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SWIMMING KINEMATICS AND RESPIRATORY BEHAVIOUR OF *XENOPUS LAEVIS* LARVAE RAISED IN ALTERED GRAVITY

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Summary

We examined the respiratory behaviours and swimming kinematics of *Xenopus laevis* tadpoles hatched in microgravity (Space Shuttle), simulated microgravity (clinostat) and hypergravity (3*g* centrifuge). All observations were made in the normal 1*g* environment. Previous research has shown that *X. laevis* raised in microgravity exhibit abnormalities in their lungs and vestibular system upon return to 1*g*.

The tadpoles raised in true microgravity exhibited a significantly lower tailbeat frequency than onboard 1*g* centrifuge controls on the day of landing (day_0), but this behaviour normalized within 9 days. The two groups did not differ significantly in buccal pumping rates. Altered buoyancy in the space-flight microgravity tadpoles was indicated by an increased swimming angle on the day after landing (day_1). Tadpoles raised in simulated microgravity differed to a greater extent in swimming behaviours from their 1*g* controls. The tadpoles raised in hypergravity showed no substantive effects on the development of swimming or respiratory behaviours, except swimming angle. Together, these results show that microgravity has a transient effect on the development of locomotion in *X. laevis* tadpoles, most notably on swimming angle, indicative of stunted lung development. On the basis of the behaviours we studied, there is no indication of neuromuscular retardation in amphibians associated with embryogenesis in microgravity.

Key words: *Xenopus laevis*, tadpole, microgravity, locomotion, swimming, respiration, hypergravity, development.

Introduction

A fundamental question in biology is how gravity affects development. Prior to the availability of orbital space flights, this subject could only be explored by raising organisms in either a centrifuge (hypergravity) or a slowly rotating clinostat (simulated microgravity) (Neff *et al.* 1993). Lately, extended space flight has made it possible to study developmental processes in true microgravity (e.g. Ijiri, 1995; Souza *et al.* 1995; Yamashita *et al.* 1995; plus earlier studies reviewed in Rahmann and Slenzka, 1994).

Most space-flight experiments directed at investigating vertebrate development in microgravity have used the African clawed frog *Xenopus laevis* as a model species (Rahmann *et al.* 1994; Snetkova *et al.* 1995; Souza *et al.* 1995). Studies to date have established that *X. laevis* can complete embryogenesis in microgravity, but the initial behaviour of hatchlings, once they are in the 1***g*** environment, is abnormal for at least two reasons. First, some yet to be identified component of the developing vestibular system appears to be influenced by the absence of gravity. Visual tracking of moving stimuli is accentuated; i.e. the tadpoles have a heightened optomotor response (Pronych *et al.* 1996). Second, tadpoles in microgravity do not appear to fill their lungs in a normal or timely fashion (Black *et al.* 1996; Snetkova *et al.* 1995). Consequently, the larvae tend to be negatively rather than positively buoyant as are control tadpoles raised in 1***g***. Some *X. laevis* tadpoles (e.g. Neubert *et al.* 1994; Snetkova *et al.* 1995), but not all (Black *et al.* 1996), raised in microgravity have had caudal lordosis.

There is contradictory information in the literature as to whether development overall, and in particular neural development, of *X. laevis* raised in actual or simulated microgravity is retarded. Black *et al.* (1996) report no change in the developmental rate of tadpoles raised in microgravity. However, *X. laevis* tadpoles from the space-flight experiment of Snetkova *et al.* (1995) were significantly smaller than controls. Studies of fishes raised in microgravity and tadpoles raised in simulated microgravity (e.g. Neubert *et al.* 1994; Rahmann and Slenzka, 1994; Slenzka *et al.* 1994) suggest that development in reduced gravity can lead to major changes in brain size and brain chemistry.

If development in microgravity does retard tadpole growth, it could be a result of respiratory insufficiency, secondary to the failure of the tadpoles to inflate their lungs. It is known, for example, that *X. laevis* larvae raised in 1***g***, but prevented from

inflating their lungs, have drastically lower growth rates than control specimens allowed to inflate their lungs (Pronych and Wassersug, 1994).

In the present study, we examined two aspects of the behaviour of *X. laevis* larvae raised in microgravity, hypergravity and simulated microgravity: basic swimming kinematics and respiratory behaviours. These behaviours were selected for several reasons. The basic kinematics of *Xenopus* tadpole locomotion is well known (Hoff and Wassersug, 1986). Under conditions of normal gravity, *X. laevis* tadpoles scull in the middle of the water column with their heads tipped downwards; they hold their position by swimming downwards against their own buoyancy. Their tailbeat frequency averages 10–12 Hz (Hoff and Wassersug, 1986), with a low-amplitude movement at the tail tip, but the frequency can be slightly higher for younger hatchlings (van Mier, 1986). Larval swimming behaviour develops quickly over a short period (Roberts *et al.* 1983; van Mier, 1986; van Mier *et al.* 1989; Sillar *et al.* 1991). Thus, changes in the kinematics of experimental *versus* control tadpoles would be some indication of either acceleration or retardation in the development of their neuromotor control.

