Variables Determining Buoyancy in Juvenile Mink Frogs, *Rana septentrionalis*, with Comparisons to Boreal Chorus Frogs, *Pseudacris triseriata maculata*, and Wood Frogs, *Rana sylvatica*.

By

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A thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
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Abstract

Aquatic organisms must optimize buoyancy to minimize energy expenditure in locomotion and in holding position. This study examined the hydrostatic role of lungs in the buoyancy regulation of larval *Rana septentrionalis* in relation to its mode of life. Wet weights in air and water, gas volume, gas-free volume and dry weight were determined in various larval stages. A newly-defined, dimensionless buoyancy index was then calculated in terms of the animal's gas-free specific gravity and gaseous lift factor (ratio of included gas volume to gas-free volume). Field and laboratory observations showed larval and early metamorphic stages to be benthic, inactive, and resident in shallow water near shore in dense vegetation. Accordingly, the buoyancy profile showed this species to remain negatively buoyant from hatchling to early metamorphic stages, although significant ontogenetic variation did occur; least buoyant were hatchlings and larvae just before and after hibernation. Buoyancy variation was due to considerable changes in gaseous lift factor (as lung volume changed) and specific gravity (varying mainly with body water content).

In laboratory experiments, *R. septentrionalis* ingested all types of substrate provided (detritus, silt and sand) during feeding. This increased their specific gravity, and they responded by increasing gaseous lift thereby maintaining buoyancy. Experiments with tadpoles placed on silt showed that the digestive tract was filled in 48 h and significant increases in weight and lung volume occurred within 6 h and 12 h respectively, maintaining buoyancy control;

consequently buoyancy levels did not vary significantly over time.

In behavioural experiments, *R. septentrionalis* did not reduce its activity, vertical distribution or buoyancy level in the presence of caged predators nor did *Rana sylvatica*. In contrast, a temporary pond species, *Pseudacris triseriata maculata*, did reduce all three variables under these conditions, but did not reduce its number of air breaths. Thus, these tadpoles were not inhibited by the predator from going to the surface but modified their behaviour to avoid predation.

In summary, buoyancy is closely linked to mode of life. For species inflating their lungs during early larval stages and inhabiting permanent ponds with an abundance of predators, the ability to maintain optimal negative buoyancy levels by regulating lung volume is a highly adaptive trait.

Acknowledgements

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List of Abbreviations

25*e*	Early stage 25 in which lungs are not yet inflated
25*l*	Late stage 25 which have lung gases present
31-33*a*	Tadpoles of stage 31-33 found in autumn - pre-hibernation
31-33*s*	Tadpoles of stage 31-33 found in the spring - post-hibernation
BI	Buoyancy index
C	Concentrated non-aqueous specific gravity
FW	Fractional water content
LF_g	Gaseous lift factor
ΔP	Pressure increment required to make the tadpole neutrally buoyant
P_{atm}	Local atmospheric pressure
PNB	Total pressure (hydrostatic plus atmospheric) at the depth neutral buoyancy is achieved
P_{pan}	Total pressure (hydrostatic plus atmospheric) at the depth of the weighing pan
P_{expt}	Total pressure (hydrostatic plus atmospheric) experienced by the tadpole, at depth, in the aquarium prior to capture
SG	specific gravity
$V_{bubble,Pexpt}$	Corrected volume of any bubbles at aquarium pressure
$V_{bubble,\ Patm}$	Volume (measured above the water) of any bubbles released after capture, at atmospheric pressure
V_{gas}	Volume of included gas corrected to aquarium pressure
V_{gf}	Gas-free body volume of the tadpole
$V_{L,\ Pexpt}$	Lung volume corrected to aquarium pressure but uncorrected for escaped bubbles.
$V_{L,\ Ppan}$	Lung volume at the depth of the weighing pan
$V_{L,\ PNB}$	Lung volume at PNB
W_{dry}	Dry weight
W_{water}	Weight of the water in body tissues
W_{wet}	Wet weight in air
W_{wg-}	Gas-free weight in water
W_{wg+}	Weight in water with lungs intact

INTRODUCTION

Generally, aquatic organisms tend to sink in either fresh water or sea water when their specific gravity exceeds that of the medium. Therefore, to overcome sinking, aquatic organisms have evolved several adaptations that provide lift (buoyancy). Maintaining an optimal buoyancy is important as it minimizes energy expended in swimming (Alexander, 1966), holding vertical position in the water column (Graham *et al.*, 1987) and maintaining position within stream environments (Gee, 1987; Wassersug and Feder, 1983).

Many strategies are available to provide lift to aquatic organisms and some are size-dependent (Alexander, 1990). Very small organisms such as protistan and other planktonic species sink very slowly and can use eddies to remain suspended in the water column. Alexander (1990) has estimated a maximum mass limit of 2×10^{-9} kg (or a maximum diameter of 150 μm for a spherical organism) for this mechanism to be successful.

Many planktonic organisms are too big to be kept suspended by eddies. Some small ciliates can hover, i.e., swim constantly upward just enough to avoid sinking (Alexander, 1990). Copepods and other planktonic crustaceans use the "hop and sink" strategy, which alternates small bursts of upward swimming with periods of sinking (Morris *et al.*, 1985). The "hop and sink" strategy requires less energy and is more economical for larger planktonic organisms than hovering (Alexander, 1990).

Large and dense aquatic organisms such as fishes or squids can use hydrodynamic lift to prevent sinking (Magnuson, 1970; O'Dor, 1988). Movement of the body, fins or caudal keels can generate lift in the same way that an aeroplane is supported by aerodynamic lift. Hydrodynamic lift can be combined with buoyancy or static lift, which is derived from one or more body components that are lighter than water: gas inclusions, lipids of low specific gravity or low density body fluids.

Gas inclusions provide lift in a variety of species including phytoplankton (Visser *et al.*, 1995), cephalopods and siphonophores (Alexander, 1990), reptiles (Graham *et al.*, 1987) and amphibians (Gee and Waldick, 1995; Bruce *et al.*, 1994). Most teleost fishes have a gas-filled swimbladder (Alexander, 1990), but gases held in the intestine (Gee and Graham, 1978), stomach (Gee, 1976), or in the mouth, pharynx, or buccal or opercular cavities (Schuster, 1989; Gee and Gee, 1991; 1995) also provide lift in a variety of species. To adjust swimbladder volume, physostomous fishes gulp air or spit gases (Alexander, 1966; Gee, 1983) while physoclistous species secrete or resorb gases. Limited adjustments can also be made by relaxing or contracting muscles in the swimbladder wall (Gee and Gee, 1976).

Lipids (with specific gravity < 1.0) provide lift in aquatic organisms, especially in fishes. Some elasmobranches possess large quantities of the hydrocarbon squalene, primarily in the liver, providing an overall body density near that of sea water (Heller *et al.*, 1957). Wax esters are used for buoyancy in

the coelacanth (*Latimeria*) and some lantern fishes (Alexander, 1990). Other lipids providing lift include diacyl glyceryl ethers and triglycerides (Gee, 1983). Furthermore, some mid-water crustaceans also acquire lift from large proportions of lipids in their body (Childress and Nygaard, 1974). Larval fish condition (tissue density) is one of the factors determining successful vertical movements in the critical stage between yolk-sac resorption and functional swimbladder development in several marine species (Sclafani *et al.*, 1993).

Squid have body fluids in which sodium ions are replaced by low-molecular weight ammonium ions (Denton *et al.*, 1969; Denton, 1974). The exclusion of sulfate ions in the mesoglea of scyphozoan jelly fish, ctenophores and some siphonophores also contributes to lift (Bidigare and Biggs, 1980). Many marine fishes acquire lift from a high water content accompanied by a reduction in skeletal structure and musculature (Gee, 1983).

Individual characteristics such as size, age, and sexual development (Gee, 1977; Pinder and Eales, 1969; Alexander, 1990) and environmental factors including photoperiod, hydrostatic pressure, water temperature, velocity and salinity (Saunders, 1965; Neave *et al.*, 1966; Gee, 1968, 1977; Machniak and Gee, 1975; Beaver and Gee, 1988; Gee and Holst, 1992) act to influence the amount of static lift from the swimbladder and, ultimately, affect buoyancy.

Lungs of larval anurans appear to have an important hydrostatic function. Although lungs have been suggested to play a role in buoyancy control of larval amphibians (Feder and Wassersug, 1984; Wassersug and Feder, 1983;

Wassersug and Seibert, 1975), the first evidence of such a role was documented by Gee and Waldick (1995). They described ontogenetic changes in static lift from the lungs, tissue density and resulting buoyancy from time of hatching to metamorphosis for five anuran species inhabiting shallow, temporary and permanent bodies of water. Of the five species studied by Gee and Waldick (1995) *Bufo americanus* (toad) did not inflate their lungs prior to metamorphosis. The other four species inflated their lungs in stages 25-26. There were differences within and among species over time in lift and weight values and in buoyancy levels. *Rana sylvatica* (wood frog), *Pseudacris triseriata maculata* (boreal chorus frog) and *Hyla chrysoscelis/versicolor* (treefrog) were near neutral buoyancy throughout their larval development while *Rana pipiens* (leopard frog) remained more negatively buoyant. Furthermore, Gee and Waldick (1995) found that nearly neutrally buoyant larval stages were mostly active in the water column while negatively buoyant stages were either active or sedentary on the bottom of aquaria.

Gee and Waldick (1995) found larvae of *R. sylvatica* to be sensitive to changes in their relative density. When larvae were placed into a solution of Percoll (1.008 g/ml), their lung volume was reduced significantly to overcome their increased buoyancy in this solution and to maintain buoyancy. Because of this observed plasticity in lung volume, they suggested that variables encountered in the field such as water current, dissolved oxygen, intensity of illumination or water turbidity may influence buoyancy levels of larval anurans as

do some of these variables in fishes.

Two important environmental factors for tadpoles are permanence of water bodies and presence of predators (Skelly, 1995; 1997; Skelly and Werner, 1990). Most temporary bodies of water have few or no predators and those that are present are usually small (Skelly, 1997). Tadpoles inhabiting temporary ponds must develop quickly and metamorphose before the pond dries up (Skelly, 1997). Since predation risk is low, tadpoles are active and emphasis is oriented toward feeding and using all of the water column.

On the other hand, a greater diversity and abundance of large predators is encountered in permanent bodies of water. As the risk of predation increases, tadpoles benefit more from being less active, thus avoiding predation (Skelly, 1997). Since tadpoles in permanent bodies of water are not restricted by time to metamorphose quickly, they can afford to become more secretive, develop more slowly, use less of the water column or even to become benthic.

Active species inhabiting temporary ponds have indeed been found to use all of the water column and to remain near neutral buoyancy during their hatchling, larval and metamorphic stages (Gee and Waldick, 1995; Gee, in prep.).

Pseudacris triseriata maculata and *Rana sylvatica* are active species and the former is known to be plastic in its activity, becoming inactive in the presence of predators (Skelly, 1997). The switch to inactivity could be facilitated by plasticity in buoyancy, allowing juveniles to become negatively buoyant when

inactive. Both *Pseudacris triseriata maculata* and *Rana sylvatica* are very close to neutral buoyancy when active and undisturbed by predators (Gee and Waldick, 1995).

Many aquatic reptiles and mammals ingest substrate particles that increase their specific gravity (Taylor, 1993). Aquatic tadpoles inhabit different environments (ponds, streams, lakes, etc.) and consequently encounter different substrates upon which they feed. Juveniles are known to feed by rasping their food off the substrate. Thus it would be possible for them to ingest particles of substrate accidentally while feeding. Since juvenile mink frogs are benthic and occur on detritus, large boulders, rocks and sand, they could potentially ingest substrate particles which could ultimately increase their specific gravity and affect their buoyancy. Buoyancy studies on the Australian frog, *Litoria peroni*, showed that tadpoles held with gravel and sand substrate did in fact ingest substrate particles that increased the weight of larvae in water (Gee, pers. comm.).

The present study examines aspects of buoyancy and its regulation by tadpoles of the mink frog, *Rana septentrionalis*, and relates this information to the mode of life of this species (Fig. 1). Mode of life refers to the way in which an organism has evolved to live within a particular environment as reflected by its physiology, morphology and behaviour. For larval amphibians this includes: vertical and horizontal distribution in the water, pattern of activity, diet and feeding behaviour, habitat preferences and reactions to environmental variables.

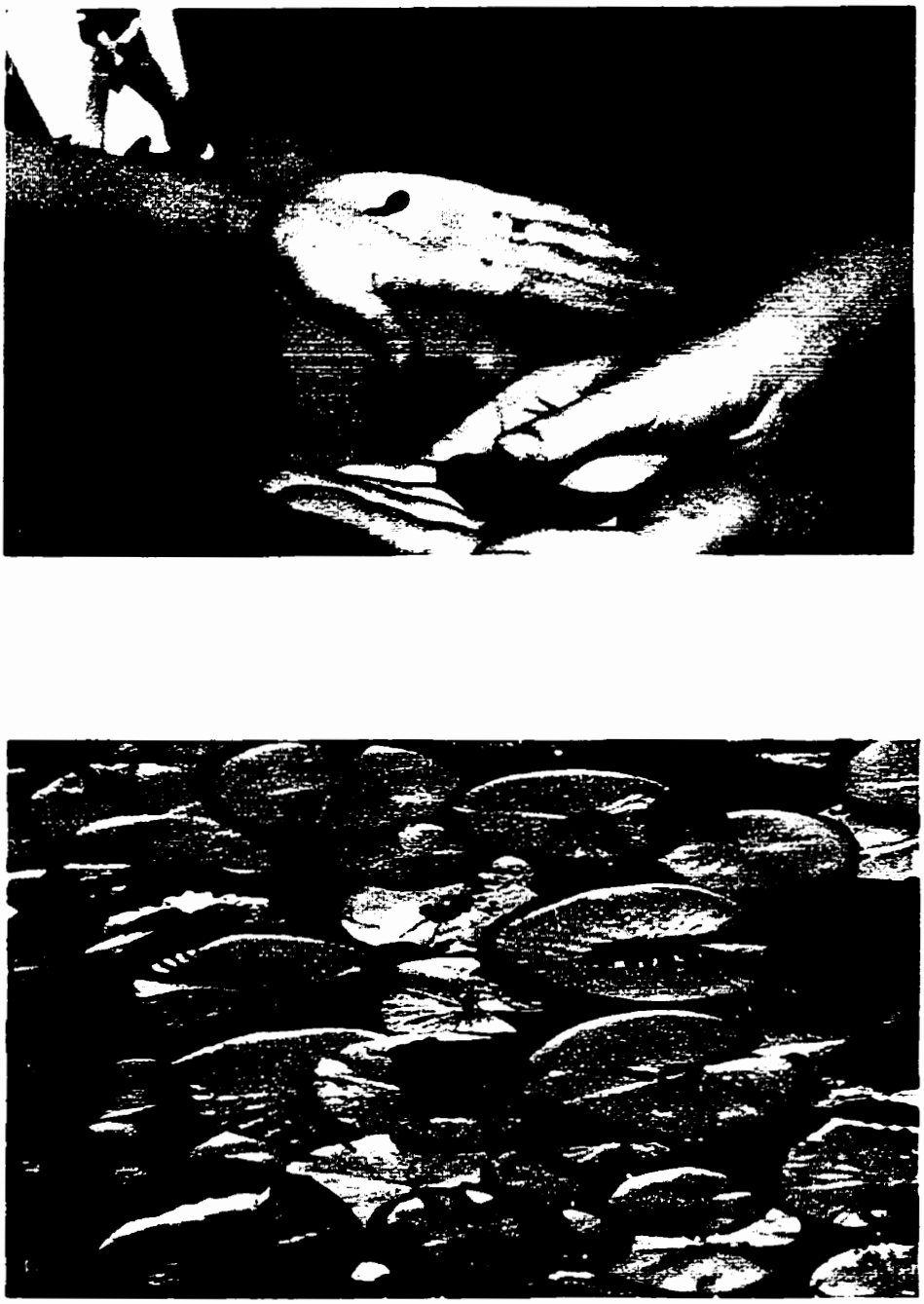


Figure 1: **A. Differences in larval size between early larval and late larval stages of *Rana septentrionalis*. B. Adult *Rana septentrionalis*.**

Variables that are important to larval anurans include: substrate, detritus, vegetation, predator presence (as well as the type of predators), dissolved oxygen, water temperature, turbidity, current depth and permanence of water (temporary vs permanent). *Rana septentrionalis* remains aquatic throughout its life span. Its distribution is restricted to the northeastern part of North America, *i.e.*, from southern Labrador and the Maritime provinces to southeastern Manitoba in Canada and from northern New York and Wisconsin to Minnesota in the United States. Isolated populations are also found in northern Québec and northern Labrador (Conant and Collins, 1991). *Rana septentrionalis* prefer still and permanent bodies of water such as ponds and lakes with dense emergent and floating aquatic vegetation, including lily pads, and with muddy and silty substrate (Courtois *et al.*, 1995). Lily pads are important to mink frogs since the leaves are frequently used as basking sites (Preston, 1982) and the stalks as attachment sites for eggs (Moore, 1952). Spawning occurs in late June or early July, and metamorphosis occurs one or, occasionally, two years later (Hedeen, 1971). Adults are rarely seen away from water except occasionally on rainy nights. Tadpoles feed primarily on algae (Hedeen, 1972a) and their predators include among others: great blue heron, larvae of the tiger salamander (*Ambystoma tigrinum*), five-spined stickleback (*Culaea inconstans*) and invertebrate predators including insects and leeches (Hedeen, 1972b).

In Manitoba, mink frogs are known only in the Whiteshell and Nopiming provincial parks (Preston, 1982), where their ideal habitats are often created by beaver dams (pers. obs). Major predators encountered in the Whiteshell include

dragon fly larvae (*Aeshna* spp. and *Cordulia* spp.), mudminnows (*Umbra limi*), and giant water bugs (*Belastoma* spp. and *Lethocerus americanus*) (Watkins, 1997).

Specific objectives of this study were to:

1. describe vertical and horizontal distribution and activity of larval and metamorphic *Rana septentrionalis* based on field and laboratory observations;
2. describe a buoyancy profile showing ontogenetic changes in gaseous lift, specific gravity and resulting buoyancy of hatchling, larval and metamorphic *Rana septentrionalis*;
3. test hypotheses that larval *Rana septentrionalis* (a) ingest substrate particles that increase their specific gravity and (b) compensate and react to this change in specific gravity by increasing gaseous lift, thereby maintaining buoyancy at a constant level; and
4. test the hypothesis that in the presence of predators, larval *Rana septentrionalis*, *Rana sylvatica* and *Pseudacris triseriata maculata* will reduce their vertical distribution in the water column, activity and buoyancy and, consequently reduce their frequency of air breathing.

MATERIALS AND METHODS

Study site

Field studies were conducted in McGillivray Creek, Whiteshell Provincial Park, Manitoba, during the summer of 1996. Observations of distribution and abundance were made in a large beaver pond 0.25 km south of Hwy. 44 that was formed in the creek channel. The pond was approximately 225 m in length and averaged 20 m in width and 1 m in depth with a maximum depth in the original stream channel of about 2 m. The average substrate depth was about 0.5 m and consisted predominantly of detritus and mud with smaller areas of sand and silt particles. Emergent and submergent vegetation included species of *Scirpus*, *Myriophyllum*, *Polygynum* and *Nymphaea*. Water lilies proliferated in summer. During summer days of 1996 (June 18 to August 22), water temperature varied from 26° to 28.8°C at the surface and from 19° to 26°C off-shore at 1m depth. Oxygen and conductivity levels were measured using a YSI Model 54 oxygen meter with a mechanical stirrer and a YSI SCT meter, respectively. Summer oxygen readings on shore ranged from 5.4 to 8.4 ppm and offshore from 6.3 to 7.8 ppm at the surface and from 2.2 to 6.4 ppm at the bottom. Conductivity was constant throughout the creek at any one time and varied from 37 to 90 µMho over the summer while salinity ranged from 0.05 to 1 ppt.

Heavy rain in late summer of 1996 raised the water level by about 0.6 m

(Fig. 2), which was followed by a drop of the same magnitude as the rain began to wash out the beaver dam. Damage became irreversible as vandals enlarged the breach in the beaver dam (Fig. 3). Water was then restricted to the original stream channel, and the pond area became contracted to < 5% of its original area (Fig. 4). Some larvae of the year could be dipnetted from the mud in fall of 1996. In spring of 1997, some overwintered larvae were dipnetted from the substrate and transformed individuals were found in the field in late June, 1997. Numbers of frogs were greatly reduced at McGillivray Creek during the summer of 1997 as erosion of the beaver dam continued (Fig. 5). In 1998, a few overwintered larvae were caught in early spring, but none were seen or captured later that summer.

