpoles into two groups: for the 'landing' group, a sloped terrain was provided in the aquarium so that tadpoles at later stages could be loaded as they emerged from water to the terrain surface; for an 'in-water' group, such terrain was not provided all through the metamorphosis so that the tadpoles had no chance to experience weight-loading until the muscle experiment.

After pithing each tadpole, the iliofibularis muscle (an anti-gravity muscle for posture) was dissected out from the right hindlimb and was soaked in a cooled oxygenated Ringer's solution (see below) in a petri dish. Tendons of both ends of the muscle were tied tightly with silk threads, with the length of the tendons kept as short as possible in order to minimize their stretch at contraction. We placed the muscle horizontally in a muscle bath (80 ml). One end of the muscle was connected to a Harvard 60-2995 force transducer (natural frequency 60 Hz) and the other end to a vertical beam of a T-lever attached to a Harvard 52-9511 rotary transducer. During isometric contraction, the muscle

Table 1. Effect of loading on locomotory development in *Rana catesbeiana* at the time of complete transformation (n=7)

Factor	Non-loaded[a]	Loaded[a] for		
		6 h	1 d	3 d
Body mass (g)	14.39 ± 4.06	13.40 ± 2.98	13.42 ± 4.45	12.26 ± 2.20
Take-off speed (m s⁻¹)	1.79 ± 0.20	2.20 ± 0.18[b]	2.15 ± 0.22[b]	2.20 ± 0.17[b]
Take-off angle (°)	36.75 ± 5.57	37.64 ± 9.40	43.60 ± 11.45	35.72 ± 9.91

Values are mean ± 1SD.
[a]Non-loaded=in deep water at stage 46; 6 h=on wet ground for 6 h at stage 46; 1 d=on wet ground for 1 d after stage 46; 3 d=on wet ground for 3 d after stage 46.
[b]Data of the loaded groups are significantly different from that of the non-loaded group (one-way Scheffe's multiple range test, $P < 0.05$).

was held immobile by a stop on a horizontal side of the T-lever. For isotonic shortening, the stop was quickly removed by a 24-W solenoid actuator. The muscle bath temperature was regulated at 10 ± 1°C by a refrigerated circulator (Kookje Scien 33-WBF-15), and was monitored using the Cole-Parmer thermocouple thermometer. The Ringer's composition (in mmol l⁻¹) was NaCl, 115; KCl 2.5; $CaCl_2$, 1.8; Na_2HPO_4 2.15; NaH_2PO_4, 0.85; and glucose, 11 (at pH 7.2; Julian et al., 1986). Muscles were stimulated with a pair of bright platinum electrodes connected to a Grass S48 stimulator that supplied a 1.0 ms square wave pulse or pulse train. All electrical signals from transducers were digitized by a Biopac MP100 A/D converter and stored in an IBM 586 compatible PC.

Procedure: For each preparation, we determined the maximum isometric tetanic tension (T_o), maximum shortening velocity (V_{max}), and maximum power (P_{max}) as described in Choi et al. (1998). We determined optimum muscle length (l_o) and supramaximal voltage to produce the maximum twitch tension. Stimulus frequency of 200 Hz was used to attain fully-fused tetanic force (F_o). A rest period of at least 20 min was allowed between the contraction trials. In after-loaded isotonic contractions, the muscle was stimulated at the same frequency. The stop on the horizontal arm of the T-lever was removed by the solenoid. The muscle then shortened against the imposed load (force), ranging 10%-80% F_o. Shortening velocity was determined from a constant length change over a time period of 10-15 ms after the shortening started. From the data of F_o and of isotonic series, a force - velocity relationship was established for that muscle using a curve-fit equation of Marsh and Bennet (1986). We calculated muscle power with every force and velocity pair to obtain a power-velocity relationship. From the two relationships, we determined a particular force (F) at P_{max}, and obtained a ratio of F/F_o. This ratio was found to be 0.45 on the average, and was used in the subsequent experiments to predict a load (F) from F_o of each muscle. Shortening velocity (V) was then determined after the muscle was loaded with F. At the end of each experiment, we measured the optimum muscle length (l_o) in place using a micrometer under a light microscope, muscle mass (M_m) with a chemical balance,

and cross-sectional area (CSA=$M_m \times l_o^{-1}$). Muscle force was converted to tension by dividing the values with CSA. Shortening velocity V was normalized with l_o, and muscle power with M_m.

Data were presented as the mean ± SD, unless otherwise noted. Statistical significance of the 'loading' effect within stage was examined by independent samples t-test. Changes in jumping capacity among the three loaded groups (6 h, 1 d, and 3 d) and changes in muscle contractility among the four stages within each group ('landing' or 'in-water') were examined by one-way analysis of variance (ANOVA) and Scheffe's multiple-comparison test. All the statistical procedures were conducted with SPSS/PC+.