It is also true that, if exposure to gravity treatments other than 1 ***g*** induced changes in tadpole locomotion, then tracking those changes over time would reveal how long it takes for tadpoles raised in altered gravity to acclimate to 1 ***g***. Previous studies of the optomotor behaviours (Pronych *et al.* 1996) and turning patterns (Neubert *et al.* 1994) of *X. laevis* tadpoles hatched in microgravity show that these behaviours normalize in the course of 2–9 days in a 1 ***g*** environment.

The respiratory behaviour of *X. laevis* larvae has been similarly well studied (Feder and Wassersug, 1984; Orlando and Pinder, 1995; Wassersug, 1996). These tadpoles utilize buccal pumping, a conspicuous lowering and raising of the floor of the mouth, to propel water through their gill slits. This aquatic respiratory frequency in normoxic water at room temperature (approximately 20 °C) is approximately 1 Hz. Shortly after hatching, *X. laevis* larvae swim to the surface to fill their lungs and thereafter supplement aquatic respiration with intermittent air breathing (Wassersug, 1996). Alterations in the position of the tadpoles in the water column or in their frequency of aerial respiration are indicative of changes in buoyancy and lung use. Changes in swimming angle, in otherwise normal tadpoles, can reflect differences in the centre of buoyancy, secondary to differences in lung volume. Thus, respiratory behaviours, like swimming kinematics, can provide insight into the overall developmental rates of *Xenopus laevis* larvae exposed to altered gravity.

Materials and methods

Ground-based simulation experiments

General

Ground-based studies were performed prior to the space-flight experiments (see below) to test the experimental protocols and equipment. *Xenopus laevis* (Daudin) larvae were raised in cultisak (Falcon) plastic bags containing 10 ml of 20 % Steinberg's or frog Ringer's solution (Neff *et al.* 1993), from a point shortly after fertilization to the feeding, free-swimming tadpole stage, in one of three different gravitational regimes: normal 1 ***g*** (vertical clinostat at 6 revs min^{-1}), simulated hypergravity on a 3 ***g*** centrifuge (120 revs min^{-1}) and simulated microgravity on a horizontal clinostat rotating at 6 revs min^{-1}. This rotational speed has been shown previously to cause the eggs to tumble gently and to mask the unidirectional influence of gravity. Full details of these procedures are given in Neff *et al.* (1993) and Pronych *et al.* (1996). The volume of water was large enough to provide the embryos with oxygen for the length of the experiment. Approximately 1 day after the tadpoles began to swim freely, they were removed from their respective gravitational environments and placed in an open aquarium at 1 ***g*** for video-taping. The tadpoles were then placed in groups of up to 15 individuals in small 40 ml plastic culture flasks (12 cm×7.3 cm×3.2 cm, height × width × depth) containing 25 ml of Ringer's solution. The animals' swimming and respiratory behaviours were filmed on two occasions: upon initial placement in the 1 ***g*** aquarium (=day$_0$) and 1 day later (=day$_1$). The films of the three tadpole groups were analyzed in a double-blind fashion.

Note that, in order to avoid collecting data on behaviours distorted by wall effects, we excluded from analysis animals that were within one body width of the flask edge. Animals occasionally swam in and out of the field of view, so it was therefore impossible to track each individual tadpole. As a result, it is probable that our data set includes multiple measurements on the same individual. However, the movement within each flask was clearly random, so we are confident our results are not biased by one particular individual. There was also a high incidence (approximately 50 %) of morphological deformity in the simulated microgravity and 3 ***g*** groups in particular. All grossly deformed tadpoles were excluded from analysis.

Locomotion

Video recordings, taken from above using a NAC high-speed camera at 200 frames s^{-1} with a strobe light for illumination, were analyzed frame by frame to assess the swimming kinematics of the tadpoles. This involved locating sequences of tadpoles swimming at a relatively constant velocity in a straight line (following Hoff and Wassersug, 1986) and measuring (1) the time and distance travelled over 10 tailbeats (one tailbeat equals the distance between consecutive ipsilateral propulsive wave crests), and (2) body length (snout to tail tip). The on-screen measurements were taken in centimetres and converted to actual values using on-screen grid lines to obtain the correct conversion factor. Velocity was calculated in s^{-1} and converted to body lengths s^{-1}. In addition, any behavioural or morphological abnormalities in the tadpoles were noted (i.e. looping, bloatedness, etc.).

The tadpoles were also filmed in side view using a JVC S-VHS camcorder at 30 frames s^{-1}, illuminated with standard

microscope fibre-optic lamps, to study posture and respiratory behaviours. Swimming angles were measured on day_0 by sampling the films at 2 min intervals. The swimming angle was measured between the long axis of the head-down tadpole and the horizontal plane, such that horizontal swimming is at 0° and a vertical tadpole with head down is at 90°.

Comparisons between the three groups in swimming velocity and tailbeat frequency were made using multiple regression analysis to determine the equations and the fit of the lines, using SAS. Complete and reduced models were calculated, and the slopes and *y*-intercepts of the lines were compared using an *F*-test (see Mendenhall and Sincich, 1988). Data on swimming angle were not normally distributed and thus were analyzed nonparametrically using Mann–Whitney *U*-tests.

Respiration

Buccal pumping rates were also measured from the side-view camcorder recordings. Pumping was measured as elevations and depressions of the buccal floor in animals that were sculling in place and not moving actively about, because tadpoles tend to suppress buccal pumping when swimming rapidly (R. Wassersug and M. Fejtek, personal observations). Sequences of at least five pumps were timed and the number of beats s^{-1} (Hz) determined.