Horizontal and vertical distribution in the field

Minnow traps were used to determine the horizontal and vertical distribution of stages 25ℓ to 45 (Gosner, 1960; see below) in littoral areas of McGillivray Creek from May 28 to August 22, 1996. Ten transects, perpendicular to shore, were established at 3 m intervals and traps were set at 0 m (i.e. partly emersed on shore) 3, 6 and 9 m from shore. Traps on-shore and at 3 m from shore were placed at the bottom in approximately 15 and 30 cm of water, respectively, in very dense emergent and submergent vegetation. Traps positioned at 6 m from shore were also set at the bottom, in 55-65 cm of water but here vegetation was sparse. At 9 m from shore, 2 traps were set on a pole,

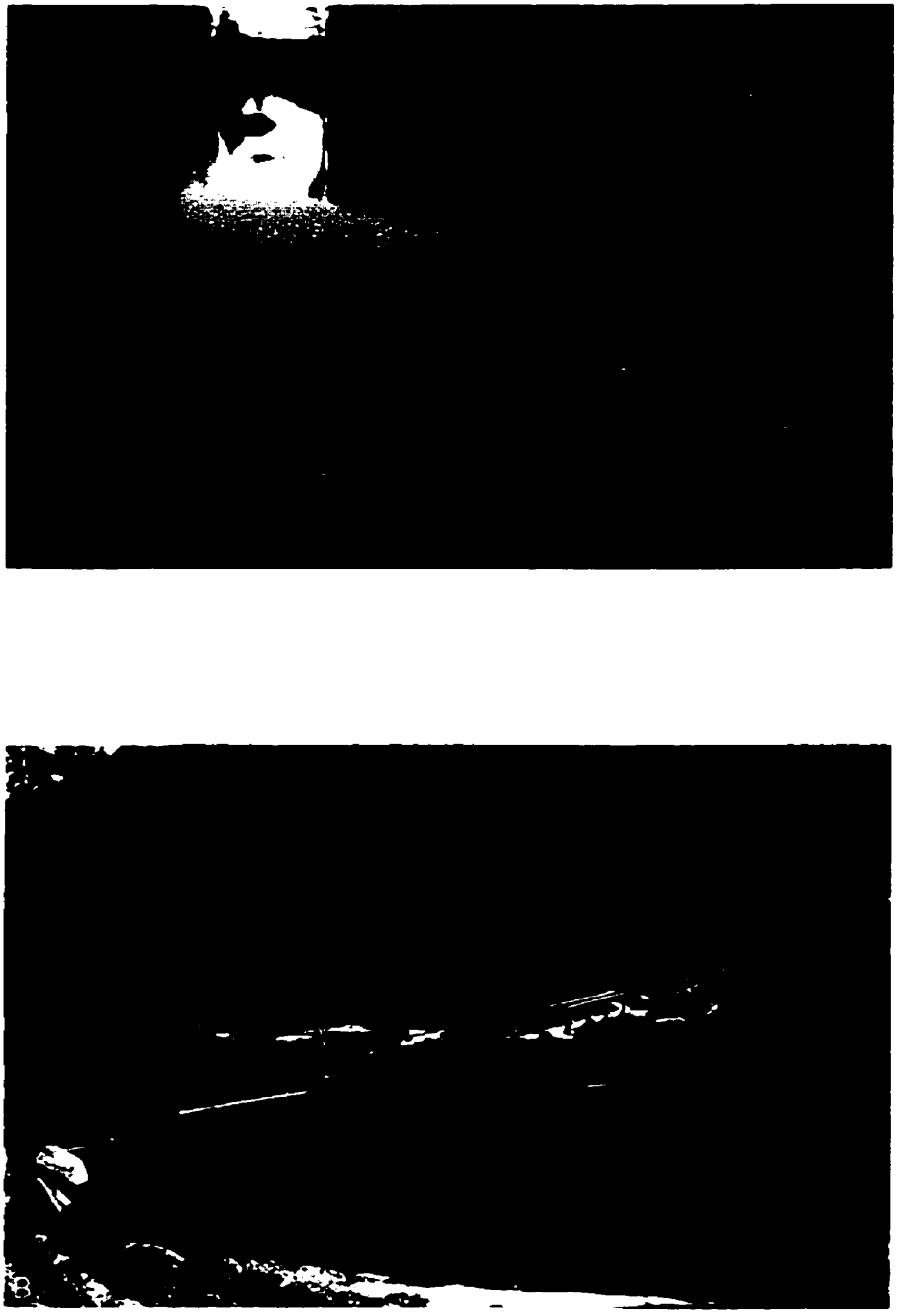


Figure 2: Study site in McGillivray Creek, Whiteshell Provincial Park, Manitoba, in summer of 1996. A. View of the channel. B. View of the pond at the beaver dam.

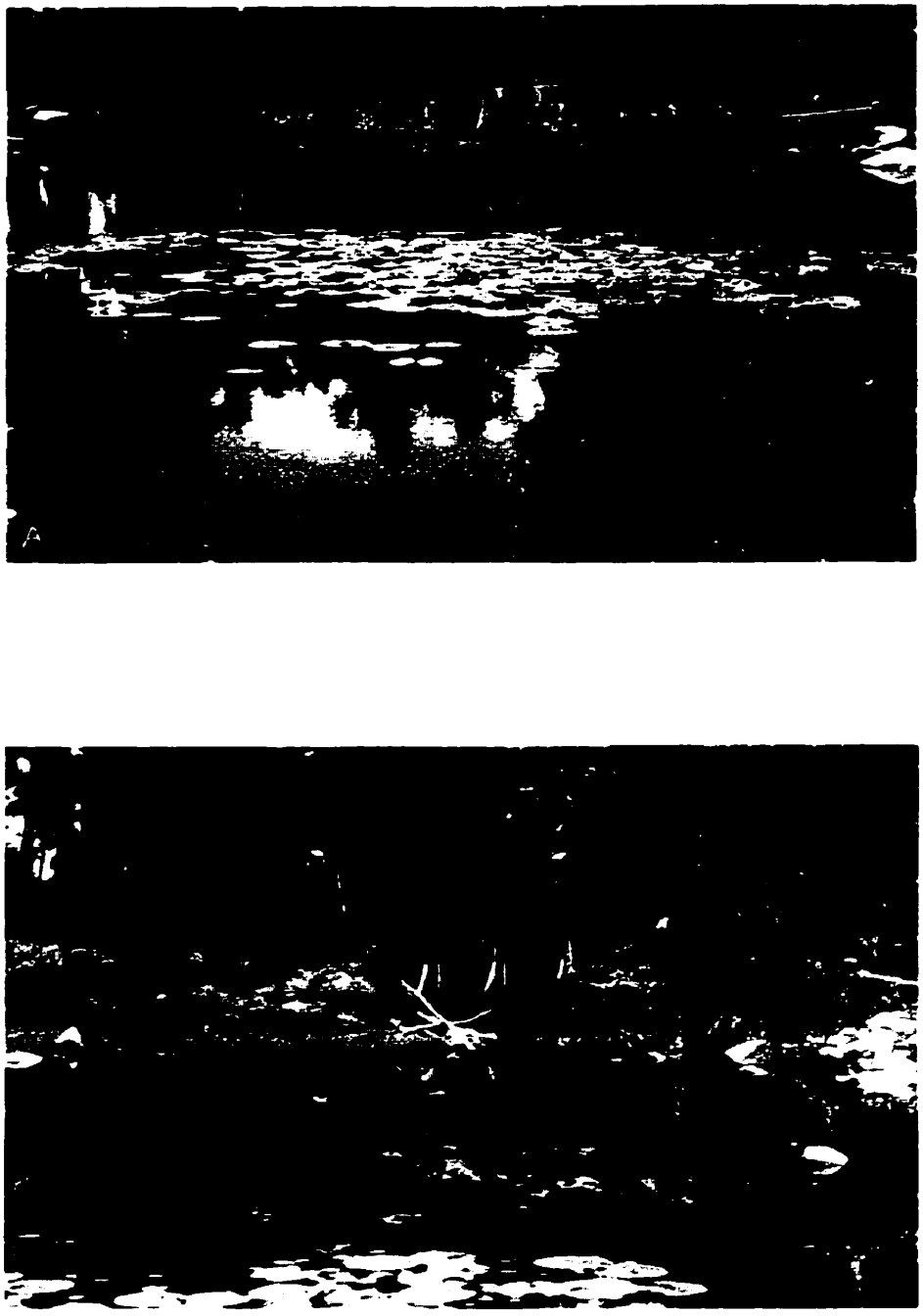


Figure 3: Study site in McGillivray Creek, Whiteshell Provincial Park, Manitoba. Breach in the beaver dam. A. Spring. B. Summer.

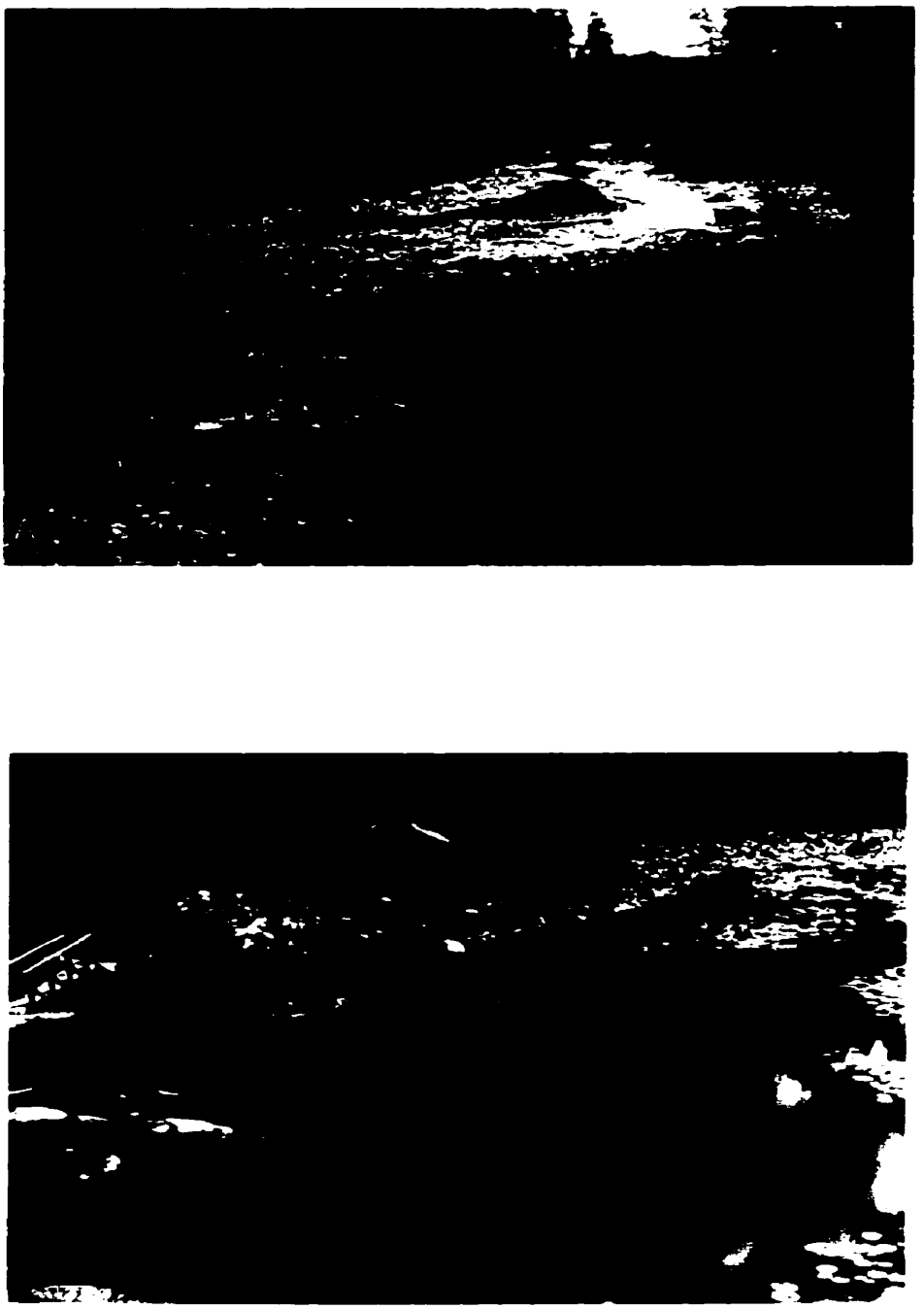


Figure 4: Study site in McGillivray Creek, Whiteshell Provincial Park, Manitoba, in fall of 1996. A. View of the channel. B. View of the pond at the beaver dam.

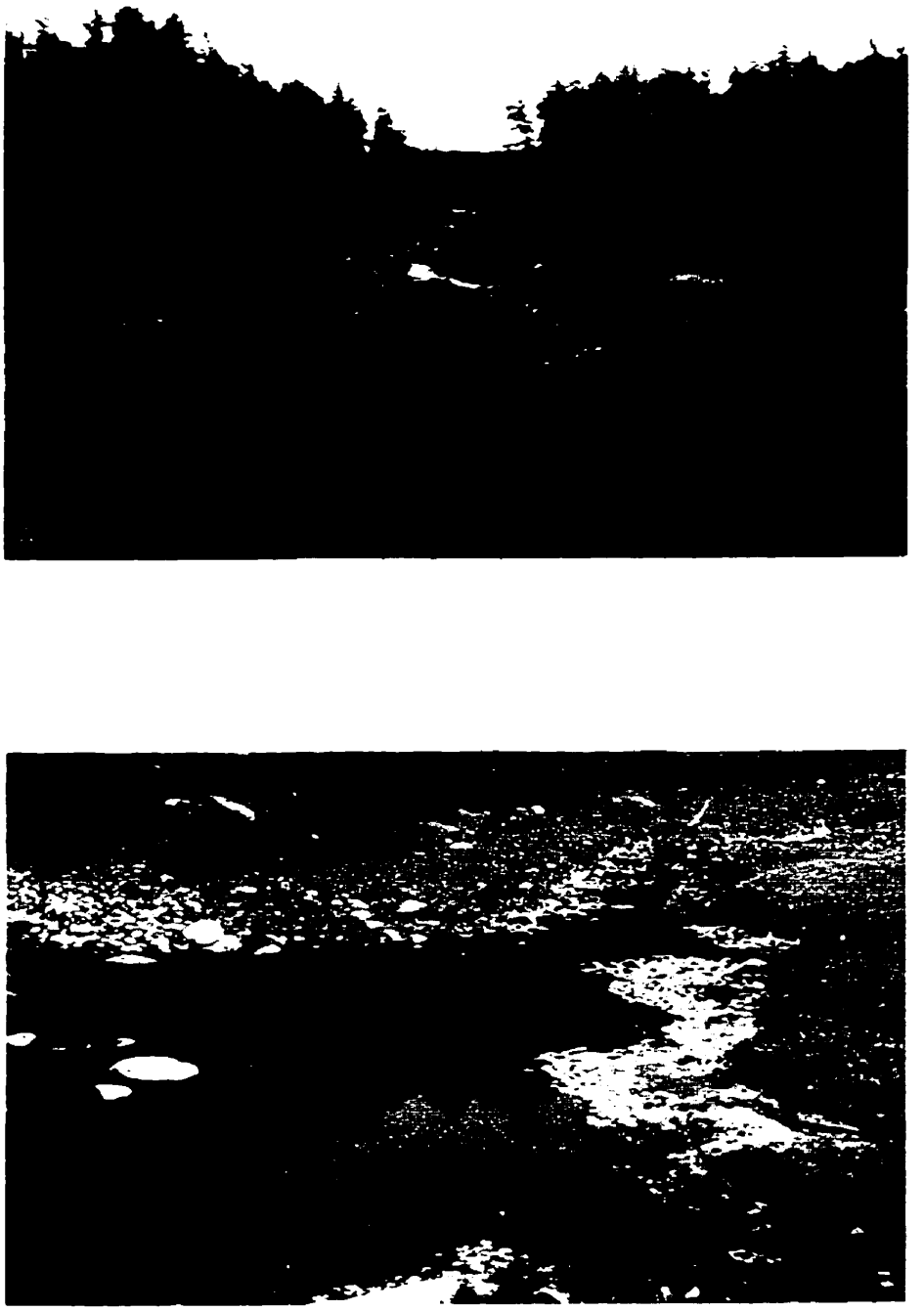


Figure 5: Study site in McGillivray Creek, Whiteshell Provincial Park, Manitoba, in summer of 1997. A. View of the channel. B. View of the pond at the beaver dam.

one at the surface, the other at the bottom in 75 - 90 cm of water. There was little vegetation off-shore. Deeper water could not be sampled because of equipment limitations. Traps were set in the morning and removed in the afternoon. The number of tadpoles captured was recorded and densities expressed as mean number caught / 10 traps / h. All individuals were released.

Field collection

Eggs, larvae, and metamorphics were collected from the field and held in the laboratory (under the Canadian Council of Animal Care guidelines) to be used for behavioural observations, completing buoyancy profiles, and conducting other buoyancy-related experiments. Developmental stages were classified according to Gosner (1960) and three developmental groups are considered in this thesis: hatchlings (stages 20 to 25*e*), larvae (stages 25*l* to 39) and metamorphics (stages 40 to 45). The notation 25*e* is used to refer to early stage 25 in which lungs are not yet inflated, while 25*l* refers to late stage 25 which have lung gases present. Similarily, stage 31/33*a* refers to tadpoles prior to hibernation in autumn, and 31/33*s* to the same stages found in the spring.

Larvae and metamorphics of *Rana septentrionalis* were collected weekly by seining and/or dipnetting from the McGillivray Creek drainage and transported in jars, with lids loosened, to the laboratory. Individuals were staged and weighed (wet weight in air - ± 0.001g), and total length (± 0.1 mm) was measured with vernier calipers. Juveniles were then segregated in aquaria at ambient

temperature according to stage of development. Aquaria were supplied with slow-flowing dechlorinated water and an air stone, and were illuminated with two 60 watt bulbs on a 10L:14D photoperiod. Metamorphics were further supplied with floating platforms for resting. Water temperature was adjusted to the experimental level of 13°C ± 1°C at a rate of 1°C/day. Mink frog larvae were fed Tetra-min flakes daily. Aquaria were cleaned every two days to prevent accumulation of faeces and fungus development (Gromko *et al.*, 1973; Licht, 1967), and dead tadpoles were removed immediately .

Egg masses from the field were held in the laboratory in still water aquaria supplied with an air stone, *R. septentrionalis* at 18°C ± 1°C and *Pseudacris triseriata maculata* and *Rana sylvatica* at 12.5°C ± 1°C until hatching, after which they were transferred to slow-flowing dechlorinated water aquaria at 13°C ± 1°C and held as described above. While *R. septentrionalis* was fed Tetra-min flakes as above, *P. triseriata maculata* and *R. sylvatica* larvae were fed 3:1 rabbit chow/ Tetra-min flakes daily (Alford and Harris, 1988).

Numbers available dictated the stages selected for each species and some stages were combined to give adequate numbers per treatment. In some procedures, individuals were euthanized and all excess tadpoles were returned to the collection site. For buoyancy-related measures, 8 individuals were used per treatment (unless otherwise mentioned), as this number gave the least variation using the fewest individuals possible.

Vertical distribution and activity in the laboratory

Vertical distribution and activity were measured in the laboratory to complement field observations and to relate these to buoyancy levels. Observations were made on stages or groups of stages throughout larval and metamorphic development (25*ℓ*, 27, 29, 31/33*a*, 40 and 42/43, 45).

Fifteen tadpoles of the above stages were placed in an aquarium (55 x 40 x 43.5 cm for stages 25*ℓ* to 33, and 85 x 45 x 58 cm for stages 40 to 45) at 13°C ± 1°C. Aquaria were supplied with slow-flowing dechlorinated water, an air stone and simulated vegetation, comprised of 11 (small tank) or 19 (large tank) groups of 8 strands of 51 cm long dark plastic strips attached to a weight to provide a refuge. Horizontal reference lines on the outside of the aquarium were used to divide the water column into three equal parts (upper, middle and lower). The larger aquarium was modified with resting platforms to accommodate stage 45. Tadpoles were fed following release into the aquarium and allowed 24 h to acclimate prior to observations. They were not fed on the morning of observation and the air stone was removed to ease observation. To prevent disturbance or stimulation of tadpoles, room lights were turned off 30 min prior to observation and only a 60W bulb over the tank was used for illumination. The following were recorded a total of 10 times at 10-min intervals: (a) vertical distribution (number of individuals in upper, middle and lower portions of aquarium), (b) swimming activity (number of active individuals at a given time), and (c) air breathing frequency (total number of air breaths taken during 5 min).