Results and Discussion

Data of morphology, locomotion, and muscle contraction are summarized in Tables 1 and 2. Body mass (M_b) of the anurans did not differ among the four groups in the jumping study, nor did M_b within and between the groups in the muscle study (one-way ANOVA, $P > 0.05$). Muscle mass and length used in the muscle study differed significantly among the four stages within each group (one-way ANOVA, $P < 0.05$) but did not differ between groups in each stage (independent samples t-test, $P > 0.05$).

The take-off speed was 1.23-fold greater in the 6 h-loaded group than in the non-loaded group at stage 46 (t=4.08, df=10, $P < 0.02$). The jumping ability then remained at a similar level (2.15 - 2.20 m s⁻¹) over the following 3 d period (one-way Scheffe's multiple-comparison test, $F_{2,12}$=0.344, P=0.72). The take-off speed of our loaded anurans was close to or slightly lower than those of other adult ranids. Two bullfrogs that grew more than 6 months after metamorphosis in our laboratory showed maximum take-off speed of 2.21 and 2.43 m s⁻¹ (27.68 g and 18.19 g, respectively) (J. Park, unpublished data). *Rana nigromaculata* (average M_b=9.2 g) and *R. rugosa* (average M_b=11.3 g) exhibited take-off speed of 2.33 - 2.35 m s⁻¹ (Choi and Park 1996).

The greater speed in the 6-h loaded group compared to the non-loaded group may be partly due to M_b reduced by 7% after landing. The reduction in body mass is quite normal at landing when the skin of the young is losing water (Shoemaker, 1992). Even if the

Table 2. Effect of loading on development of the iliofibularis muscle contractility in *Rana catesbeiana* during late stages of metamorphosis (n=5)

Factor	In-water group[+]				Landing group[+]			
	37[d]	40	43	46	37	40	43	46
Body mass (g)	29.22 ± 4.24	31.42 ± 4.83	28.57 ± 2.92	25.95 ± 3.27	26.92 ± 2.55	29.16 ± 3.57	29.58 ± 0.79	20.27 ± 5.52
Muscle mass (10^{-3} g)	8.56 ± 1.94	33.7 ± 7.33	35.0 ± 24.5	44.70 ± 0.85	8.60 ± 1.77	16.0 ± 10.3	27.7 ± 7.78	39.5 ± 12.6
Muscle length (mm)	7.88 ± 1.26	13.58 ± 1.64	15.18 ± 3.81	17.66 ± 1.52	8.04 ± 0.82	10.92 ± 1.91	14.70 ± 1.79	16.50 ± 1.99
T_o[a]	32.60 ± 14.42	65.31 ± 13.00	118.64* ± 12.01	162.50 ± 20.18	43.09 ± 18.02	63.87 ± 7.97	150.85* ± 17.76	183.14 ± 19.62
P_{max}[b]	27.11 ± 10.18	56.90 ± 9.96	104.82 ± 5.47	134.71* ± 9.95	26.15 ± 8.94	52.72 ± 6.01	132.28 ± 28.04	174.30* ± 17.71
V^c at P_{max}	2.03 ± 0.25	1.98 ± 0.19	1.99 ± 0.14	1.86 ± 0.15	1.57 ± 0.73	1.89 ± 0.13	1.98 ± 0.18	2.13 ± 0.15

Values are mean ± SD.
[a]Maximum tetanic tension (kN m^{-2}); [b]Maximum power (W kg^{-1}); [c]Shortening velocity (muscle length s^{-1}); [d]Stage.
[+]Muscle mass, muscle length, T_o, and P_{max} in the in-water group, and body mass, muscle mass, muscle length, T_o, and P_{max} in the landing group were significantly different among stages (one-way Scheffe's multiple range test, $P < 0.05$).
*Data between in-water and landing groups were significantly different for that variable (independent samples t-test, $P < 0.05$).

reduction in M_b is taken into account, the major part of the increase in take-off speed could be brought by adjustment of the internal mechanism (e.g., neuromuscular system) to loading.

The take-off angle was not statistically different between the two transitional groups (t=0.02, df=10, P=0.982; Table 1), despite the fact that the non-loaded animals were expected to display substantially low postural response at take-off. The average take-off angle of 37° in our animals was similar to those seen in adult bullfrogs (35° - 40°) and other species (Olson and Marsh, 1998; Choi et al., 2000).