The incidence of aerial respiration was also noted as the tadpoles attempted to take a breath at the air–water interface. A successful breath was marked by the expulsion of an air bubble. Buccal pumping and aerial respiratory rates in the simulated microgravity, 3 ***g*** and 1 ***g*** groups were compared using nonparametric analysis (Mann–Whitney *U*-tests).

Activation of the strobe light used with the NAC high-speed camera (see above) caused the tadpoles to swim rapidly and dart about, and suppressed respiratory behaviours. Therefore, data from the camcorder were collected only on day_0 in the ground-based experiments, and an alternative lighting design was used for the subsequent space-flight experiments.

Space-flight experiments

General

Four adult female *X. laevis* were launched on the Space Shuttle *Endeavour* (STS-47) in September 1992 and injected with human chorionic gonadotropin 18 h into their orbital flight. The eggs were fertilized with a sperm suspension obtained from male *Xenopus laevis* prior to launch. Groups of 15–30 fertilized eggs were then placed into specially designed growth chambers containing 50 ml of 20 % frog Ringer's solution, with very little air space. Half of these chambers were placed in an incubator and the other half in an onboard 1 ***g*** centrifuge (c-1***g***); both groups of embryos were raised at the same temperature and in the dark (see Black *et al.* 1996; Souza *et al.* 1995; Pronych *et al.* 1996, for details). The *X. laevis* tadpoles hatched on the Shuttle and were available for postflight behavioural observations 3–4 h after landing, 8 days later. These tadpoles were approximately 1 day less advanced in their development than the day_0 ground-based animals. This difference can be accounted for by our earlier access to the space-flight tadpoles. Nevertheless, for clarity, we refer to them similarly as day_0 tadpoles, i.e. when we first observed their behaviour postflight. Films of these tadpoles were made in 1 ***g*** on two additional occasions: 1 day postflight (=day_1) and 9 days postflight (=day_9). The films were again subjected to double-blind analysis, examining the swimming and respiratory behaviours of the tadpoles raised in microgravity and on the 1 ***g*** centrifuge.

Locomotion

The tadpoles were filmed with both the NAC high-speed and VHS video cameras as described above (with lighting from fibre-optic microscope lamps). In addition to the previous variables, position in the water column (bottom, middle, top) was noted for each tadpole sampled for swimming angle. Three positions were recognized: bottom, tadpoles in contact with the flask bottom; top, tadpoles directly below or contacting the water surface; middle, tadpoles in the remaining space between. These data are presented graphically as percentages of individuals at each position. Multiple analysis of variance (MANOVA) was used on the raw data to compare overall and single position differences between the two groups. Results of the swimming data were analyzed statistically as described above.

Respiration

Buccal pumping rates and the incidence of aerial respiration were noted for the space-flight microgravity and c-1***g*** tadpoles (measured from the camcorder recordings). The microgravity and c-1***g*** groups were compared by nonparametric analysis (Mann–Whitney *U*-tests) across all three test periods.

Results

Ground-based simulation experiments

General

There were no significant differences in body length (Mann–Whitney *U*-test, P=0.5) within or between any combination of simulated microgravity, 1 ***g*** and 3 ***g*** tadpoles over the 2 days of testing (Table 1).

A few cases of looping behaviour were seen in all three tadpole groups on day_0. This involved swimming in tight, repetitive, forward-outside loops. Some tadpoles were lying on the bottom of the flask in the simulated microgravity and 1 ***g*** groups. However, most of the 1 ***g*** tadpoles were normal and swimming actively. Approximately half the simulated microgravity tadpoles exhibited some morphological anomalies, such as a bloated abdomen or an upwardly bent tail, with the latter contributing to cases of backward looping observed in this group. Similar abnormalities were noted in approximately two-thirds of the 3 ***g*** tadpoles but were rare in the 1 ***g*** controls.

Locomotion

Mean swimming velocity was significantly different

Table 1. *Comparison of mean swimming variables for ground-based tadpoles*

	s-μ***g***		1***g***		3***g***	
Variable	Day_0	Day_1	Day_0	Day_1	Day_0	Day_1
Body length (cm)	1.08±0.01 (*N*=52)	1.09±0.01 (*N*=55)	1.10±0.02 (*N*=27)	1.10±0.01 (*N*=28)	1.11±0.01 (*N*=42)	1.10±0.01 (*N*=40)
Tailbeat frequency (Hz)	10.74±0.09[b]	11.42±0.09[a,c]	11.90±0.15	11.85±0.16	11.61±0.14	11.78±0.12
Velocity ($L\,s^{-1}$)	1.86±0.04[b]	2.19±0.07[a]	2.16±0.12	2.41±0.12	2.18±0.05	2.36±0.07
Swimming angle (degrees)	44.2±4.5 (*N*=30)	–	30.9±4.3 (*N*=17)	–	24.9±4.4[d] (*N*=13)	–

Values are means ± S.E.M.

N values for body length also apply to tailbeat frequency and velocity.

s-μ***g***, simulated microgravity on a clinostat; *L*, body length.

[a]Significantly different from day_0, [b]significantly different from 1***g*** on day_0, [c]significantly different from 1***g*** on day_1, [d]significantly different from s-μ***g*** on day_0; $P \leq 0.05$ in all cases.

between day_0 and day_1 only in the simulated microgravity tadpoles (Table 1).