Observations for all stages were done in the morning between 10:00 - 12:00 h.

Buoyancy-related measurements

1. Capture methods

Prior to making any measurements on tadpoles, it is necessary to capture and euthanize them. During this process, tadpoles can gulp air or release lung gases resulting in inaccurate measurements of buoyancy. Tadpoles must, therefore, be handled in such a way as to prevent either loss or gain of lung gases, or be able to correct for them when they occur. Hatchlings (lungs not inflated) were captured in a clear plastic tube with a suction bulb fitted to one end. Later stages (lungs inflated) were captured below the surface in a plastic vial (70 mm long and 32 mm diam for stages 25ℓ to 33; 95 mm long and 44 mm diam for stages 34-43) which was closed with a cap while under water. The cap was fitted with a fine mesh screen (289 meshes/cm^2) that retained any gases released by the tadpole. When the vial was removed from the aquarium in an upright position (cap up) and transferred to a 5% solution of 2-phenoxyethanol, the anesthetic passed through the screen and the tadpole was euthanized. The volume of any trapped gases was measured (see measurement procedures below).

2. Physical principles and analysis

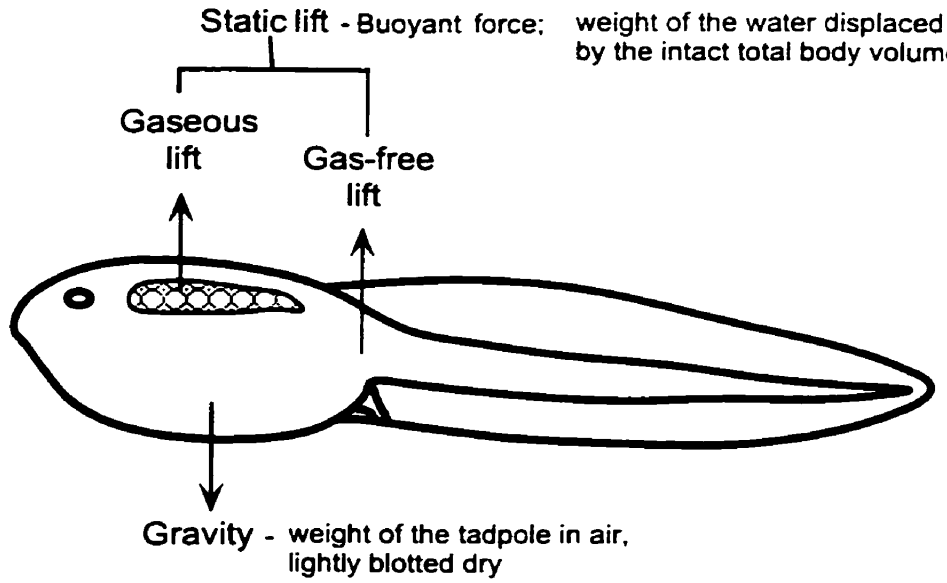
Two forces act upon a motionless tadpole in water, (1) the downward force of gravity (*i.e.*, the weight of the tadpole in air, unsupported by water) and

(2) the upward buoyant force, which I will call static lift (*i.e.*, the weight of the water displaced by the intact total body volume). The difference between these two forces is the net force defined as the upward static lift minus the downward force of gravity. The volume of the water displaced can be divided into two components: the volume displaced by the gases enclosed within the tadpole's body and the volume displaced by the gas-free body volume. Accordingly, static lift (weight of the water displaced) includes two components referred to as (1) gaseous lift and (2) gas-free lift components. The net force can be summarized as the gaseous lift plus gas-free lift minus the weight of the tadpole in air (Fig. 6).

To facilitate comparisons among individuals, these forces are converted into dimensionless ratios by dividing each one by the weight of the water (with a specific weight taken to be 1.000 gwt/ml) displaced by the gas-free body volume of the tadpole. These conversions imply new terminology and definitions as follows:

- the net force acting on a submerged tadpole divided by the weight of water displaced by the gas-free body volume of the tadpole will be called the buoyancy index (BI). The buoyancy index will be either zero for a neutrally buoyant tadpole when static lift equals weight in air, positive when static lift exceeds weight in air (resulting in a net upward force on the tadpole), or negative when static lift is less than weight in air (resulting in a net downward force on the tadpole).

A.

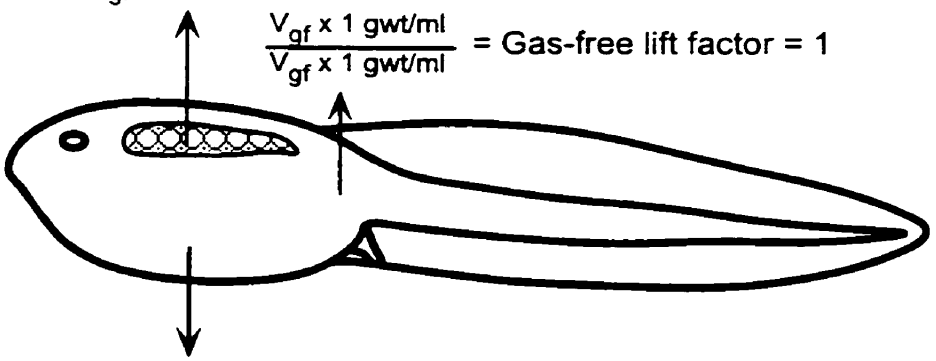


Net force = Gaseous lift + Gas-free lift - weight of the tadpole in air

B.

$\frac{V_{gas} \times 1\ gwt/ml}{V_{gf} \times 1\ gwt/ml}$ = Gaseous lift factor (LF_g)

$\frac{V_{gf} \times 1\ gwt/ml}{V_{gf} \times 1\ gwt/ml}$ = Gas-free lift factor = 1



$\frac{W_{wet}}{V_{gf} \times 1 gwt/ml}$ = Specific gravity (SG)

$$\frac{\text{Net force}}{V_{gf} \times 1\ gwt/ml} = BI = LF_g + 1 - SG = LF_g - (SG - 1)$$

Figure 6 : A. Forces acting upon a motionless tadpole in water
B. Conversions of forces into dimensionless ratios.

- the gaseous lift (weight of the water displaced by the enclosed gases -- V_{gas} x 1 gwt/ml) divided by the weight of water displaced by the gas-freebody volume of the tadpole (V_{gf} x 1 gwt/ml) equals the ratio of the tadpole's total gas volume to its gas-free body volume; this ratio (V_{gas} / V_{gf}) will be called the gaseous lift factor (LF_g).
- gas-free lift (weight of the water displaced by the gas-free body volume of the tadpole -- V_{gf} x 1 gwt/ml) divided by the weight of water displaced by the gas-free body volume of the tadpole is called the gas-free lift factor and equals 1 because they are equal by definition.
- the tadpole's wet weight, blotted dry, in air (W_{wet}) divided by the weight of water displaced by its gas-free body volume (V_{gf} x 1 gwt/ml) equals its specific gravity (SG) as specific gravity by definition is the ratio of its density to that of pure water.

Thus as described above:

Net force = gaseous lift + gas-free lift - weight of tadpole in air

When all four terms of the equation are divided by V_{gf} x 1 gwt/ml, it becomes:

Net force / (V_{gf} x 1 gwt/ml) = V_{gas} / V_{gf} + V_{gf} / V_{gf} - W_{wet} / (V_{gf} x 1 gwt/ml).

Substituting the terms defined in the preceding, we get:

$BI = LF_g + 1 - SG$, or

$BI = LF_g - (SG - 1)$ as summarized in Fig. 6B.

Note: (SG - 1) is an index of the net downward force on gas-free tissue (weight - buoyant force) while LF_g is an index of gaseous lift.

In addition, two other quantities related to the tadpoles' buoyancy were calculated:

- the fractional water content (FW) can be determined from wet weight (W_{wet}) and dry weight (W_{dry}). If only water is driven off during desiccation (see below), then its weight (W_{water}) would equal $W_{wet} - W_{dry}$ and the fractional water content by weight would be:

$$FW = W_{water} / W_{wet} = (W_{wet} - W_{dry}) / W_{wet} = 1 - (W_{dry} / W_{wet}).$$

- the concentrated non-aqueous specific gravity (C) represents the specific gravity of the animal's dry tissue concentrated into the hypothetical body volume remaining after desiccation and equal to the original gas-free body volume (V_{gf}) <u>minus</u> the volume of water driven off:

$$C = W_{dry} / [V_{gf} - (W_{water} /1 \text{ gwt/ml})] /1 \text{ gwt/ml}$$

$$= W_{dry} / [V_{gf} - (W_{wet} - W_{dry}) /1 \text{ gwt/ml}] /1 \text{ gwt/ml}.$$

It can be shown that

$$C = [SG \times (1 - FW)] / [1 - (SG \times FW)]$$

It is important to note that the BI to be determined is that of the tadpole in its surroundings before capture, in the present case, at the bottom of an aquarium in the laboratory. For practical purposes, however, BI must be calculated from experimental variables measured on tadpoles at or near the top of the water column, where hydrostatic pressure is lower or absent. Taking into account local atmospheric pressure and hydrostatic pressure at specific depths,

all measured gas volumes were corrected herein to the values they would take on at the depth of the aquarium floor where the tadpole, in this case a benthic species, was found. Local atmospheric pressure (P_{atm}) values were obtained for each experimental period from Environment Canada at the Winnipeg Airport and corrected for elevation differences between the airport and laboratory sites in the Duff Roblin building (+ 0.1 mmHg for the lab (Z310) and + 0.8 mmHg for the lab in the Animal Holding Facilities). Hydrostatic pressure (P_H) was calculated in mmHg simply by dividing the water depth above the tadpole (in mm) by 13.6, the specific gravity of mercury. Gas volumes were corrected for total pressure at three specific depths used in two procedures described in the following section. (Details and definitions of abbreviations that follow immediately below are given in the descriptions of these procedures.) These specific corrected pressures were:

P_{expt} = total pressure at depth in the experimental aquaria prior to capture (210 or 275 mm, depending on the tank used) -- Procedures 2 and 3

= atmospheric pressure + hydrostatic pressure at that depth (210/13.6 or 275/13.6 mmHg)

= P_{atm} + (15.4 or 20.2) mmHg, respectively.

P_{pan} = total pressure at the depth of the weighing pan (15 mm)

= atmospheric pressure + hydrostatic pressure at that depth (15/13.6 mmHg) -- Procedure 3

= P_{atm} + 1.1 mmHg

In procedure 2 (see below), I applied an additional external pressure (ΔP) to compress or expand the lungs in order to produce neutral buoyancy resulting in total pressure at that depth denoted by PNB:

PNB = total pressure at the depth neutral buoyancy was achieved (50 mm)

= atmospheric pressure + hydrostatic pressure at that depth (50/13.6 mmHg) + applied pressure ΔP -- Procedure 3

= P_{atm} + 3.7 mmHg + ΔP

The corrected variables included among others: the volume, measured at atmospheric pressure, of any bubbles released after capture ($V_{bubble,\ Patm}$) and corrected to the pressure in the aquarium ($V_{bubble,\ Pexpt}$); and the measured lung volume corrected to aquarium pressure but uncorrected for escaped bubbles ($V_{L,\ Pexpt}$). Thus the total volume of included gases that generate the tadpole's gaseous lift in the aquarium before capture (V_{gas}) is determined as follows:

$V_{gas} = V_{L,\ Pexpt} + V_{bubble,\ Pexpt}$

BI can then be calculated as:

$BI = LF_g - (SG - 1)$

$= V_{gas} / V_{gf} - [W_{wet} /(V_{gf} \times 1\ gwt/ml)] + 1$

$= [(V_{L,\ Pexpt} + V_{bubble,\ Pexpt}) / V_{gf}] - [W_{wet} /(V_{gf} \times 1\ gwt/ml)] + 1$

Different forms of this equation will be used depending on the size of the tadpole because size determines the measurements that can be made.

3. Measurement procedures

After euthanasia, if lung gases were released into the vial, they were freed

under water and trapped in a water-filled inverted funnel. Their volume was then measured in an attached pipette ($V_{bubble,\ Patm}$; ± 0.001 ml), and later corrected (see procedures below) for compression by the additional hydrostatic pressure the tadpole experienced in the aquarium before capture. Total body length was measured (± 0.1 mm) and stage of development was recorded. Three different procedures, summarized in Fig. 7, were then used to measure buoyancy-related variables for individual tadpoles, depending on stage of development.

Procedure 1 was used for hatchlings (stages 20-25*e*, lungs not inflated). Specific gravity (± 0.001) was measured by trial and error using a calibrated series of Percoll solutions, each of known density. Individuals were immersed in different solutions until the one in which they were neutrally buoyant was found. If an individual required immersion in several solutions, it was carefully blotted dry between immersions to prevent contamination of the solutions. Tadpoles were removed, blotted lightly prior to being weighed (yielding W_{wet}, ± 0.0001 gwt), and then placed in a drying oven at 60°C for at least 24h, after which W_{dry} (± 0.0001 gwt) was measured.

Procedure 2 was used for early larval stages (25*e*-29, lungs inflated). These small larvae were too small to use the direct approach in procedure 3 and lung volume was measured indirectly through a determination of the pressure of neutral buoyancy (PNB, the ambient pressure required to make an intact tadpole, with lungs inflated, neutrally buoyant). PNB (± 2 mmHg) was determined by the procedure of Beaver and Gee (1988), based on the Cartesian diver principle (as the ambient pressure is increased above atmospheric, the

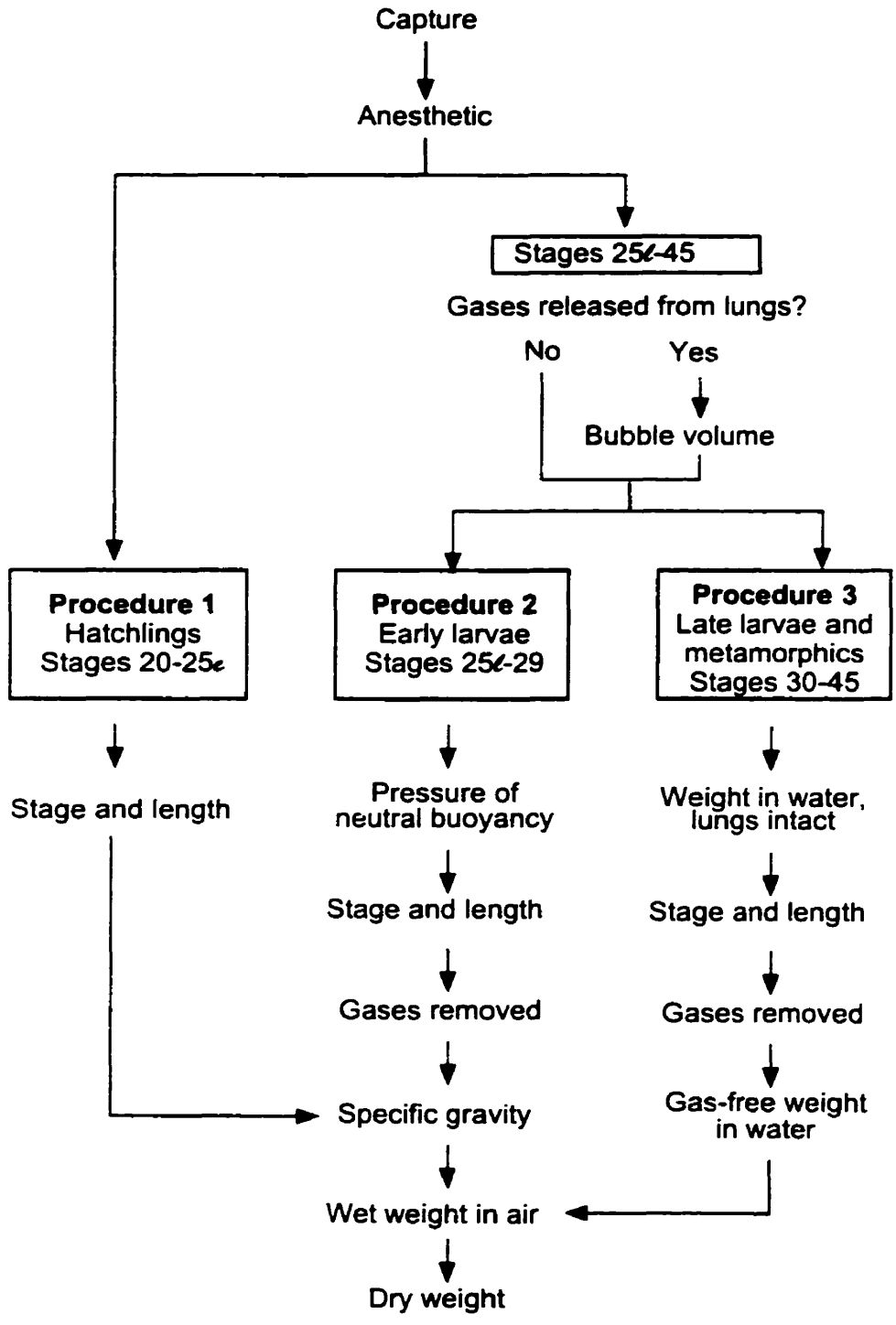


Figure 7: Procedures used to measure buoyancy-related variables on hatchlings, early larvae and late larvae and metamorphics of mink frogs, *R. septentrionalis*.

volume of gas decreases and displaces less water, causing the diver (tadpole) to sink; in contrast, when the pressure is reduced below atmospheric, the volume of gas increases, displacing more water and causing the diver to rise in the water column). Air pressure above the water in a sealed chamber was raised or lowered (accommodating both positively or negatively buoyant tadpoles) relative to ambient atmospheric pressure by an amount ΔP, thus compressing or expanding the lung in accordance with Boyle's law (which states that volume varies inversely with pressure in a gas at constant temperature). At neutral buoyancy, the weight of water displaced by the lung volume ($V_{L.\ PNB}$) is equal to the tadpole's gas-free weight in water (W_{wg-}), so lung volume at PNB equals W_{wg-} / 1 gwt/ml. By Boyle's law, the lung volume at the tadpole's normal ambient pressure (at depth in the aquarium, before capture) is then:

$V_{L.\ Pexpt} = V_{L.\ PNB} \times PNB / P_{expt} = (W_{wg-} / 1\ gwt/ml) \times PNB / P_{expt}$

Using a binocular microscope, small incisions were made dorsally on either side of the vertebral column to remove lung gases. Care was also taken to remove any gases that might be trapped in the oral and gill cavities and the intestine. Specific gravity was determined on the gas-free tadpole, as well as W_{wet} and W_{dry}, all as in Procedure 1.

Procedure 3 was used for late larval and metamorphic stages (30-43) as it was possible to weigh these larger specimens directly under water. First, the intact tadpole (after removal of all trapped bubbles from oral and gill cavities) was placed on a submerged pan connected to a balance by a below-the-balance hook and weighed (± 0.001g) to determine its weight in water with lungs intact

(W_{wg+}). All lung gases were removed as in procedure 2 and the gas-free weight in water (W_{wg-}) was measured. Lung volume at the depth of the weighing pan ($V_{L.\ Ppan}$) could then be determined as the difference between W_{wg-} and W_{wg+} divided by the specific weight of water (1.000 gwt/ml). This volume was corrected to obtain the lung volume at depth in the aquarium ($V_{L.\ Pexpt}$) as follows:

$V_{L.\ Pexpt} = V_{L.\ Ppan} \times P_{pan} / P_{expt}$

Wet weight in air and W_{dry} were measured as in procedure 1.