These results demonstrate that, within 6 h of loading during the first landing trials in their lives, the transitional frogs attained take-off speed that was fairly close to the highest value exerted by their adult form. The similarity in the take-off angle between the non-loaded and loaded anurans suggests that the juvenile responded to threats with a similar jumping posture for the 'best' escape performance even if the actual take-off outcome differed between them. Considering that anatomical features of the skeleto-muscular system determine jumping posture and kinetics, the unchanged take-off angle might in turn imply that there is no inequality in development of the system between the two groups. The locomotory adjustment by our froglets can be compared with recovery response exhibited by spaceflight hylid frogs (*Hyla japonica*, 2 - 3 g) (Yamashita et al., 1997). The hylids that returned from 8 d exposure to microgravity on the Russian Space Station Mir displayed slower leaping speed and more delayed post-leap retraction of hindlimbs than a ground control group. Abnormal posture and locomotion started to fade out after 2.5 h in the flown hylids. This fast recovery time was supposed to be dominated mainly by re-adaptation process in the neural system, that would be imposed on the change in the muscular system. Adjustment of the locomotory system to loading (or reloading) within 2.5 h to 6 h, however, is faster in anurans than in mammals. From Spacelab Life Sciences-1 and -2 experiments, it was found that post-flight rats (Sprague-Dawley) required 4 - 9 d to restore normal behavior and pedal locomotion (Riley et al., 1996).

In the muscle study, we tested whether loading also affects contractile properties of the hindlimb anti-gravity muscle through developmental stages from 37 to 46 (Table 2). In this case, loading was provided by their own landing behavior occurring naturally at stages 41 - 42. For the in-water group, individuals were forced not to land at those stages, that might be a stress in reducing their growth. However, we did not see any evidence of such an adverse effect on animal growth in that a ratio of the M_b-specific muscle length was 0.99 between the in-water and the landing groups.

Our results for the in-water group showed that muscle tension and power changed significantly across the four stages (one-way ANOVA, $F_{3,16}$=71.04, $P < 0.001$ for tension; $F_{3,16}$=139.41, $P < 0.001$ for power) while shortening velocity did not ($F_{3,16}$=0.72, P=0.78). Both muscle tension and power increased 5-fold during the developmental period (Table 2). For the landing group, muscle tension and power also changed significantly during the same period ($F_{3,16}$=83.43, $P < 0.001$ for tension; $F_{3,16}$=77.83, $P < 0.001$ for power) while shortening velocity did not ($F_{3,16}$=1.87, P=0.20). Muscle tension (183.14 kN m^{-2}) of the landing group at stage 46 was fairly close to tension of the 1-d loaded (181.73 ± 71.05 kN m^{-2}, n=7) and 3-d loaded (202.31 ± 70.09 kN m^{-2}, n=7) young bullfrogs examined in our preliminary study.

Contractile values of the landing group at stages 43 and 46 may attest to the effect of loading in addition to the developmental process of the muscle tissue. There was a significant inter-group difference in tension at stage 43 (independent samples t-test, t=3.36, df=8, P=0.01) and in power at stage 46 (t=4.36, df=8, P= 0.002). Difference in muscle shortening velocity, however, was not detectable between the two groups at any stage (Table 2). Thus, loading potentially enhanced contractile capacity of the larval hindlimb muscles exclusively after the landing behavior occurring around stage 43.

It was in fact the muscle tension and power that increased dramatically during development of the larval hindlimbs through the metamorphic climax. Hindlimbs of anuran larvae, including our animals, grow simply without any particular activity before stage 37, and

show the first sign of movement (e.g., stepping, synchronized kicking in fast swimming) at stages 37 - 39 (Gosner 1960). Thus, the 5-fold increase in tension and power from stage 37 to 46 in the in-water group seems to be in accordance with the locomotory activities of hindlimbs that increased during the late metamorphic stages. The rates of increment in these variables were greater in the landing group than in the in-water group, as the former had opportunities to access the ground. It is well documented that juvenile animals are slower in movements, with lower rate of muscle shortening than adults (Swynghedauw, 1986, also see Wassersug and Sperry, 1977). Surprisingly, this was not the case in the hindlimb muscle of our anuran larvae (Table 2). Shortening velocity of the muscle at stage 37 was almost identical with that at stage 46 in both the in-water and the landing groups. Muscle mass and length at stage 37 were significantly smaller than at later stages. Hence, it is likely that the neuromuscular and myofibrillar mechanisms associated with shortening rate (e.g., neural input patterns, myosin ATPase activity, and Ca^{2+}-sarcoplasmic interaction) were much differentiated in this very first stage of hindlimb movements (stage 37), while the limbs were still growing rapidly. Myofibrillar differentiation may arise in association with development of fast twitch fibers at this stage, that would be needed for the synchronized kicking behavior during fast swimming.

In conclusion, loading on hindlimbs by their own weight indeed affected both locomotory and muscle performance of our subjects examined during the last stages of metamorphosis. As reported by other studies (e.g., Lutz and Rome, 1994), muscle power, rather than shortening velocity, would be the major variable that is associated with the animal locomotory speed. The 1.23-fold increase in take-off speed may be partly explained by the 1.29-fold increment in muscle power after the motor system was loaded (Tables 1 and 2). Transition of the anuran larvae from water to land thus seems to come with the well-prepared motor system by its functional development at the right time as well as its adjustment to loading. It is worth addressing signal transduction of anuran muscle cells in the future, which would provide information on an internal regulatory basis of locomotory adjustment in the variable loading status.

Acknowledgements

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