All three tadpole groups showed a significant correlation (P=0.06 or better) between tailbeat frequency and velocity on both day_0 and day_1 (Fig. 1). On day_0, the relationship between these variables in the simulated microgravity and 3***g*** tadpoles differed significantly from that of the 1***g*** tadpoles (*F*-test, $P<0.0001$), but this difference disappeared on day_1. Only in the simulated microgravity group was there a significant difference between the two test days ($P<0.0001$). Mean tailbeat frequency increased significantly only in the simulated microgravity tadpoles between day_0 and day_1 (Mann–Whitney *U*-test, P=0.0006) (Table 1).

Swimming angle was only measured on day_0 in the ground-based experiments. The single significant difference occurred between the simulated microgravity and 3***g*** tadpoles (Mann–Whitney *U*-test, P=0.007), with the simulated microgravity tadpoles having a higher mean angle (Table 1).

Respiration

There were no significant differences in buccal pumping rate on day_0 between any of the groups (Mann–Whitney *U*-test, P=0.9); all three had a mean rate of approximately 1 Hz. There were also no significant differences in the aerial respiratory rates between the three groups (Mann–Whitney *U*-test, P=0.7).

Space-flight experiments

General

Although the tadpoles grew over the course of the experiment, there were no significant differences in body length (Mann–Whitney *U*-test, P=0.4) between the microgravity and c-1***g*** tadpoles on day_0, day_1 or day_9 (Table 2).

Table 2. *Comparison of mean swimming variables for space-flight tadpoles*

	Microgravity			c-1***g***		
Variable	Day_0	Day_1	Day_9	Day_0	Day_1	Day_9
Body length (cm)	1.02±0.02 (*N*=22)	1.07±0.01 (*N*=33)	1.28±0.04[c,d] (*N*=11)	0.98±0.02 (*N*=24)	1.04±0.02[c] (*N*=12)	1.26±0.03[c,d] (*N*=14)
Tailbeat frequency (Hz)	12.99±0.17[a]	13.17±0.14	11.24±0.15	13.72±0.22	12.79±0.18	11.60±0.21
Velocity ($L\,s^{-1}$)	2.42±0.10	2.50±0.08	2.28±0.16	2.66±0.15	2.32±0.12	2.52±0.13
Swimming angle (degrees)	42.1±1.8 (*N*=98)	36.0±1.6[b,c] (*N*=113)	28.5±1.6[c,d] (*N*=53)	45.2±2.1 (*N*=62)	28.6±2.4[c] (*N*=53)	25.1±1.9[c] (*N*=52)
Buccal pumping rate (Hz)	1.37±0.10 (*N*=7)	2.03±0.10[c] (*N*=2)	1.85±0.11[c] (*N*=9)	1.63±0.04 (*N*=3)	1.53±0.01 (*N*=2)	2.13±0.11[d] (*N*=7)
Aerial respiratory rate incidences (per flask)	4.33±0.76 (*N*=6)	4.43±0.75 (*N*=7)	4.75±0.25 (*N*=4)	4.0±1.18 (*N*=6)	3.0±0.45 (*N*=5)	5.0±0.58[d] (*N*=4)

Values are means ± S.E.M.

N values for body length also apply to tailbeat frequency and velocity.

c-1***g***, simulated normal gravity in onboard centrifuge; *L*, body length.

Aerial respiratory incidence is the mean number of successful attempts at taking a breath at the air–water interface per fixed observation period.

[a]Significantly different from c-1***g*** on day_0, [b]significantly different from c-1***g*** on day_1, [c]significantly different from day_0, [d]significantly different from day_1; $P \leq 0.05$ in all cases.