4. Calculation of buoyancy index and related variables.

Calculations for each of the three procedures described above were then carried out as follows:

Procedure 1 - stages 20-25*e*

- no gases are present, so the gaseous lift factor $LF_g = 0$.
- specific gravity is measured directly by immersion in Percoll
- W_{wet} is measured directly
- W_{dry} is measured by weighing after desiccation

Procedure 2 - stages 25*e* - 29

- any escaped air bubbles are trapped and their volume is measured ($V_{bubble.\ Patm}$)
- equivalent bubble volume in the experimental aquarium is calculated as

  $V_{bubble.\ Pexpt} = V_{bubble.\ Patm} \times P_{atm} / P_{expt}$

- the pressure increment ΔP required to make the tadpole neutrally buoyant at a 50 mm depth is measured, and PNB is determined.
  $PNB = P_{atm} + 3.7mmHg + \Delta P$
- specific gravity of gas-free body volume is measured directly by immersion in Percoll
- W_{wet} is measured by weighing and W_{dry} is measured by weighing following desiccation
- V_{gf} is calculated as W_{wet} / SG / 1 gwt/ml
- gas-free weight in water, W_{wg-}, is calculated as W_{wet} - (V_{gf} x 1 gwt/ml)
- $V_{L.\ Pexpt}$, lung volume excluding any gases lost as bubbles, is calculated as $V_{L.\ Pexpt} = (W_{wg-}/ 1\ gwt/ml) \times PNB / P_{expt}$
- V_{gas} is calculated as $V_{L.\ Pexpt} + V_{bubble.\ Pexpt}$ (if bubble released prior to capture)
- the gaseous lift factor is calculated as $LF_g = V_{gas} / V_{gf}$

Procedure 3 - stages 30 - 43

- escaped air bubbles are measured for volume ($V_{bubble.\ Patm}$)
- equivalent bubble volume in the experimental aquarium is calculated as $V_{bubble.\ Pexpt} = V_{bubble.\ Patm} \times P_{atm} / P_{expt}$
- weight in water with lungs intact, W_{wg+}, is measured
- gases are released from lungs and gas-free weight in water, W_{wg-} is measured

- W_{wet} is measured by weighing and W_{dry} is measured by weighing following desiccation
- V_{gf} is calculated as $(W_{wet} - W_{wg-}) / 1$ gwt/ml
- SG is calculated as $W_{wet} / V_{gf} / 1$ gwt/ml
- $V_{L.\ Ppan}$ is calculated as $(W_{wg-} - W_{wg+}) / 1$ gwt/ml
- $V_{L.\ Pexpt}$ is calculated as $V_{L.\ Ppan} \times P_{pan} / P_{expt}$
- V_{gas} is calculated as $V_{L.\ Pexpt} + V_{bubble.\ Pexpt}$
- gaseous lift factor LF_g is calculated as V_{gas} / V_{gf}

Using these measurements, BI, FW and C were calculated with the formulae given previously.

Buoyancy profile

The purpose of the buoyancy profile was to describe changes in gaseous lift factor (LF_g) and specific gravity and resulting buoyancy indices at particular stages of development prior to metamorphosis. Hatchlings were held in an aquarium (30.5 x 15 x 21 cm) in still dechlorinated water supplied with an air stone at 18°C. Eight hatchlings of the appropriate stages were randomly selected from the holding tank for buoyancy-related measures. For each of the larval and metamorphic stages used in the profile, 10 to 12 tadpoles were held in an aquarium (41 x 21.5 x 26 cm - stages 25ℓ to 31/33 and 51 x 26.5 x 32 cm - stages 36 to 43) at 13°C ± 1°C. Tadpoles were fed Tetra-min fish flakes and allowed 24 h to acclimatise. The aquarium was supplied with slow-flowing

dechlorinated water and an air stone. Tadpoles were not fed the morning prior to measuring buoyancy. Eight tadpoles were randomly selected for buoyancy-related measurements.

Effect of substrate on buoyancy and related variables

To test hypotheses that larval mink frogs ingest dense substrate particles and that this increases their specific gravity initiating a buoyancy correction by increasing gaseous lift factor in a timely manner, it is first necessary to determine (a) if tadpoles do ingest substrate particles, and, if so, to determine (b) if this has a significant effect on specific gravity, (c) if gaseous lift factor is adjusted accordingly, and (d) how the buoyancy index is ultimately affected. The times required for the digestive tract to become completely filled with substrate and for responses to any increase in specific gravity to occur (through changes in volume of included gases and buoyancy index) must also be assessed.

1. Effect of substrate ingestion

The purpose of this experiment was to determine if specific gravity, gaseous lift factor or buoyancy index of mink frog larvae are altered in response to the ingestion of substrate particles. Larval stages 31/33 were held for 24 h at 12.5°C in aquaria (40 x 21.5 x 26 cm) on 5 cm of silt, 2.5 cm of sand (0.2-1.4 mm diam), 12-15 cm of detritus (fragmented vegetation from previous year), or no bottom substrate (control). All three test substrates are found in McGillivray

Creek. Aquaria were supplied with slow-flowing dechlorinated water, an air stone, and Tetra-min flakes. Eight larvae were examined in each of the treatment and control groups.

2. Transit time of silt particles through the digestive tract

The purpose of this experiment was to determine the time required to fill the digestive tract with substrate particles. Larval stages 27-28 were held in aquaria (40 x 21.5 x 26 cm) and supplied with 5 cm of silt (< 0.1 mm in diam) substrate. They were provided with slow-flowing dechlorinated water, an air stone, and Tetra-min flakes. At times 0, 1.5, 3, 6, 12, 24, and 48 h, 8 randomly selected larvae were removed, anesthetized and staged, and total length was recorded. For each individual the entire digestive tract (from oesophagus to anus) was removed and placed on a paper towel, and the following measurements were made: (1) the length (mm ± 0.5) of the entire digestive tract; (2) the distance (mm ± 0.5) travelled by silt and (3) the actual total length (mm ± 0.5) of the digestive tract filled with silt particles, correcting for empty segments along the length of the gut. The length of the digestive tract was also felt with tweezers to get a feeling for the particle size ingested.

3. Response time to increased weight from silt ingestion

The purpose of this experiment was to determine the response time of buoyancy adjustment of tadpoles to an increase in specific gravity caused by

ingestion of silt particles. Larval stages 27-28 were held in aquaria (40 x 21.5 x 26 cm) and supplied with 5 cm of silt, slow-flowing dechlorinated water, an air stone and Tetra-min flakes. Buoyancy-related measures were performed at 0, 1, 1.5, 3, 6, 12, 24, 48 and 96 h on 8 randomly selected tadpoles.

Behavioural responses to predators

1. Experiment I: activity, vertical distribution and buoyancy index

To test the hypothesis that the presence of predators will bring about a reduction in activity, vertical distribution and buoyancy, I tested two groups of boreal chorus frogs (stages 28-31 and 37-40), two groups of mink frogs (stages 26-27 and 28-29), and one group of wood frogs (stages 28-31), resulting in a total of 5 tests. In each test, comparisons were made between a control treatment (larvae without exposure to predators) and an experimental treatment (larvae with predators). Activity, vertical distribution and buoyancy indices were measured.

Three tadpoles of the appropriate stages and species were randomly selected and transferred from the holding aquarium to each of 20 aquaria, of which 10 were randomly selected to receive either the control treatment or the experimental treatment. Each aquarium held a cylindrical transparent plastic vial (a 9 x 2.8 cm cylindrical vial with 14 drilled holes (2 mm) on the sides and 5 at the bottom for *Cordulia* (dragon fly larvae) for tests 1 (small boreal chorus frogs), 2 (large boreal chorus frogs), 3 (small mink frogs) and 5 (wood frogs), or a 300

ml plastic cup (9.5 cm x 7.5 to 5.3 cm) with 5 melted holes (3 mm) on the side and 5 on the bottom for *Belostomatida* (giant water bug) for test 4 (large mink frogs). Horizontal reference lines on the outside of each aquarium divided the water column into three equal strata (upper, middle and lower) to permit observations of vertical distribution.

Tadpoles were placed in aquaria on day 0 and care was taken not to injure any tadpoles as detection of injured conspecifics via chemical cues elicits an alarm response which could affect activity (Hews and Blaustein, 1985). After an acclimation period of 24 h, a single predator recently fed a conspecific tadpole (Wilson and Lefcort, 1993) was placed in each of the predator treatment aquaria. In control aquaria, a small rock of approximately 1 cm^3 volume was added to the vial instead of a predator to simulate disturbance caused by the addition of a predator in the experimental treatments. The predator or rock was replaced on day 3, halfway through the trial. Over the 5-day trial, the number of active tadpoles per 3 sec interval and the vertical distribution was recorded for each aquarium, at 10:30, 12:30, 14:30 and 16:30, respectively, for a total of 16 observations per replicate.

On day 5 of the experiment, 4 tanks for both control treatments and predator treatment were randomly selected and buoyancy measurements were made on all larvae (12) in these aquaria.

2. Experiment II: frequency of air breathing

Two larger aquaria, each containing 12 tadpoles (one with 4 predators (as per experiment I) and the other predator-free), were set up to measure frequency of air breathing. Twice a day, at 12:00 and 16:00, for the length of the experiment (5 days), tadpoles in each aquarium were observed for 20 min, and time and number of air breaths were recorded.

Between trials, water in all aquaria was changed and extra care was taken to eliminate carryover of chemical predator cues (Petranka *et al.*, 1987). Furthermore, aquaria assigned to the predator treatment remained as such for all tests (for all species and all stages tested) and the positions of aquaria were altered.

Difficulties were encountered in obtaining predators and in getting them to feed. This resulted in differences among the 5 tests (Table 1).

Due to technical problems within the laboratory, I could not control either water temperature or ambient temperature. As a result, water temperature fluctuated with ambient temperature (Table 1) causing oxygen levels to drop in 4 of the 5 tests. To correct the situation, partial water changes were made in the mink frog and wood frog tests on days 2, 3 and 4. The partial water changes also allowed the removal of fungus growth on the bottom of the aquaria. One litre of water was replaced in the "activity" aquaria while 4 liters of water were replaced with well oxygenated water in the "frequency of air breathing" aquaria.

Table 1: Differences in procedures among activity experiments.

Test	Species and stages	Predator used	Predators ate conspecifics offered				Water Temperature
			Exp I		Exp II		
			Day 1	Day 3	Day 1	Day3	
1	Chorus frog (stages 28-31)	*Cordulia* sp.	yes	yes	yes	yes	16.5 to 19°C
2	Chorus frog (stages 37-40)	*Cordulia* sp.	yes	no*	yes	no*	22 to 24°C
3	Mink frog (stages 26-27)	*Cordulia* sp.	yes	yes	yes	yes*	23 to 24°C
4	Mink frog (stages 28-29)	*Belostoma* sp.	yes	yes	yes	yes	22 to 24°C
5	Wood frog (stages 28-31)	*Cordulia* sp.	no	no	yes	no	18.5 to 23°C

* Original predators used over 5 days

Statistical analysis

1. Horizontal and vertical distribution in the field

Data were expressed as mean numbers of tadpoles caught per 10 traps per hour at each depth from May 28 to August 22, 1996.

2. Vertical distribution and activity in the laboratory

Because of pseudoreplication, the ten successive observed distributions of individuals at particular stages were averaged to allow comparison (Hurlbert, 1984). In each stage, the mean numbers of tadpoles observed in the upper, middle, and lower portion of the aquarium were calculated and than analysed using a R x C contingency table (stages 25ℓ through 42-43). The late metamorphic stage (stage 45) was also analysed using a R x C contingency table, but separately.

For both activity and frequency of air breaths, means were calculated as the mean number (n=10) of active tadpoles per 15 tadpoles and mean number of air breaths per 5 minutes per 15 tadpoles, respectively, with one standard deviation.

3. Buoyancy profile

All results are presented as means from 8 randomly selected individuals with 95% confidence limits. Variation among stages in specific gravity-1, gaseous lift factor and buoyancy index were compared using one-way ANOVA

combined with the Student-Newman Keuls test on SPSS for Mac, version 6.1. The assumption of homogeneity of variance (Levene Test) was met for all variables except specific gravity-1 (stages 25ℓ to 43), which subsequently was transformed using a square root transformation.

4. Effect of substrate on buoyancy and related variables

All results are shown as means from 8 randomly selected individuals with 95% confidence limits. Significance of variation among substrate and mean buoyancy related variables over time were determined by one-way ANOVA using SPSS for Mac, version 6.1. The assumption of homogeneity of variance (Levene Test) was met for gaseous lift factor and buoyancy index; however, in the substrate ingestion experiment, specific gravity-1 had to be transformed using square root (arcsine). As for the experiment on the response time to increased weight from silt ingestion, specific gravity-1 did not met the assumption of homogeneity of variance.

For the experiment on transit time of silt particles through the digestive tract, the distance travelled by silt through the digestive tract (from mouth to anus) as well as the length of the digestive tract compacted with silt for the 8 randomly selected individuals were converted to percentages. Mean values (n=8) for these two distances were then calculated along with their respective 95% confidence limits.

5. Behavioural responses to predators

Some tadpoles did not survive. Tanks in which tadpole mortality occurred were excluded from the analyses to avoid bias in case healthy tadpoles perceived the death of one of their conspecific as a result of a predator encounter.

Experiment I:

a) Activity: For activity, the mean numbers of active tadpoles per tank per day were calculated. Repeated Measures ANOVA using SPSS for Mac, version 6.1, was first performed to determine if the predator exchange mid-way through the experiment interfered with the tadpoles' activity. As no effect was found, except for the predator tanks in test 5, Repeated Measures ANOVA was than conducted on each test to determine the significance of the presence/absence of predators on tadpoles' activity.

b) Vertical distribution: Prior to any analysis, data were transformed into distribution scores. A score of 1 was given to tadpoles in the lower portion of the aquarium, a score of 2 for those in the middle portion, and a score of 3 for those in the upper portion. Thus the total score per tank ranged from 3 when all three tadpoles were at the bottom, to 9 when they were all in the upper portion. The scores were then averaged over each tank each day. Repeated Measures ANOVA using SPSS for Mac version 6.1 was first performed to determine if the

mid-week predator exchange, mid-way through the experiment, interfered with the tadpoles' distribution score. As no effect was found, Repeated Measures ANOVA was then conducted for each test to determine the significance of the presence/absence of predators on the tadpoles' distribution score.

c) buoyancy index: All results are presented as means of 12 randomly selected individuals (4 tanks) with 95% confidence limits. Comparisons between experimental and control treatments were made for each test for buoyancy index, specific gravity and gaseous lift factor using a by one-way ANOVA using SPSS for Mac, version 6.1.

Experiment II

Frequency of air breathing - Test 1.

The number of air breaths in test 1 was tested using the Fisher exact test. The two categories were determined using the mean number of air breaths/ sample (36/16 = 2.25) which eliminates bias for either the control or the experimental results.

RESULTS

Horizontal and vertical distributions in the field

Larval and metamorphic stages were seen frequently on the substrate, in or on vegetation, near shore. Minnow trap data corroborated these observations as most individuals were caught within 3 m of shore in vegetation (Fig. 8). Catch per unit effort was greatest in early spring and overall numbers declined with each sampling. No late metamorphic stages or adults were captured in minnow traps.

Vertical distribution and activity in the laboratory

1. Larvae and early metamorphic (stages 25ℓ to 43)

R x C contingency table analysis showed that observed distributions of individuals among upper, middle and lower sections were similar for all 6 stages ($X^2 = 1.29$; df = 10; $p > 0.05$) and observed distributions differed from a uniform one (1:1:1) ($X^2 = 109.15$; df = 2; $p < 0.05$) (Table 2); larvae in these stages were observed much more frequently in the bottom third of the aquarium than elsewhere. These data support field observations that mink frog larvae are benthic.

2. Late metamorphic (stage 45)

R x C contingency table analysis showed that late metamorphic tadpoles

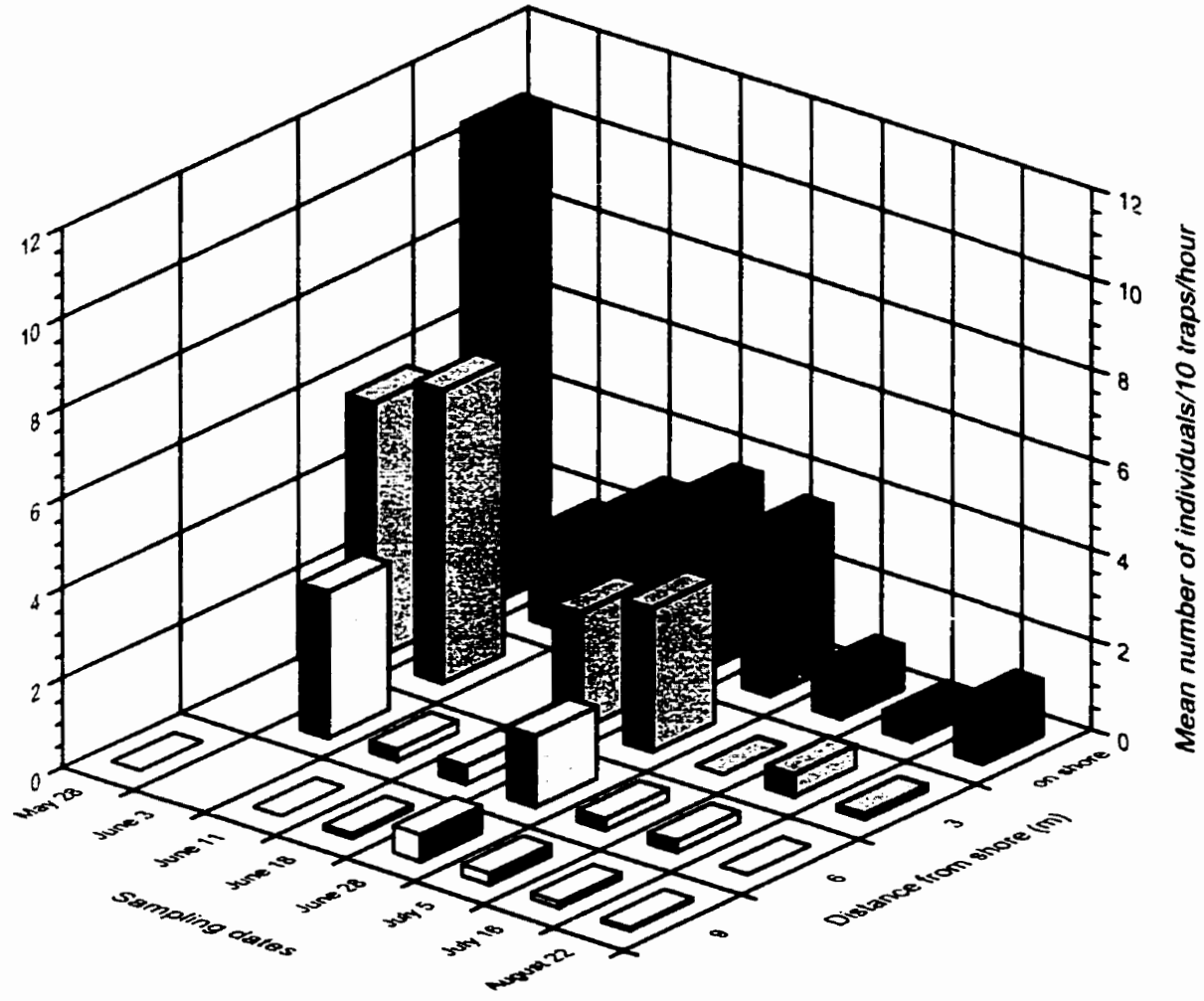


Figure 8 : Horizontal distribution of juvenile (larval and metamorphic) mink frogs, *Rana septentrionalis*, at McGillivray Creek from May 28 to August 22, 1996. Represented is the mean number of individuals per 10 minnow traps per hour.

Table 2: Distribution of mink frogs by stage of development within three sections (upper, middle and lower) of the aquarium. Data are the means of 10 observations made on 15 tadpoles.

Stages	Distribution			Total
	Upper	middle	lower	
25ℓ	0.40	1.5	13.1	15
27	0.9	1.5	12.6	15
29	1.3	1.9	11.8	15
31-33ɑ	0.9	1.1	13.0	15
40	0.7	1.1	13.2	15
42-43	1.2	0.8	13.0	15
45	13.5	0.3	1.2	15

spent significantly more time in the upper portion of the aquarium ($X^2 = 21.76$; $df = 2$; $p < 0.05$) (Table 2). Furthermore, they were found resting more frequently than swimming in the water column ($X^2 = 13.07$; $df = 2$; $p < 0.05$). This agrees with field data where no stage 45 individuals were collected in minnow traps. They were observed either resting on shore or on lily pads.

On average, over stages 25ℓ to 43, 2.9 ± 0.5 tadpoles out of 15 were active at any given time and frequency of air breathing was 5.82 ± 1.76 for 15 tadpoles / 5 min or 4.65 air breaths / tadpole / hour (Table 3; Fig. 9) .