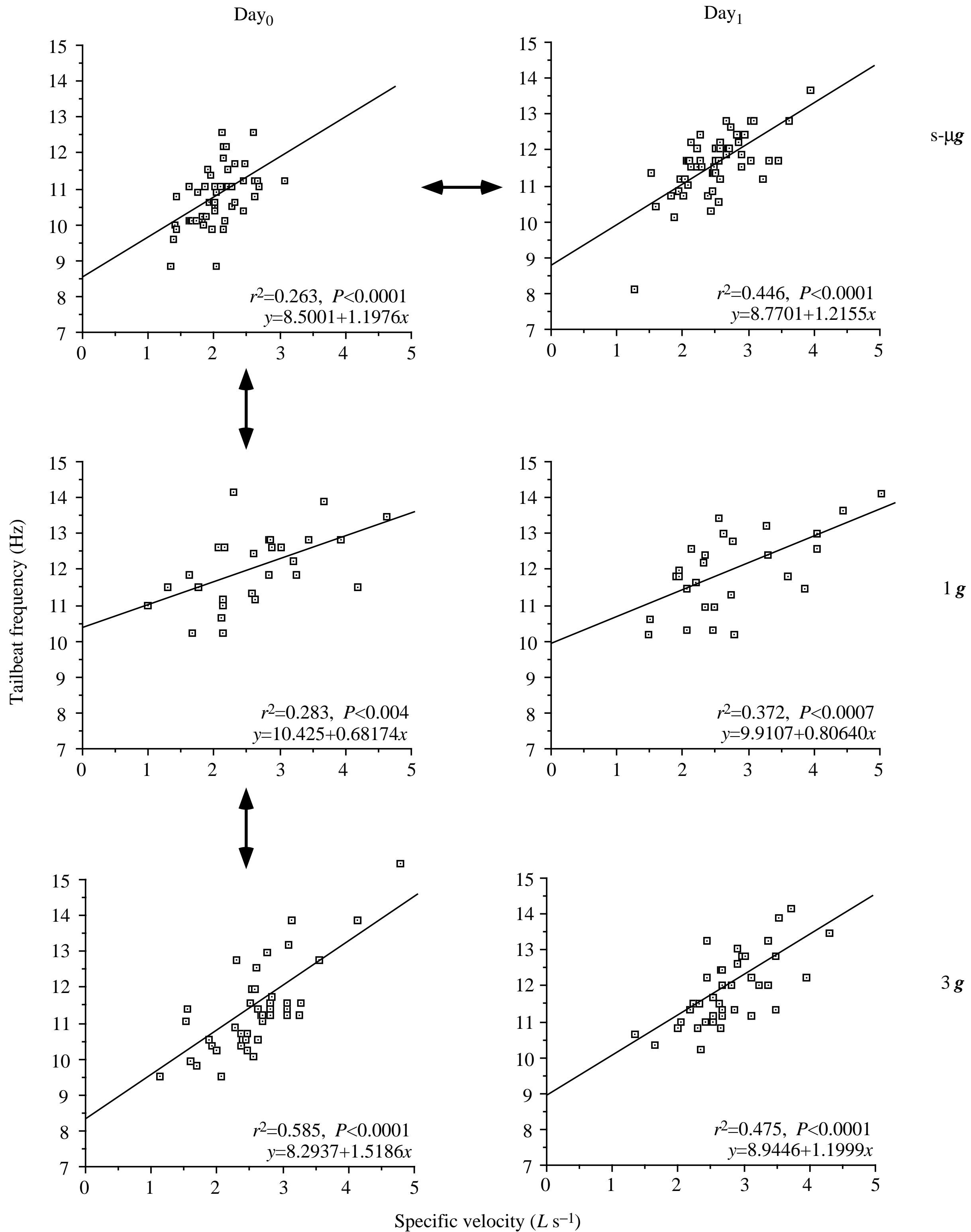

Fig. 1. The relationship between tailbeat frequency and specific swimming velocity in the clinostat-raised (simulated microgravity, s-μ***g***), normal gravity (1 ***g***) and centrifuge-raised (3 ***g***) ground-based tadpole groups during the two periods of testing. Day$_0$ represents 1 day after the start of the free-swimming stage and day$_1$ is 24 h later. *L*, body length. Here and in Fig. 2, regression lines are shown only where *P*=0.06 or better. Arrows indicate significant differences between groups (vertical) or between test periods (horizontal). Regression for both altered-gravity tadpole groups differed from that for the control (1 ***g***) group on day$_0$ but did not 24 h later. Only the simulated microgravity group exhibited a difference in mean tailbeat frequency (Table 1) and in the regression lines between the two test periods.