Buoyancy profile

1. Profile description

Gaseous lift factor and specific gravity changed greatly during development, resulting in considerable fluctuations in buoyancy index. At stage 20, specific gravity-1 was highest with a mean value of 0.050. Subsequently it dropped rapidly to 0.019 in stage 25ℓ (Fig. 10). Specific gravity-1 then increased gradually from stage 25ℓ to stage 43 when it reached 0.040. The lungs were inflated during stage 25ℓ and gaseous lift factor increased from 0.008 in stage 25ℓ to 0.018 in stage 29. Gaseous lift factor dropped dramatically in autumn to 0.005 in stages 31/33ℓ. In spring, stages 31/33ℓ were still present and gaseous lift factor increased from 0.009 to 0.019 in stage 36 and varied about this level during stages 40 and 43 (Fig. 10). As a result of these changes, buoyancy index increased from -0.050 to -0.019 during the hatchling phase due to the decrease

Table 3 : Mean number ± 1 standard deviation (n = 10) of active tadpoles/15 tadpoles and mean number of air breaths/5 min/15 tadpoles throughout the larval and early metamorphic stages of mink frog larvae, *Rana septentrionalis*.

Larval stages	Mean number of active tadpole / 15 tadpoles ± one standard deviation	Mean # of air breaths/ 5 min / 15 tadpoles ± one standard deviation
25ℓ	2.9 ± 1.37	3.7 ± 1.337
27	3.2 ± 1.62	6.2 ± 2.39
29	2.6 ± 1.43	3.7 ± 1.42
31/33ɑ	2.9 ± 1.66	6.1 ± 1.91
40	3.7 ± 1.767	7.7 ± 2.263
42/43	2.2 ± 0.919	7.5 ± 2.014

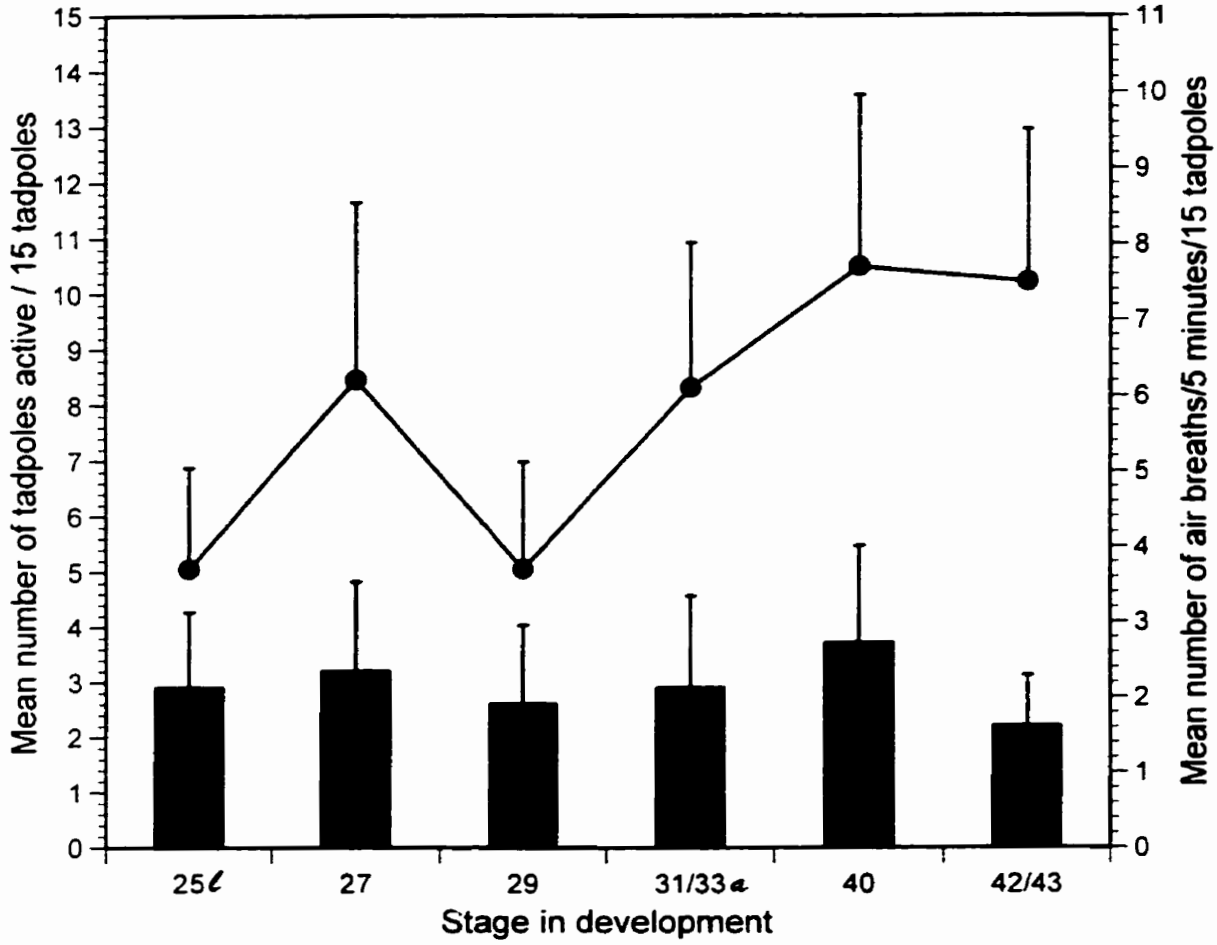


Figure 9: Mean activity (solid bars) and number of air breaths (solid circles) of juvenile mink frogs, *Rana septentrionalis*. Vertical bars denote 1 standard deviation of mean (n = 10 in all cases).

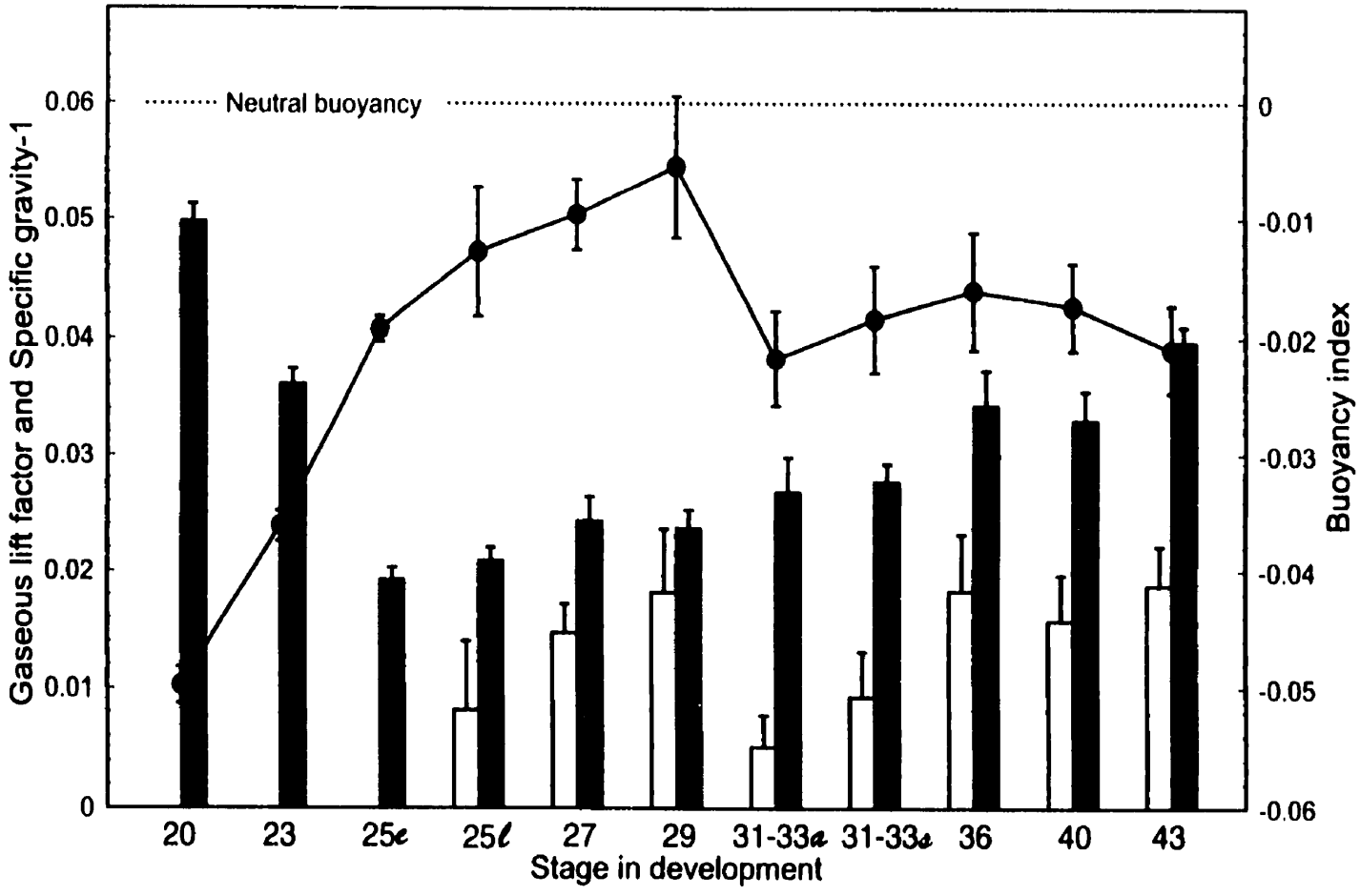


Figure 10: Buoyancy profile of *Rana septentrionalis*. Mean values (n = 8) of specific gravity -1 (solid bars) and gaseous lift factor (open bars) and resulting buoyancy index (solid line) are shown with 95% confidence limits on the means.

in specific gravity. In early larval stages buoyancy index increased to -0.006 in stage 29 as lungs were filled and as gaseous lift factor increased more rapidly than specific gravity. In autumn, stages 31/33*a*, buoyancy index was reduced to - 0.022 as gaseous lift factor dropped dramatically. Buoyancy index levels remained low throughout late larval and metamorphic stages (Fig. 10).

The variation described above resulted in significant differences between mean values of these variables at different stages (Table 4). There were significant differences in specific gravity-1 among all 3 hatchling stages (20, 23, 25*e* - SNK test, Table 4). Larval and metamorphic stages can be divided into 5 different homogeneous subsets resulting in each group having a significantly different specific gravity-1. These subsets are: stages 25*l*, 27 and 29, 31/33*a* - 31/33*s*, 36 - 40, and 43 (Table 5).

As for gaseous lift factor, the SNK test showed that levels of this variable in stage 25*l* and stages 31/33*a* and 31/33*s*, pre- and post-hibernation respectively, are significantly lower than those of other larval and metamorphic stages (Table 5).

The SNK test on buoyancy index levels showed that each of the 3 stages of hatchlings are significantly different from each other (Table 5). In addition, buoyancy indices of stages 25*l*, 27 and 29 are significantly higher than most of the later stages (Table 5). Furthermore, there are no significant differences among stages 31/33*a* and later stages.

Table 4: Changes in gaseous lift factor and (specific gravity-1) and buoyancy index of the developmental stages of mink frog, *Rana septentrionalis*. F and P values are given for each variable and stage.

Variables	Stages (Gosner, 1960)	Statistical data	
		F	p
Specific gravity -1	20-25*e*	$F_{(2,21)} = 756.0169$	<0.001
	25*e*-43	$F_{(7,56)} = 47.4955$	<0.001
Gaseous lift factor	25*e*-43	$F_{(7,56)} = 8.7708$	<0.001
Buoyancy index	20-25*e*	$F_{(2,21)} = 756.0169$	<0.001
	25*e*-43	$F_{(7,56)} = 8.7860$	<0.001

Table 5: Mean (n = 8) specific gravity-1, gaseous lift factor and buoyancy index of mink frogs during development, showing significant differences among means. Within columns, means that share the same letter are not significantly different (SNK test); hatchlings are analysed separately from the larval and early metamorphic stages. CL = 95% confidence limits of means.

Stages	Specific gravity -1 ± 95% CL	Gaseous lift factor ± 95% CL	Buoyancy index ± 95% CL
20	0.04975^{A} ± 0.00153	---	-0.04975^{A} ± 0.00153
23	0.03610^{B} ± 0.00130	---	-0.03613^{B} ± 0.00130
25*c*	0.01925^{C} ± 0.00107	---	-0.01925^{C} ± 0.00107
25*ℓ*	0.02087^{A} ± 0.00113	0.00811^{A} ± 0.00591	-0.01276^{BC} ± 0.00546
27	0.02425^{B} ± 0.00213	0.01466^{B} ± 0.00247	-0.00959^{CD} ± 0.00298
29	0.02362^{B} ± 0.00161	0.01813^{B} ± 0.00541	-0.00550^{D} ± 0.00610
31-33*a*	0.02677^{C} ± 0.00299	0.00506^{A} ± 0.00268	-0.02172^{A} ± 0.00397
31-33*e*	0.02767^{C} ± 0.00157	0.00924^{A} ± 0.00385	-0.01843^{AB} ± 0.00446
36	0.03431^{D} ± 0.00300	0.01827^{B} ± 0.00487	-0.01604^{ABC} ± 0.00491
40	0.03308^{D} ± 0.00247	0.01571^{B} ± 0.00396	-0.01737^{AB} ± 0.00364
43	0.03970^{E} ± 0.00125	0.01875^{B} ± 0.00339	-0.02095^{A} ± 0.00363

2. Variations in gaseous lift factor and specific gravity.

Changes in gaseous lift factor through the different stages of development (Fig. 10) reflect variation in the volume of included gases relative to gas-free body volume (Fig. 11). From stage 25ℓ to 27 and from stage 27 to 29, gas volume increased at a faster proportional rate than gas-free body volume (9.3-fold compared to 5.93-fold, and 2.21-fold compared to 1.79-fold, respectively) resulting in an increase in gaseous lift factor. At stage 31/33a, gas volume dropped while gas-free body volume increased resulting in a drastic decrease in gaseous lift factor in autumn. In spring (stage 31/33a and 36), gaseous lift factor increased as gas volume increased relatively more than gas-free body volume. Following stage 36, gas volume and gas-free body volume showed relatively little change and thus gaseous lift factor showed little difference between stages 36 and 43 (Fig. 11).

Body water content varied greatly from 75.2 to 94.1% and increases were closely related to decreases in specific gravity (Fig. 12). That is, the proportion of dry weight increased with specific gravity. Unexpectedly, however the concentrated non-aqueous specific gravity decreased from 1.48 to 1.24 with increasing specific gravity (Fig. 12). Thus the observed changes in specific gravity are being driven by changes in body water content which are greater than oppositely-directed changes in concentrated non-aqueous specific gravity.

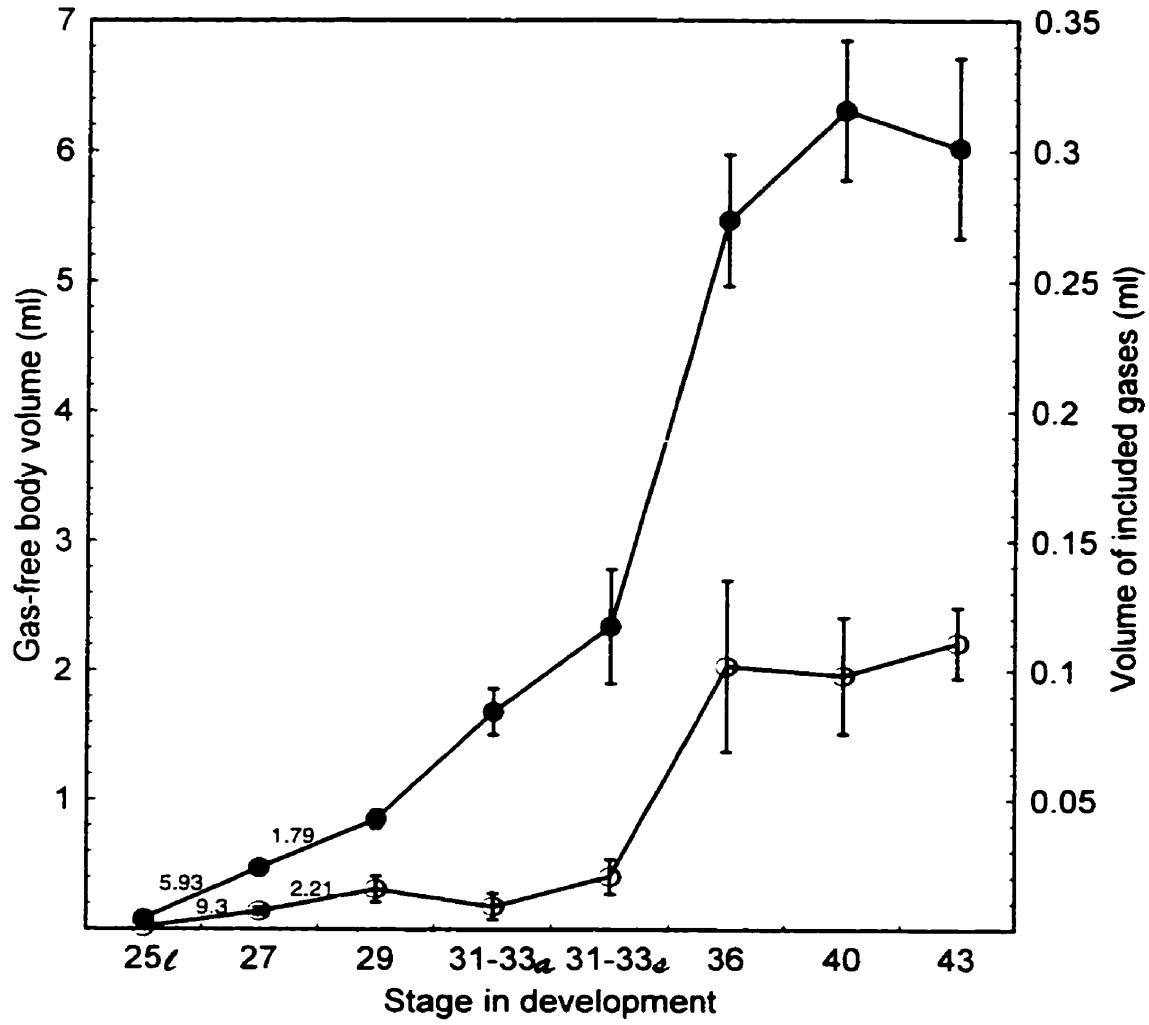


Figure 11: Ontogenetic changes in volume of gases relative to gas-free body volume in mink frogs, *Rana septentrionalis*. Mean values (n = 8) of gas-free body volume (solid circles) and volume of gases (open circles) are shown with 95% confidence limits. Numbers on graph represent the proportional increase between two successive stages of development.

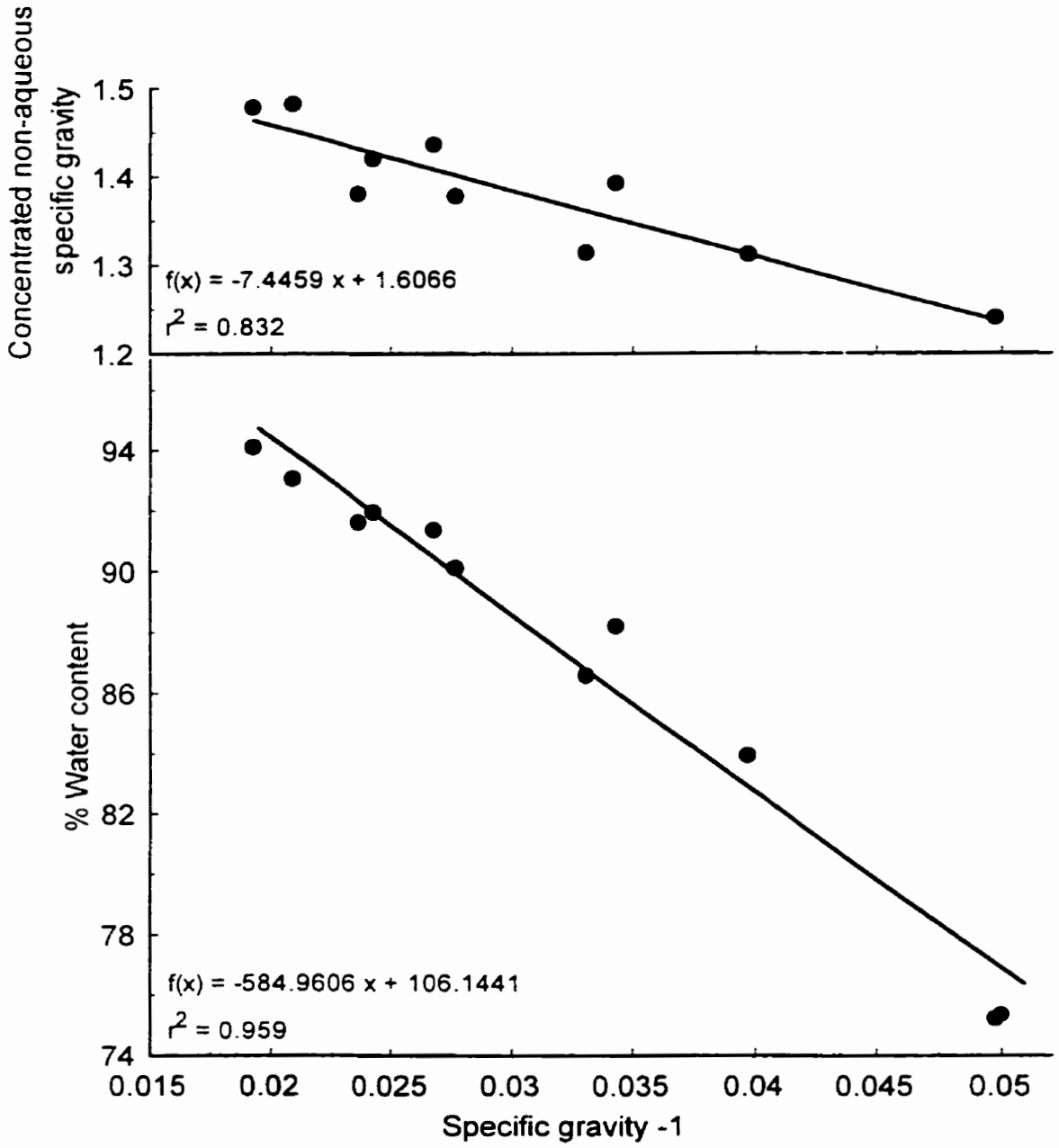


Figure 12: Variation in concentrated non-aqueous specific gravity and percent water content with specific gravity-1 in mink frogs, *Rana septentrionalis*, stages 20 to 43. All values are means (n = 8) and regression lines were fitted by the method of least-squares.