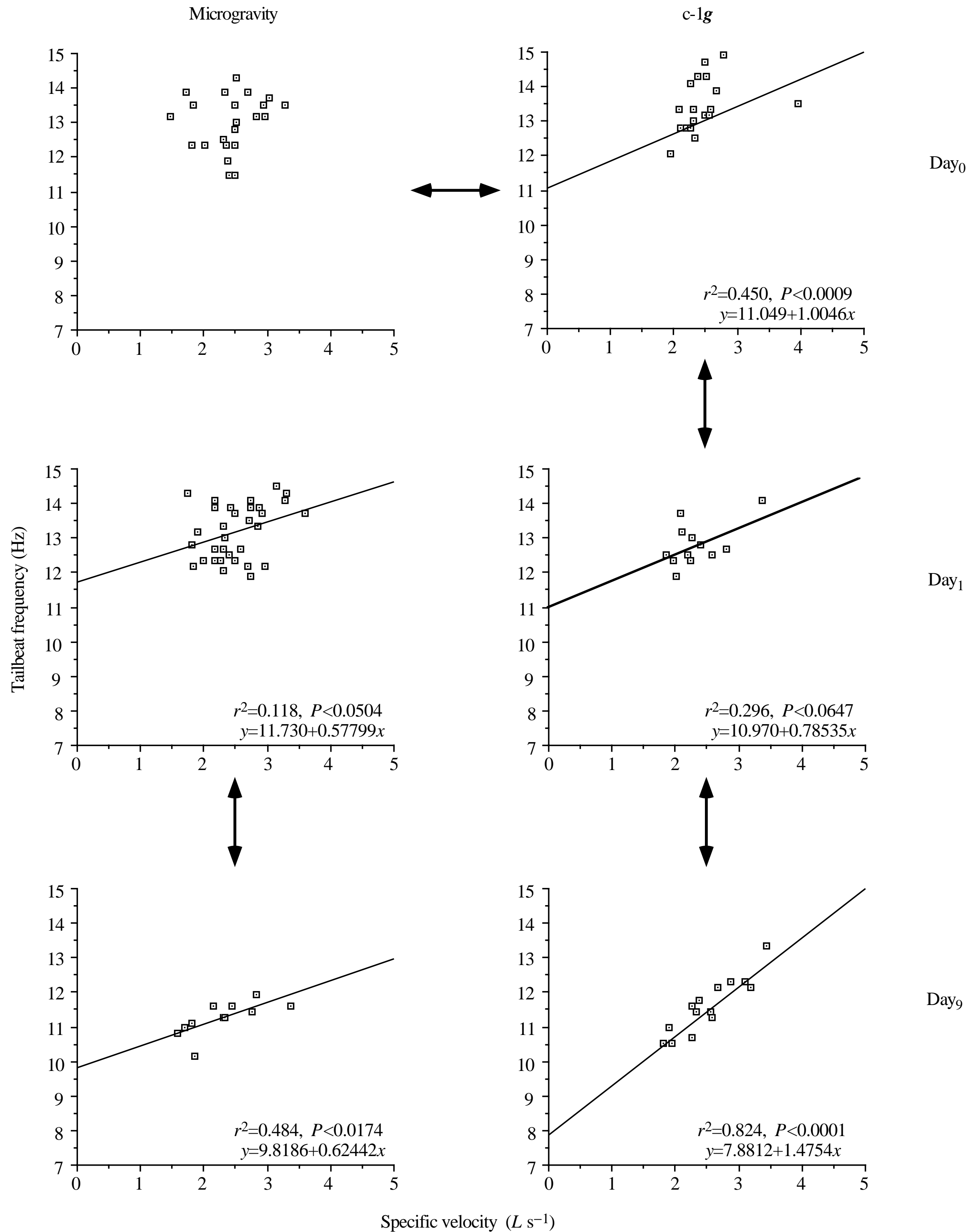

Fig. 2. The relationship between tailbeat frequency and specific swimming velocity in the space-flight (microgravity) and c-1***g*** onboard centrifuge tadpole groups over the three periods of testing. Day_0 represents the day of Shuttle landing, day_1 is 1 day postflight and day_9 is 9 days postflight. *L*, body length. Arrows indicate a significant difference between groups (horizontal) or between test periods (vertical). There was a difference between the regressions for the groups on day_0. The microgravity tadpoles only developed a significant relationship between frequency and velocity on day_1, after which there were no longer any significant differences between the two groups.

A few isolated cases of looping behaviour were seen in the microgravity tadpoles on day_0 and in both groups on day_1. No looping was observed on day_9. Several morphological anomalies were noted; however, none was severe or common in either treatment group: bloated abdomen (microgravity), dark pigmentation (microgravity), upwardly bent tail (microgravity, c-1***g***), elongated body (c-1***g***), stunted size (c-1***g***). By day_9, tadpoles in both groups swam in a normal, consistent pattern and external abnormalities were no longer evident (see Pronych *et al.* 1994).

Locomotion

Velocity did not differ significantly (Mann–Whitney *U*-test, P=0.2) between the microgravity and c-1***g*** tadpoles in any of the three postflight filming periods (Table 2).

On day_0, there was no correlation between tailbeat frequency and velocity in the microgravity tadpoles (*F*-test, r^2=0.027, P>0.46); however, these variables were highly correlated in the c-1***g*** tadpoles (P<0.0009) (Fig. 2). The two variables became significantly correlated in the microgravity tadpoles on day_1 (*F*-test, P<0.05) (Fig. 2), which differed significantly from day_9 (P<0.0001) (Fig. 2). On day_9, the two variables were significantly correlated in both the microgravity (P<0.017) and c-1***g*** (P<0.0001) tadpoles, but the regressions did not differ between the two groups (P>0.55). Initially, the microgravity and c-1***g*** tadpoles differed significantly in the regression between tailbeat frequency and velocity, but this difference disappeared by the second day postflight (P>0.26) (Fig. 2). There was also a significant difference between all three periods within the c-1***g*** tadpoles (P<0.02 day_0–day_1; P<0.0001 day_1–day_9). The c-1***g*** tadpoles also had a significantly higher mean tailbeat frequency (Mann–Whitney *U*-test, P=0.04) on day_0 (Table 2); however, after day_0, mean tailbeat frequency and velocity between the microgravity and c-1***g*** tadpole groups were indistinguishable.

It was only on day_1 that the microgravity and c-1***g*** tadpoles differed significantly in mean swimming angle (Mann–Whitney *U*-test, P=0.006) (Table 2). The mean angle decreased over time in both groups, with the microgravity group having the greater angle in all cases except day_0. Within groups, the microgravity tadpoles differed significantly in mean angle between all three test periods (P=0.01 day_0–day_1, P=0.008 day_1–day_9, P<0.0001 day_0–day_9), while the c-1***g*** tadpoles were significantly different between day_0 and day_1 (P<0.0001) and between day_0 and day_9 (P<0.0001) (Table 2).

The difference in position in the water column between the microgravity and c-1***g*** tadpoles on day_0 and day_1 (MANOVA, P<0.0001) disappeared by day_9. In general, the percentage of both microgravity and c-1***g*** tadpoles on the bottom of the flask was higher on day_0 and day_1 then decreased by day_9 (Fig. 3). Numbers at the top of the water column remained relatively constant at a low percentage for both the microgravity and c-1***g*** groups. On the basis of the raw data, the only significant difference in specific position between the microgravity and c-1***g*** groups was in the number of tadpoles at the bottom of the water column on day_1 (P<0.003).

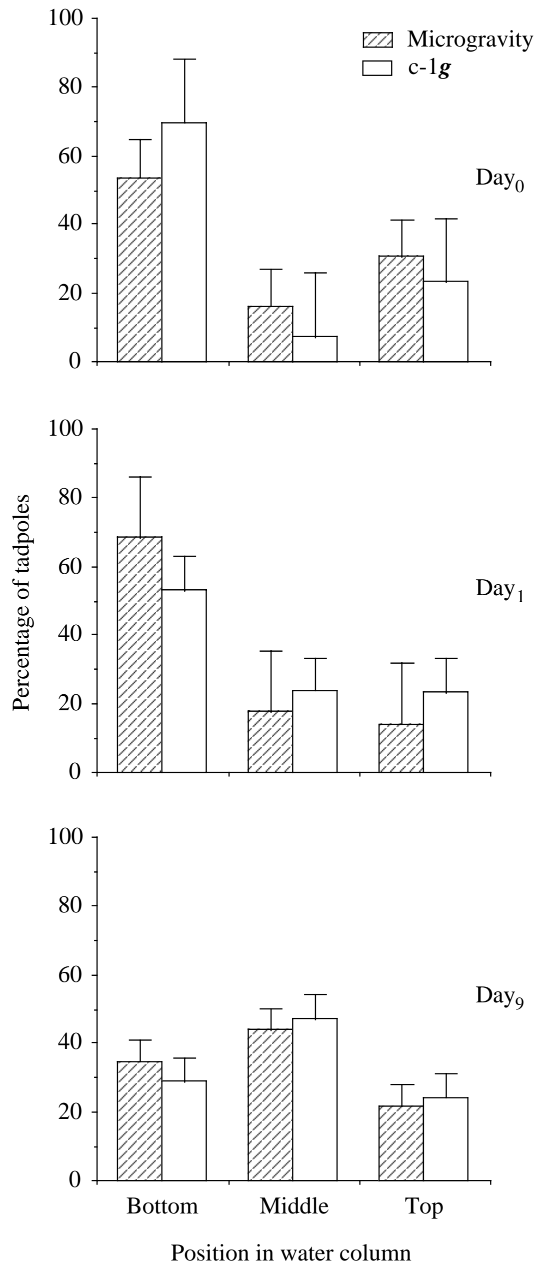

Fig. 3. Percentage of space-flight (microgravity; hatched bars) and c-1***g*** onboard centrifuge tadpoles (open bars) at various positions in the water column over the three periods of testing. The percentage of tadpoles at a particular position was calculated and corrected for the number of specimens in each flask. Day_0 is the day of Shuttle landing, day_1 is 1 day postflight and day_9 is 9 days postflight. Error bars show 1 S.E.M. For the microgravity group, N=35 on day_0 and day_1, and N=20 on day_9. For the c-1***g*** group, N=25 on day_0, N=24 on day_1 and N=20 on day_9.

Respiration

Initially the tadpoles had relatively high activity levels (i.e. swam rapidly) and thus pumped sporadically or did not stay in the field of view long enough to determine a buccal pumping rate. Hence, day_1 lacked a suitable number of data points (N=2 for each group) and measurable periods of buccal pumping

were few on day_0 in both postflight groups. On day_1, more tadpoles were observed pumping sporadically, and by day_9 the majority of tadpoles exhibited more consistent pumping behaviour. Mean buccal pumping rate (Table 2) increased in both the microgravity (Mann–Whitney U-test, P=0.01) and the c-1g groups (P=0.09) between day_0 and day_9, but only significantly so in the microgravity treatment. The c-1g group had a higher mean rate in both cases, although not significantly so (P=0.2 on day_0, P=0.1 on day_9).

The incidence of successful aerial respiration was initially low in both the microgravity and c-1g groups (Table 2). On day_0, tadpoles made mostly unsuccessful attempts at breaking the surface at the air–water interface. Attempts at lung inflation increased by day_9 and those tadpoles succeeded in taking a breath.

Discussion

Development on the Space Shuttle caused temporary changes in tadpole locomotor patterns. Both the c-1g centrifuge- and microgravity-raised tadpoles had a high tailbeat frequency on the first day back in Earth's normal gravity (day_0) relative to values reported in Hoff and Wassersug (1986), but this decreased to the predicted value by the ninth day postflight as the larvae adapted to their new environment. The onboard c-1g tadpoles maintained a consistent relationship between swimming speed and tailbeat frequency across the 9 day filming period, whereas the tadpoles raised in microgravity swam erratically (i.e. episodically dashing about rather than sculling midwater at a constant rate) on day_0 but developed a constant swimming pattern on day_1. The three ground-based tadpole groups (simulated microgravity, normal gravity and hypergravity) maintained normal tailbeat frequencies throughout the two test periods.

As just noted, the space-flight microgravity larvae were initially more fitful with respect to swimming than their c-1g controls. However, the tadpoles 'normalized' their swimming behaviours over time such that the differences observed early on disappeared by day_9. The clinostat-raised, simulated microgravity tadpoles exhibited significant differences in the tailbeat frequency and velocity from the 1g ground-based controls on one or both of the two test days. This may relate to the larger size and more developed appearance of the ground-based tadpoles, which were approximately 1 day older at each observation period than the space-flight animals. Given this fact, it may be more appropriate developmentally to compare day_0 tadpoles from the ground-based experiment with day_1 space-flight animals. Fig. 2 shows that tadpoles exposed to true microgravity in space flight show no significant relationship between tailbeat frequency and velocity on day_0, whereas both the day_0 and day_1 ground-based simulated microgravity tadpoles do show a significant relationship (Fig. 1). We infer from this that the clinostat's effect on developing amphibians does not mimic fully the effect of true microgravity obtained through space flight.