Effect of substrate on buoyancy and related variables

1. Effect of substrate ingestion

Larvae in experimental treatments ingested substrate particles causing their specific gravity to increase (Table 6; Fig. 13). The gaseous lift factor and specific gravity-1 in all treatments were significantly higher than those of controls (one-way ANOVA, $p < 0.001$: lift: $F_{[3, 28]} = 23.4670$, weight: $F_{[3, 28]} = 41.1366$). Buoyancy indices of larvae on sand and silt were similar to controls but the buoyancy index on detritus was significantly higher than the control (one-way ANOVA, $F_{[3, 28]} = 3.4268$, $p = 0.0306$; SNK < 0.05). This indicates that (1) specific gravity increases on ingestible substrates and (2) volume of included gas increases and compensates for the increase in specific gravity with sand and silt treatments, (with a slight overcompensation occurring in the detritus treatment (Table 6; Fig. 13)). Body water content of experimental treatments was not similar to the control (one-way ANOVA, $F_{[3, 28]} = 9.8942$, $p < 0.006$). The following experiment supports the inference that both increased specific gravity and decreased water content occur because the tadpoles have ingested a volume of weighty material containing almost no water, namely sand and silt grains.

2. Ingestion and progression of silt particles through the digestive tract

Although the length of the digestive tract varied greatly (range = 84-188 mm; mean = 127.90 ± 21.32 mm) among individuals in stages 27/28, the increase in

Table 6 : Effect of feeding on detritus, sand and silt on buoyancy index and related variables. Control treatment has no substrate. Values presented are means (n = 8) with 95% confidence limits. Levels of p are from single factor ANOVA.

Variables	Treatments				p
	Control	Detritus	Sand	Silt	
Specific gravity-1	0.02766 ±0.0016	0.03363 ±0.00284	0.03430 ±0.00328	0.05005 ±0.00540	<0.0001
Gaseous lift factor	0.00924 ± 0.00385	0.02404 ±0.00661	0.02063 ±0.00324	0.03641 ±0.00708	<0.0001
Buoyancy index	-0.01843 ±0.00446	-0.00959 ±0.00493	-0.01367 ±0.00384	-0.01365 ±0.00513	0.0270
% body water*	90.1447 ±0.3940	89.5948 ±0.3968	89.6386 ±0.7404	87.7138 ±1.3136	0.0060

* Variance test failed

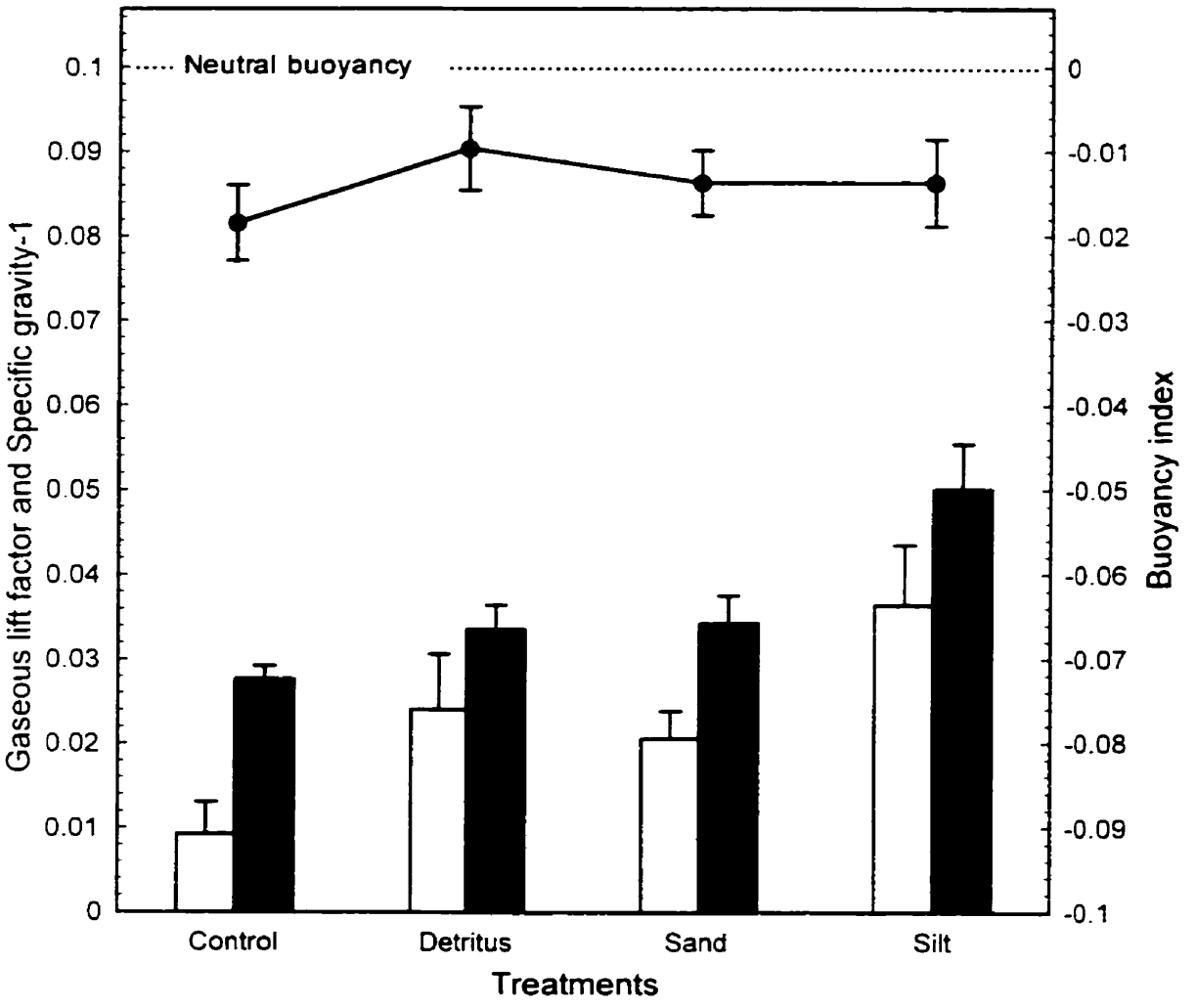


Figure 13: Buoyancy response to ingested substrate particles in larval (stages 31-33) mink frogs, *Rana septentrionalis*. Mean values (n = 8) of specific gravity -1 (solid bars) and gaseous lift factor (open bars) and resulting buoyancy index (solid line) are shown with 95% confidence limits.

body weight parallelled the rate of ingestion and compaction of substrate particles. Individual tadpoles started to ingest substrate particles upon their placement in the aquaria. Silt travelled slowly through the digestive tract for the first 6 h (16.63%;Table 7; Fig. 14). Between 6h and 12h, silt travelled its greatest distance through the digestive tract, i.e., from 16.63% to 77.22%, and tadpoles started to excrete silt particles after 24h. Compaction of silt within the digestive tract increased with time. Although silt was excreted after 24 h, the digestive tract was only 95.5% filled with silt particles. At 48 h, 99.2 % of the digestive tract was filled. From 24 h on, tadpoles started to ingest coarser substrate particles as these could be felt with tweezers along the length of the digestive tract.

Thus, the increase in specific gravity following 24 h (Fig. 14) is due to a combination of ingesting coarser particles and silt compaction, and the increased specific gravity of Fig. 13 is thus shown to be due to substrate ingestion.

3. Latency of buoyancy increase in response to increased weight from silt ingestion

Gaseous lift factor and specific gravity of larvae placed on silt increased over time until stabilization (Fig. 15). Although comparison of lines of best fit indicated that the overall rate of increase in gaseous lift factor was very similar to that of specific gravity (gaseous lift factor = 0.00748 * log(x+1) + 0.0119;

Table 7 : Travel rate of silt through digestive track and degree of compaction in digestive tract. Values are means (n = 8) with 95% confidence limits.

Time (h)	% distance travelled	% silt compaction
0	0.00± 0.00	0.00 ± 0.00
1.5	8.31 ± 2.59	5.70 ± 3.21
3	10.53 ± 5.81	9.06 ± 4.67
6	16.63 ± 8.44	12.80 ± 7.34
12	77.22 ± 20.36	64.38 ± 17.70
24	100.00 ± 0.00	94.24 ± 4.87
48	100.00 ± 0.00	99.21 ± 0.73

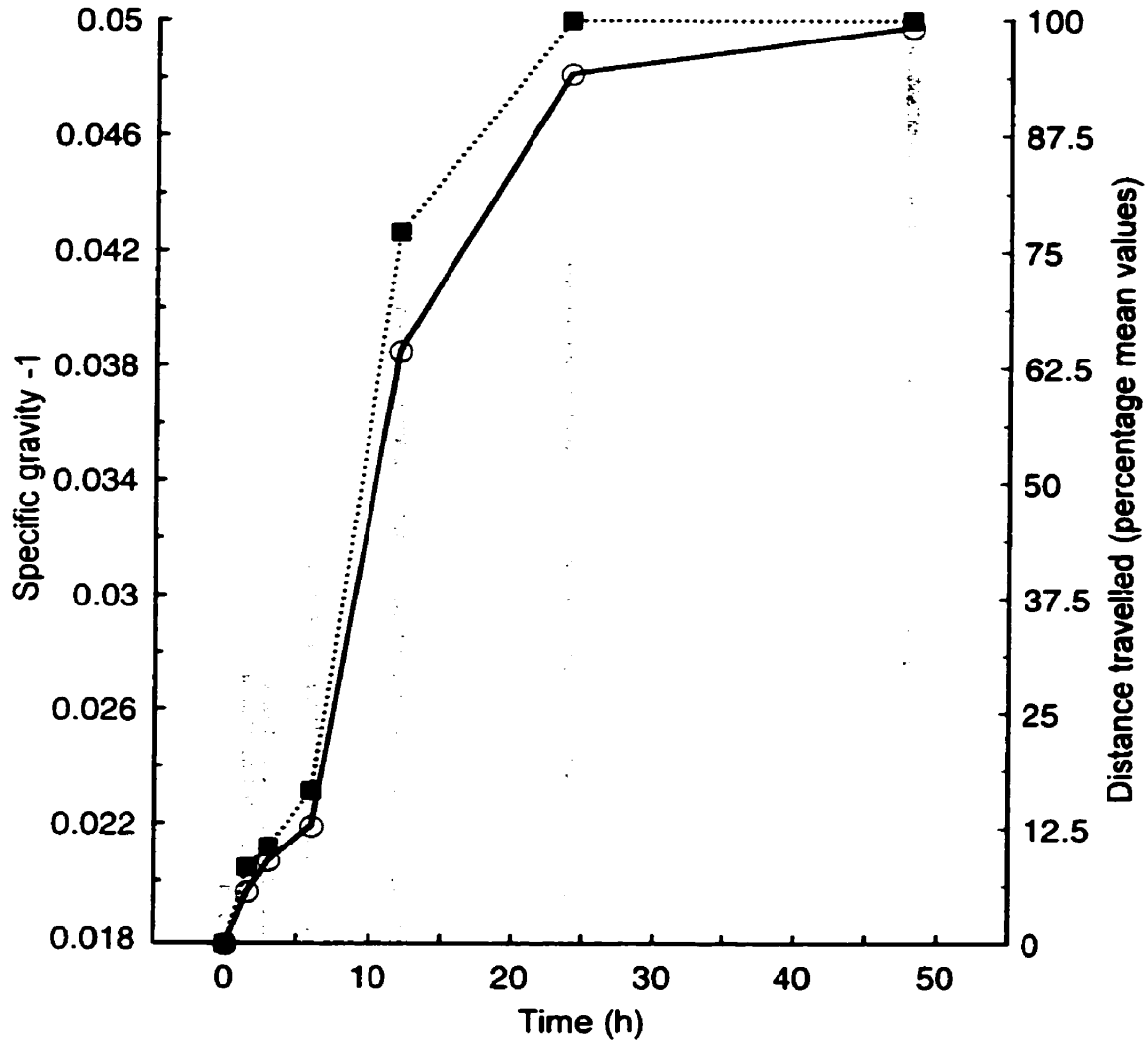


Figure 14: Filling of digestive tract correlated with specific gravity increase in juvenile (stages 27-28) mink frogs, *Rana septentrionalis*, placed on silt substrate over a 48 h period. Percentage mean values (n = 8) of the distance travelled by silt through the digestive tract (solid squares) and silt compaction (open circles) are shown against the specific gravity-1 (bars).

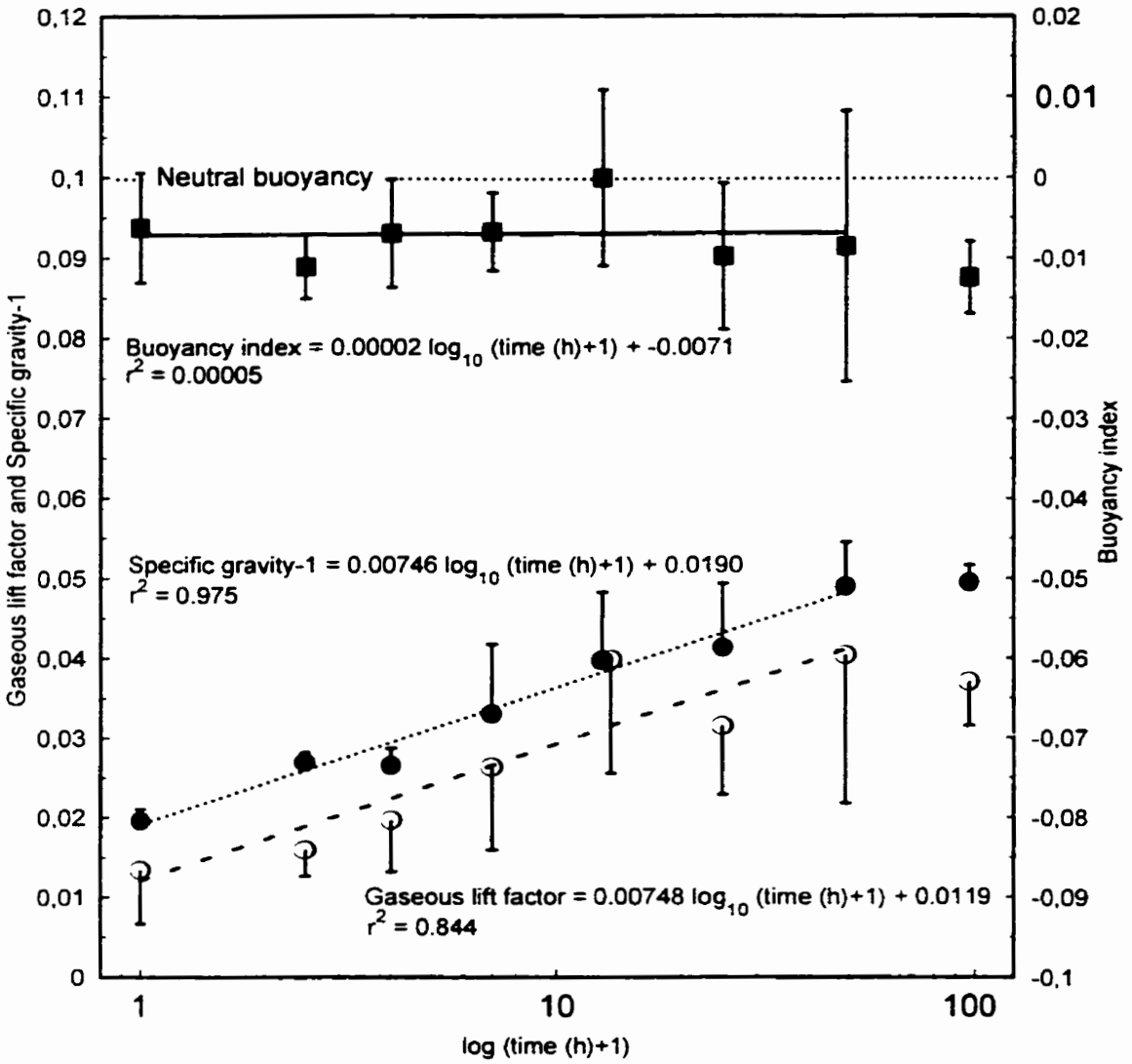


Figure 15: Time-dependence of buoyancy-related variables in mink frogs, *Rana septentrionalis*, feeding on a silt substrate. Mean values (n = 8) of specific gravity-1(solid circles) and gaseous lift factor (open circles) and resulting buoyancy index (solid squares) are shown with 95% confidence limits on the means and the lines of best fit. The independent variable was transformed by log (time (h)+1) as per Gee, 1988 and Gee and Holst, 1992.

r^2 = 0.844; specific gravity-1 = 0.00746 * log(x+1) + 0.0190; r^2 = 0.975; Fig. 15) there was considerable variation among means as well as a difference in the time at which the two variables first departed significantly from control values. Overall variance in specific gravity is less than that of gaseous lift factor, but both variables expressed the greatest variation between 6 and 48 h (Fig. 15), times which correspond to the greatest distance travelled by silt (Fig. 14). Specific gravity increased within 6 h following exposure to silt while gaseous lift factor did not increase significantly until 12 h (one-way ANOVA, $p < 0.001$: $F_{[7, 56]} = 21.1044$ for specific gravity, $F_{[7, 56]} = 5.9951$ for gaseous lift factor). Buoyancy indices did not differ over time (one-way ANOVA, $F_{[7, 56]} = 1.0172$, $p = 0.4294$). Thus larvae compensate for changes in specific gravity, caused by substrate ingestion, by altering volume of included gases maintaining buoyancy level.

Behavioural responses to predators

Tadpole mortality occurred in tests 1, 3 ,4, and 5, and numbers of replicates (tanks) varied from 8 to 10 in each treatment (appendix C). Replicates with mortality were excluded from the analysis. Data were analysed over the entire 5 days for each test. Replacement of predators on day 3 did not significantly alter the behaviour of tadpoles (Appendices A and B).

1. Experiment I

a) Activity: There was a significant effect of predation on activity with small *Pseudacris triseriata maculata* (test 1; Table 8) where tadpoles in the experimental treatment (predators present) were less active than those in the control (Fig. 16; Appendix C). There was no effect of time but the interaction of predation and time was significant (Table 8). On each day, tadpole activity in the experimental treatment was less than the control but the difference varied significantly among days (Fig. 16; Appendix C). In tests 2 and 4, with large *P. triseriata maculata* and *Rana septentrionalis* respectively, there was no significant effect of either factor (time or predation) or their interaction (Table 8). In small *R. septentrionalis* (test 3) there was both a significant effect of time and interaction of time and predation (Table 8). Activity varied among days as did the difference between control and experimental treatments. On day 1 activity was much greater in the control treatment than in the experimental treatment. This difference was reversed on day 5 (Fig. 16; Appendix C). Small *Rana sylvatica* (test 5) showed only a significant effect of time on activity (Table 8), in which activity remained low on days 2 and 3 but was higher on the remaining days (Fig. 16; Appendix C).

b) Vertical distribution: Both predation and time had significant effects on distribution of small *P. triseriata maculata* (test 1; Table 8). Tadpoles in the control treatment were distributed higher in the water column than those in the experimental treatment on each day and the mean distribution of tadpoles in

Table 8: Repeated measures ANOVA for activity and distribution score for the five tests. Replicates with tadpole mortality were excluded.

Species (Test)	Factor		Effect			
			Activity		Distribution	
		df	F	p	F	p
P. triseriata maculata Stages 28-31 (Test 1)	Time	4,60	1.85	ns	3.63	0.010
	Predation	1,15	24.71	<0.001	5.14	0.039
	Interaction	4, 12	4.72	0.016	1.34	ns
P. triseriata maculata Stages 37-40 (Test 2)	Time	4,72	1.96	ns	18.7	<0.00
	Predation	1,18	0,00	ns	0.79	ns
	Interaction	4, 15	0.17	ns	2.33	ns
R. septentrionalis Stages 26-27 (Test 3)	Time	4,64	3.37	0.015	1.02	ns
	Predation	1,16	1.55	ns	2.32	ns
	Interaction	4, 13	5.57	0.008	6.05	0.006
R. septentrionalis Stages 28-29 (Test 4)	Time	4,64	1.43	ns	0.59	ns
	Predation	1,16	0.36	ns	0.27	ns
	Interaction	4, 13	0.47	ns	0.42	ns
R. sylvatica Stages 28-31 (Test 5)	Time	4,64	7.59	<0.001	24.1	<0.00
	Predation	1,16	1.89	ns	0.41	ns
	Interaction	4, 13	0.82	ns	1.71	ns

"ns" for p values > 0.05

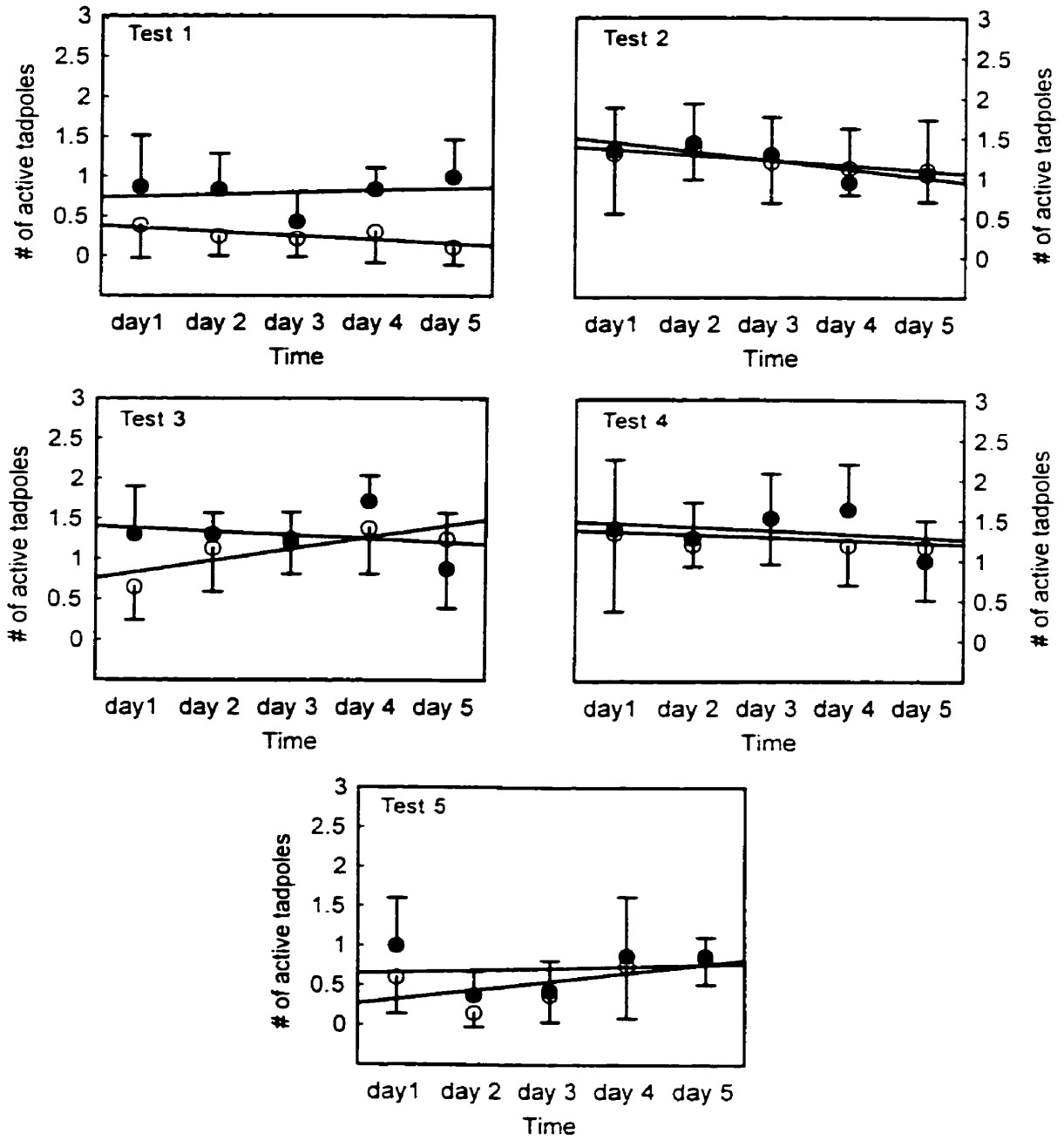


Figure 16: Number of active tadpoles per test over a 5 day period. Only tanks in which no mortality occurred were used. Mean values are shown with the standard deviation and lines of best fit. The solid circles are the control tanks while the open circles represent the predator tanks. Test 1: *P. triseriata maculata* (stage 28-31); test 2: *P. triseriata maculata* (stage 37-40); test 3: *R. septentrionalis* (stages 26-27); test 4: *R. septentrionalis* (stages 28-29) and test 5: *R. sylvatica* (stages 28-31).

both treatments on days 3, 4, and 5 was higher in the water column than on days 1 and 2 (Fig. 17; Appendix D). In large *P. triseriata maculata* (test 2), only the effect of time was significant (Table 8) and here tadpole distribution in the water column increased in height over time (Fig. 17; Appendix D). There was no effect of time or predation in small *R. septentrionalis* (test 3) but their interaction was significant (Table 8). On day 1, tadpoles in the control treatment were distributed higher in the water column than those in the experimental treatment. This difference was reversed by day 5 (Fig. 17; Appendix D). There was no effect of either predation or time in large *R. septentrionalis* (Test 4; Table 8). Only time had a significant effect on distribution in the water column in small *R. sylvatica* (Test 5; Table 8). The mean distribution of tadpoles in both treatments was lower on days 2, 3, and 4 than on days 1 and 5 (Fig. 17; Appendix D).

c) Buoyancy index: Only small *P. triseriata maculata* (test 1) showed an effect of predation on buoyancy index and related variables (Table 9). In the experimental treatment these tadpoles displayed a significantly lower buoyancy index, specific gravity and gaseous lift factor (Fig. 18; Appendix E). Although tadpoles in the presence of predators had a specific gravity lower than those in the control treatment, the reduction in buoyancy was due primarily to the major decrease in lung volume (about 50%). Thus in small *P. triseriata maculata* the reduction in activity and the decrease in selected depth in the water column

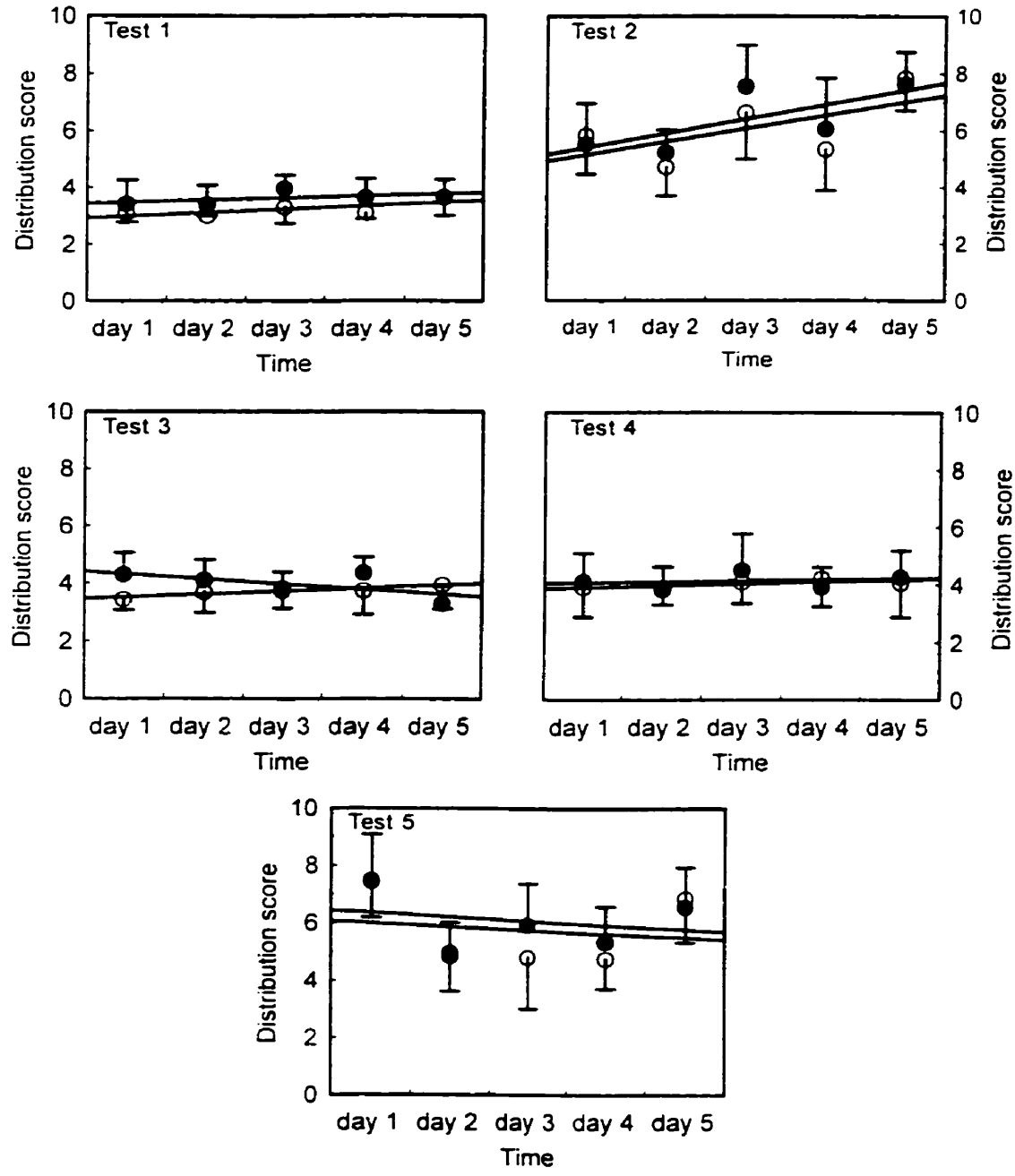


Figure 17: Distribution score per test over a 5 day period (score reflects height in tank - see p. 40). Only tanks in which no mortality occurred were used. Mean values are shown with the standard deviation and lines of best fit. Solid circles - control; open circles - predator present. Test 1: *P. triseriata maculata* (stage 28-31); test 2: *P. triseriata maculata* (stage 37-40); test 3: *R. septentrionalis* (stages 26-27); test 4: *R. septentrionalis* (stages 28-29) and test 5: *R. sylvatica* (stages 28-31).

Table 9: Comparisons of changes in buoyancy-related variables between control and experimental treatments for 5 tests measured on 3 different species and 2 age groups. F and P values for each variables are given.

Species (Test)	Buoyancy-related variables	Statistical data		
		df	F	p
P. triseriata maculata Stages 28-31 (Test 1)	Specific gravity-1	1,22	6.2762	0.0201
	Gaseous lift factor	1,22	14.0594	0.0011
	Buoyancy index	1,22	8.1052	0.0094
P. triseriata maculata Stages 37-40 (Test 2)	Specific gravity-1	1,22	0.2536	ns
	Gaseous lift factor	1,22	0.00	ns
	Buoyancy index	1,22	0.0014	ns
R. septentrionalis Stages 26-27 (Test 3)	Specific gravity-1	1,22	0.1097	ns
	Gaseous lift factor	1,22	0.0500	ns
	Buoyancy index	1,22	0.0672	ns
R. septentrionalis Stages 28-29 (Test 4)	Specific gravity-1	1,22	1.0464	ns
	Gaseous lift factor	1,22	1.1566	ns
	Buoyancy index	1,22	1.9191	ns
R. sylvatica Stages 28-31 (Test 5)	Specific gravity-1	1,22	0.0641	ns
	Gaseous lift factor	1,22	1.3118	ns
	Buoyancy index	1,22	1.2228	ns

"ns" for p values > 0.05

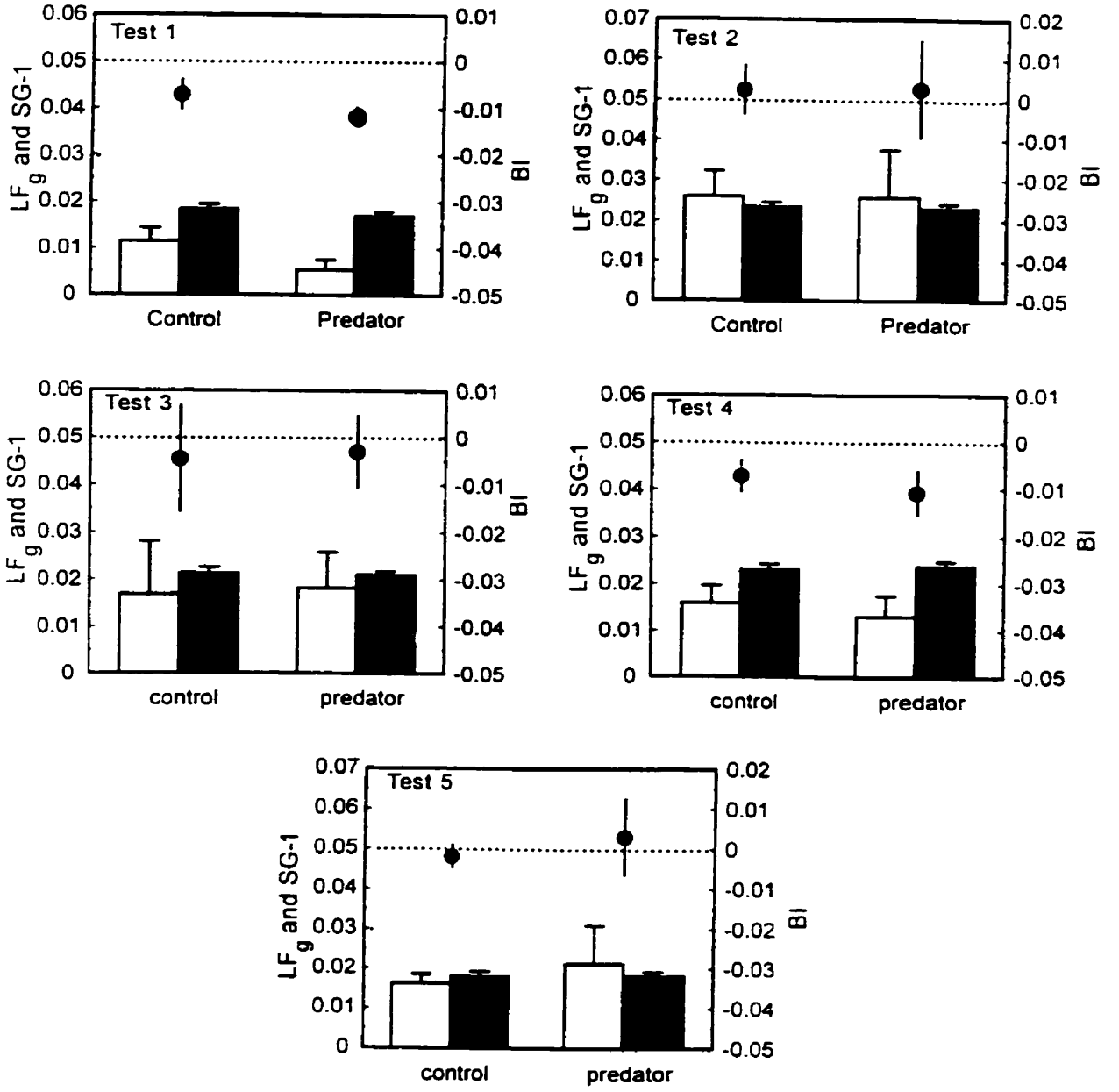


Figure 18: Buoyancy index per test over a 5 day period. Only tanks in which no mortality occurred were used. Mean values (n = 12) of specific gravity-1 (solid bars), gaseous lift factor (open bars) and resulting buoyancy index (solid circles) are shown with 95% confidence limits on the means. The horizontal dotted line indicates neutral buoyancy. Test 1: *P. triseriata maculata* (stage 28-31); test 2: *P. triseriata maculata* (stage 37-40); test 3: *R. septentrionalis* (stages 26-27); test 4: *R. septentrionalis* (stages 28-29) and test 5: *R. sylvatica* (stages 28-31)

were accompanied by a reduction in buoyancy index. There was no effect of predation in the remaining tests.

2. Experiment II:

a) Frequency of air breathing

In small *P. triseriata maculata* (test 1), the number of air breaths for the control treatment (tadpoles with predator absent) exceeded slightly that of the experimental treatment (tadpoles with predator present) for 5 of the 8 observations (Table 10). However, the Fisher exact test showed no significant difference ($p = 0.6958$) in number of air breaths between tadpoles in the presence or absence of a predator in small *P. triseriata maculata* (Table 11).

Table 10: Number of air breaths /12 tadpoles /20 minute period over 92 hours for small *Pseudacris triseriata maculata* in test 1

Treatment	Time (h)								Mean (SD)
	0	20	24	44	48	68	72	92	
Control	2	3	2	5	2	3	2	1	2.5 (1.2)
Predator	3	1	1	3	0	2	2	4	2 (1.3)

Table 11: Number of times that the number of air breaths exceeded the mean number of air breaths/sample for small *Pseudacris triseriata maculata* in test 1 (2.25).

	Category		Total
	< 2.25	> 2.25	
Control	5	3	8
Experimental	5	3	8
Total	10	6	16

DISCUSSION

Horizontal and vertical distribution in the field

Mink frog larvae and metamorphics were benthic and found concentrated in on-shore shallow waters within dense vegetation. Hatchlings were not seen or captured and only one clutch of eggs was found, located near shore. These results complement existing observations of mink frog distribution for stages 25 to 45. Watkins (1997) found more mink frogs (stages 25 to 45) in vegetation than in areas without vegetation when seining at McGillivray Creek in the summers of 1995 and 1996. This preference was confirmed by Watkins (1997) in a laboratory experiment with larvae (stages 26-28 and 37-42). A distribution survey by Courtois *et al.* (1995) within oligotrophic lakes of the Precambrian Shield in Québec also found mink frog larvae to be more abundant in vegetation near shore on silty or muddy substrates.

Mink frogs usually attach their eggs to lily pad stalks growing in deep water (Moore, 1952), a region that I was unable to sample. The gelatinous egg mass requires well-oxygenated water so that embryos in the center of the egg mass can survive (Moore, 1949). When embryos in the center of the mass die, their decomposition produces lethal substances affecting peripheral embryos. Thus adult mink frogs must submerge the egg mass in deeper, colder water, ensuring a sufficient concentration of oxygen for all embryos. Since the amount of oxygen in the water is temperature-dependent, Hedeen (1986) hypothesised that the mean monthly temperature should not exceed 21°C during egg

development for the embryos to survive. The water temperature taken on a weekly basis (1 m depth) during sampling at McGillivray Creek, from June 18 to July 5, 1996, ranged from a maximum of 28.3°C during the day to a minimum of 15.5°C at night, a range that Hedeen (1986) suggested ensures good survivorship rate of embryos. As a result, embryos and hatchlings develop off-shore and, when they become active (stage 25), they move on-shore.