Xenopus laevis larvae extract oxygen from the water that they draw into their mouths and pump out through their gill filters. Immature or neurologically retarded tadpoles show uneven or sporadic pumping patterns (Orlando and Pinder, 1995). A suppression of pumping, which occurs when tadpoles dart about, is indicative of agitation. Both factors apply to the microgravity and c-1g tadpoles raised on the Space Shuttle. Initially, tadpoles in both groups swam erratically and buccal pumping occurred only sporadically. There was an increase in mean pumping rate in the microgravity group between day_0 and day_9. The c-1g group showed a similar tendency, although there was no statistically significant change. The rapid swimming and lack of continuous pumping indicate that both groups were agitated, possibly due to the stress of re-entry, a new gravity environment, or simply to the disturbance associated with filming. In contrast, all three ground-based tadpole groups had a more stable buccal pumping rate of approximately 1 Hz. Slight differences in neurodevelopment may be a factor in the more erratic pumping behaviours observed in at least some of the space-flight tadpoles.

Xenopus laevis lungs initially are inflated by taking a breath at the air–water interface (Pronych and Wassersug, 1994). In microgravity, there is no up or down, so that tadpoles may not be able to find the surface to take a breath of air. During the first period of postflight filming, more than half the tadpoles from both space-flight groups stayed on the bottom of the flask (Fig. 3), and attempts at air breathing were infrequent. This is consistent with the results obtained by Pronych *et al.* (1996) using tadpoles from the same space flight. Often, when they attempted to breathe air, the young tadpoles did not break the surface of the air–water interface successfully. Tadpoles in both groups also swam at abnormally steep angles immediately upon landing (day_0). The onboard c-1g tadpoles, however, normalized their angle within the first day back in the 1g gravitational environment, whereas the microgravity tadpoles took longer to acquire a lung volume large enough to normalize their swimming posture. Similarly, the tadpoles in both groups moved to the more normal, even distribution throughout the water column by day_9. It is worth noting that the tadpoles had grown substantially between day_0 and day_9 (Table 2), and the relatively shallow flask (3.2 cm deep) may have affected the position and angle of the larger tadpoles.

A few anomalies were observed in the morphology and behaviour of a small number of space-flight tadpoles in the first postflight filming period. In particular, several of the microgravity-raised tadpoles exhibited looping behaviour, i.e. swimming in forward-outside loops, which has been noted previously in tadpoles and fishes raised in normal gravity and then observed in microgravity (de Jong *et al.* 1996; Moorman *et al.* 1997; Pronych *et al.* 1996; Rahmann and Slenzka, 1994). This behaviour in our tadpoles was no longer apparent by day_9 of filming, as the tadpoles adapted to Earth's normal gravity. The ground-based tadpoles exhibited a higher percentage of morphological abnormalities; this may result from the smaller growth chambers (plastic bags) and different spawnings.

There are several conclusions that can be drawn from this

study. Development in microgravity does affect some aspects of swimming but not respiratory behaviours of *X. laevis* tadpoles, once they have inflated their lungs. Most differences appear greater on the second day postflight than on the first day postflight. We believe this was due to the fact that the c-1***g*** onboard centrifuge specimens were unavoidably exposed to various exotic gravity regimes (first microgravity then hypergravity), during Shuttle re-entry. Pronych *et al.* (1996) similarly found a greater difference in optomotor behaviour between microgravity- and c-1***g***-raised tadpoles in the second day postflight.

The most consistent but transient effect of development in microgravity is a decreased tailbeat frequency relative to the c-1***g*** group, but no retardation in neuronal development is evident. Locomotor behaviours began to normalize after the second day postflight and were indistinguishable between the microgravity and c-1***g*** tadpoles by day_9.

Differences in swimming angle between microgravity and c-1***g*** raised tadpoles seen on day_1 are in concordance with differences in the centre of buoyancy. This behavioural observation fits well with the significant decrease in lung volume reported by Black *et al.* (1996) in microgravity tadpoles from the same flight.

The results from the clinostat (simulated microgravity) experiments were not identical to those from the space-flight microgravity tadpoles. The differences may be, in part, attributable to the fact that the ground-based simulated microgravity tadpoles were raised in smaller containers yet were slightly larger when tested. For example, the higher overall tailbeat frequency of the space-flight experiment animals compared with the ground-based experiment animals is explained by their slightly younger stage of development (van Mier, 1986). However, when tadpole age differences were taken into consideration, there still appeared to be a difference in the effect of true microgravity *versus* simulated (clinostat) microgravity.

Finally, ontogeny in hypergravity (3***g***) does not affect the neuromotor development of swimming behaviours. The only significant effect of early development in hypergravity was on swimming angle, which can be related to buoyancy and lung development. For small aquatic organisms, such as young *Xenopus laevis* tadpoles, early development in modest hypergravity (i.e. 3***g***) has less impact than development in microgravity.

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