Temperature and food availability are the two most important factors affecting larval growth (Duellman and Trueb, 1994), and these variables, in turn, can be used to explain larval distribution. Throughout the summer of 1996, mean water temperature on shore in the afternoon was approximately 5°C warmer than water off-shore, resulting in faster growth for larvae as the growth rate of larvae is determined by temperature (Calef, 1973). Furthermore, the dark colour of the body contributes additional warming due to solar radiation. Thus by optimizing heat absorption, they can maximize metabolism and growth. In permanent bodies of water, most larval mortality can be accounted for by predation, and survivorship to metamorphosis rarely exceeds 10%; in fact it is usually closer to 5% (Calef, 1973). The survivorship curve of larval anurans appears to be Type III (concave), as most of the mortality occurs in the early stages of life (Calef, 1973; Crump, 1984). Maximizing metabolism and growth is adaptive because larvae that grow quickly reduce risk of predation from fish and aquatic insects as a result of their larger size (Calef, 1973; Caldwell *et al.*, 1980; Brodie and Formanowicz, 1983; Travis *et al.*, 1985). Mink frog larvae inhabit

permanent ponds, which usually are much more heavily populated with predators (fish and aquatic insects) than temporary or seasonal waters (Skelly, 1997). At McGillivray Creek, mudminnows, dragon fly larvae, water scorpions and giant water bugs are well established and were observed preying upon mink frog larvae (Watkins, 1997). But because risk of predation in larval amphibians is size-dependent, tadpoles can reduce their vulnerability to predators faster by seeking warmer shallow water when their own growth is faster.

Not only does a fast growth rate and large size reduce vulnerability to predators, but it is also essential for successful and timely metamorphosis (Wilbur and Collins, 1973). Although a minimum size is required for metamorphosis, species in permanent bodies of water tend to remain in ponds until the optimum size for metamorphosis is reached (Wilbur and Collins, 1973). Mink frog remain as larvae for a minimum of 11 months (Hedeen, 1971) and metamorphose at a much larger size than other species, especially those of temporary ponds. Thus mink frog larvae would hasten their metamorphosis by seeking shallow, warmer water to increase growth rate. Furthermore, near-shore waters provide larvae with other advantages: cover and protection from predators and a rich foraging habitat (Folsom and Collins, 1984; Rozas and Odum, 1988; Stauffer and Semlitsch, 1993).

How can larvae locate these optimal areas? Most likely phototaxis plays a major role. Positive phototaxis of larvae has been demonstrated in tadpoles of many species including some species of the genus *Rana*. Beiswenger (1977) demonstrated in *Bufo americanus* that activity, swimming and feeding increased

with light intensity and that once larvae became active they are attracted to shallow and warmer water in sunlit areas of the pond. Observed diel cycles were also closely correlated with cloud cover. Activity was higher on sunny days than overcast days. Thus initially, light attracts larvae and directs their movements. Being positively phototaxic ensures that stage 25 larvae could move from hatching sites in deep water to warmer on-shore waters in July, and stage 31 larvae could migrate from overwintering sites in deep water to on-shore waters in May. This would allow larvae to anticipate the heating of shallow areas during the spring and summer, so they can move into these areas as they warm up and take full advantage of the additional time for feeding and of the sun's warmth to maximize metabolism and growth (Duellman and Trueb, 1994).

Vertical distribution and activity in the laboratory

Laboratory experiments clearly indicated that mink frog larvae are benthic and relatively inactive. Activity was limited to feeding and swimming to the surface where air breaths were taken. A benthic and inactive mode of life combined with cryptic colouration decreases the chances of larvae being detected by predators.

Larvae are faced with a trade-off between growing slowly while avoiding the risk of being eaten by a predator and rapid growth at the risk of being eaten (Skelly, 1997). Since predation risk is lower in temporary bodies of water, species inhabiting these ponds are usually more active and favour rapid growth to attain metamorphic size prior to the pond drying. In contrast, more and larger

predators are found within permanent bodies of water and predation pressure is higher, so species in these ponds tend to favour inactivity reducing predation risk. By being inactive, mink frog larvae reduce their risk of giving away their position by creating pressure waves, and thus enhance their chances of survival. However, being inactive also reduces their feeding rate (and in turn their growth and development) which ultimately means that a longer time is required in the face of predation to attain the minimum size required prior to metamorphosis. Species which larvae are inactive and less plastic in their activity response to predators could either exploit the complexity of the environment as it reduces predator foraging efficiency (Babbitt and Tanner, 1997) or they could avoid detection by feeding within the substrate (*e.g.*, flocculent detritus).

In contrast, metamorphic stages spend significantly more time in the upper portion of the aquarium resting partially or totally out of the water. In the field, metamorphics were frequently seen resting partially out of the water in or on vegetation near shore, where they can escape aquatic predators (Crump, 1984). When frightened by a terrestrial predator they quickly darted into the water (pers. obs.).

Buoyancy profile

The mink frog remains negatively buoyant from hatching to metamorphosis as shown by its buoyancy profile (Fig. 10). Significant changes in buoyancy index levels occur within the negative range during development as a result of changes in both specific gravity and gaseous lift factor. Variations in

gaseous lift factor were due only to changes in lung volume relative to body volume (Fig. 11) while fluctuations in specific gravity were due to changes in body water content. Figure 12 shows that specific gravity varied inversely with body water content whereas there was no relationship between concentrated non-aqueous specific gravity and specific gravity. As changes in water content determine ontogenetic variation in specific gravity, water content ultimately plays an important role in determining the amount of gas-free lift.

Specific gravity was highest at hatching but decreased throughout the hatchling phase due to water uptake. Rappaport (1954) also observed water uptake to occur during hatchling development. Hatchlings do not feed, and therefore the decrease in specific gravity resulted from an increase in body volume due to water uptake without a corresponding increase in their weight which ultimately increased non-gaseous lift. Gee and Waldick (1995) found similar decreases in specific gravity during the hatchling phase in four other species of anurans. At the beginning of larval stages (stage 25*ℓ*), the mouth forms, lungs are inflated and tadpoles start feeding. From this point on, specific gravity increases gradually until metamorphosis, primarily due to a gradual decrease in water content. There was no relationship between density of dry tissues and specific gravity to suggest that this affected the increase in specific gravity. Brown *et al.* (1988) found with *Rana sylvatica* that both water gain (during hatchling stages) and loss (during larval and metamorphic stages) occurred during development. *Rana sylvatica* and *Pseudacris triseriata maculata* showed a similar increase in specific gravity during larval stages to that

of *R. septentrionalis* but the remaining three species studied by Gee and Waldick (1995) showed no distinct change. Campeny and Casinos (1989) also found that specific gravity did not change during larval development in *Alytes obstetricans* although their measurements did not have the same degree of precision as those reported here.

Ontogenetic variation occurs in gaseous lift factor during development of mink frogs. Lungs were inflated by stage 25ℓ allowing gaseous lift factor to increase during the summer (to stage 29). In autumn (stages 31-33ⓐ), prior to hibernation, gaseous lift factor dropped drastically. In spring, gaseous lift factor initially was similar to that in autumn but subsequently increased during late larval stages and stabilized prior to metamorphosis. Except for stage 25ℓ. when lung inflation commenced, gaseous lift factor values were significantly lower just prior to and following hibernation than during other larval and metamorphic stages.

There is variation among species in the time at which lungs are inflated. Many species of anurans inflate their lungs during stages 25-26 while Bufonidae, as well as some torrent-adapted species, do not inflate their lungs until late in metamorphosis (Feder, 1981). Among species that inflate their lungs in early larval stages, there are also ontogenetic differences in the pattern of change in gaseous lift factor. In addition to the pattern described for *R. septentrionalis*, some species display a steady increase in gaseous lift factor during development *(Pseudacris triseriata maculata* and *Rana pipiens*- Gee and Waldick, 1995),

while others show an increase until mid-larval stages followed by a decline in late larval or metamorphic stages *(Rana sylvatica* - Gee and Waldick, 1995), and still other species show no significant variation over time (*Hyla* sp. from stages 26 to 37 - Gee and Waldick, 1995; *Alytes obstetricans* - Campeny and Casinos,1989).

Thus ontogenetic variation in buoyancy indices of the mink frog from stages 20 to 43 can be accounted for quantitatively by changes in both specific gravity (non-gaseous lift) and gaseous lift factor. The buoyancy index increased during hatchling and early larval stages including stage 29 first because specific gravity decreased and then because the lungs inflated and enlarged. Prior to hibernation (stages 31-33*a*), buoyancy index dropped significantly due to the reduced gaseous lift factor. Upon emergence from the substrate in spring, buoyancy index remained low as increasing gaseous lift counteracted increasing specific gravity. Buoyancy indices of the mink frog are lower during early larval development than those of other species studied by Gee and Waldick (1995) that inflate their lungs and are similar to that of *Bufo americanus* (lungs deflated) during stages 31 - 43 (Fig. 19). This could be related to differences in predation pressure (see other sections).

During hatchling stages, increases in buoyancy indices were similar for *R. septentrionalis* and all species studied by Gee and Waldick (1995). However, during larval and early metamorphic stages, mean buoyancy indices at particular stages varied within species with some species showing considerable variation during development (Fig. 19). *Rana septentrionalis* displayed considerable

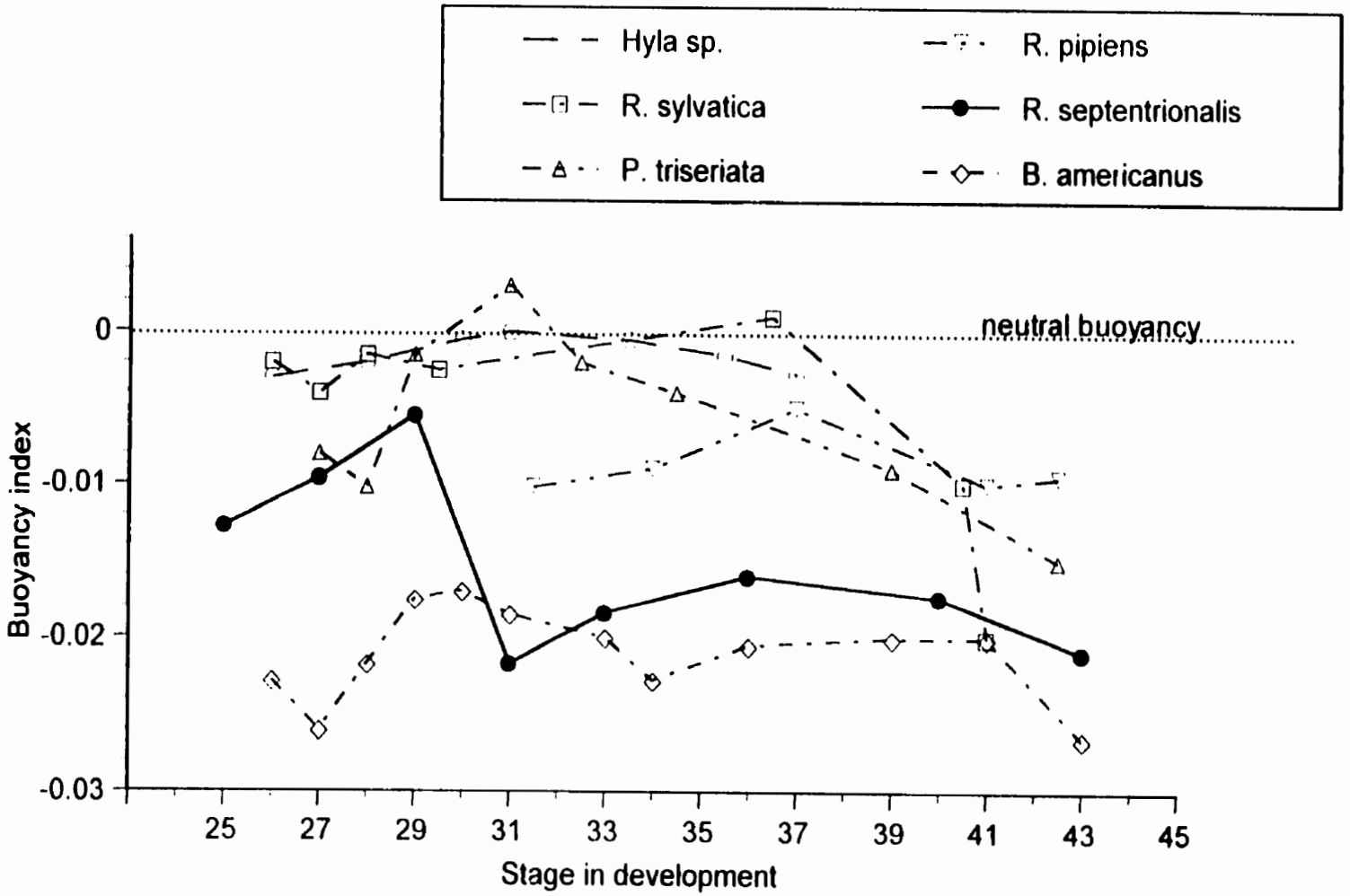


Figure 19: Buoyancy profiles of *Rana septentrionalis* (solid line) and the 5 species studied by Gee and Waldick (1995).

variation over a range of negative buoyancy levels during larval and metamorphic development, as did *Bufo americanus* (Gee and Waldick, 1995). *Rana sylvatica* showed similar ontogenetic variation but over a range of higher buoyancy levels including near-neutral levels. In contrast, other species showed little variation, including *Hyla* sp., which remained at near-neutral levels of buoyancy, and *R. pipiens*, which held somewhat constant buoyancy at negative levels.

There are striking differences in the pattern of buoyancy change among species. Some species increase their buoyancy in early larval stages and then decrease it during mid-larval stages (*Pseudacris triseriata maculata* - Gee and Waldick, 1995); other species decrease their buoyancy only at metamorphosis (*Rana sylvatica* - Gee and Waldick, 1995), and still others show repetitive increases and decreases (*Bufo americanus* - Gee and Waldick, 1995). Gee (unpub data) found similar results in an examination of buoyancy profiles of 8 species of Australian anurans from a variety of habitats.

A knowledge of ontogenetic variation in buoyancy is important in understanding changes in mode of life that occur during development, such as hibernation in *R. septentrionalis*. There are indeed great differences among species in the range of buoyancy variation experienced during development, and these can be related to differences in mode of life. Ranges of mean buoyancy indices from stages 25 to 42 are summarized for *R. septentrionalis*, species

studied by Gee and Waldick (1995) and eight Australian species studied by Gee (unpub data) in Fig. 20. Species are separated into two groups based on whether or not lungs are inflated in early larval stages and three buoyancy zones are distinguished. Among species that inflate their lungs in early larval stages (group 1), there are some species that are near neutral buoyancy (Zone A) over all stages, others that are negatively buoyant (Zone B) throughout all stages, and species that are near neutral buoyancy during part of their development and negatively buoyant during the remainder of their development. Among species that do not inflate their lungs in early larval stages (group 2), there are those at negative buoyancy (Zone B) and others at extreme negative buoyancy (Zone C). Among species with lungs inflated (group 1), variation in gaseous lift factor accounts for most of the observed stage-specific buoyancy differences as body water content at particular stages is similar over all species. Buoyancy differences among species with no lung gases present (group 2) are caused by differences in non-gaseous lift due to interspecific variation in body water content: bufonids have much higher body water content than species with lungs inflated while the other two species in this group have a greatly reduced body water content (Gee, unpub data). The relationship of these buoyancy differences to mode of life is discussed below.

Buoyancy profiles allow interspecific comparisons of buoyancy levels attained at particular stages of development under a similar set of conditions, namely in an oxygenated, still-water aquarium, with conspecifics present, no substrate, and no predators present. However, lung volume is plastic, resulting

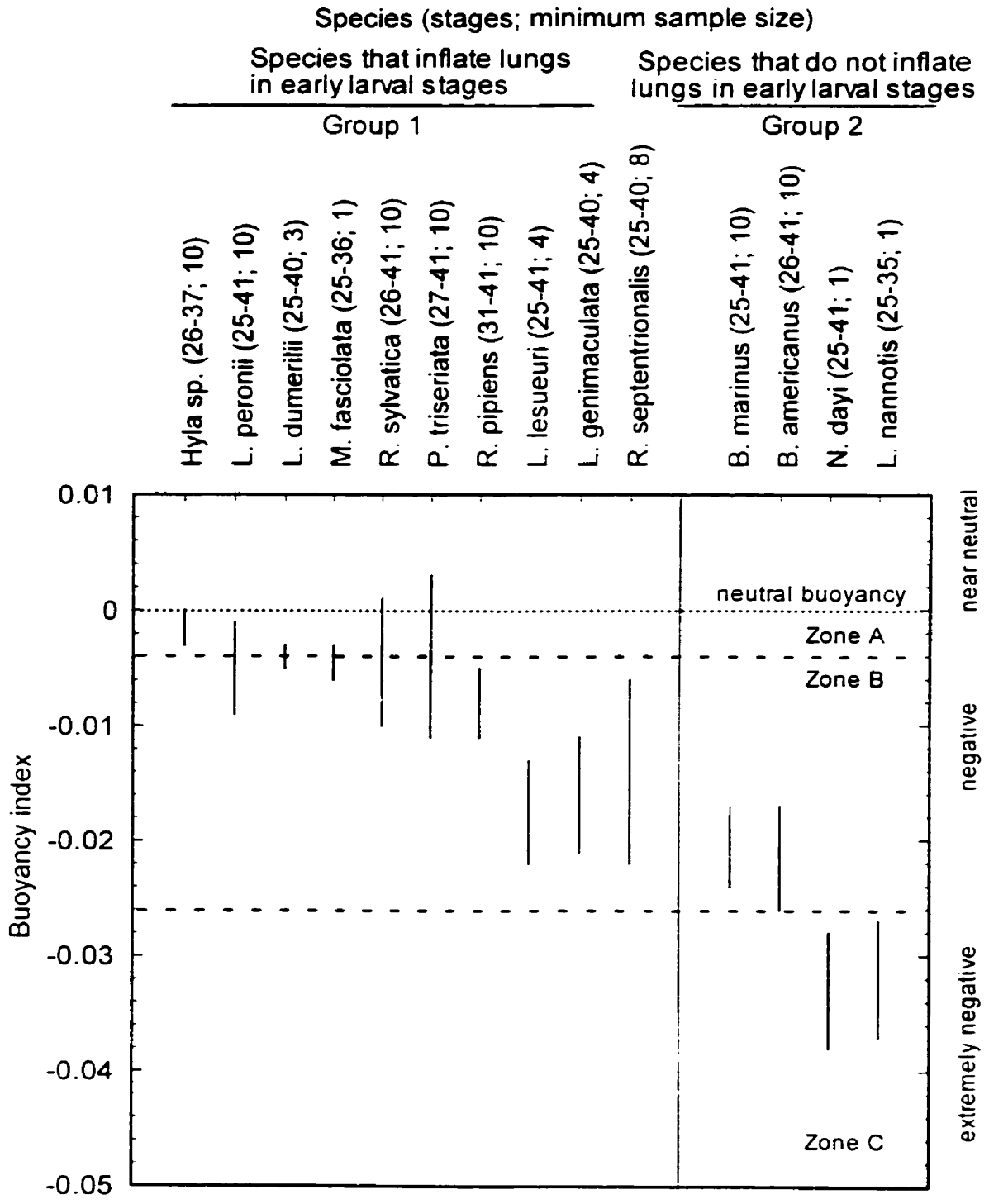


Figure 20: Ranges of mean buoyancy indices for *Rana septentrionalis*, as well as the five species studied by Gee and Waldick (1995) and eight Australian species (Gee, unpub data). Stages of development and minimum sample size are given in brackets for each species. Dashed lines delimit the three buoyancy zones.

in different levels of gaseous lift. Such plasticity permits (a) a change in buoyancy as during hibernation or the presence of predators and (b) the maintenance of a particular buoyancy level when there are changes in specific gravity due to ingested materials when feeding. As a result, buoyancy profiles measured under different sets of conditions may be very different from each other. In fishes the swimbladder volume is plastic and different levels of buoyancy are achieved with variation in environmental variables that include water temperature and water velocity (Gee, 1983; Beaver and Gee, 1988).

Effect of substrate on buoyancy and related variables

1. Effect of substrate ingestion

Rana septentrionalis larvae ingested all types of substrate particles present during feeding in experimental treatments, causing both specific gravity and subsequently gaseous lift factor to increase significantly. Gaseous lift factor increased and compensated for the increase in specific gravity caused by silt and sand ingestion leading to similar buoyancy indices among control, silt and sand treatments. However tadpoles on detritus increased gaseous lift factor to such an extent that they overcompensated for the increased specific gravity resulting in a significantly higher buoyancy index than controls.

Maintaining an optimal buoyancy is important as it minimizes energy expenditure in locomotion, holding position, predator avoidance and feeding. Furthermore, changes in the environment can happen gradually or suddenly and tadpoles must be able to react to them in a precise and controlled manner. A

limited number of mechanisms are known to adjust gaseous lift factor as means of maintaining buoyancy in aquatic vertebrates, including gulping and spitting gases, secreting and resorbing gases, altering internal pressure of gases in gas inclusions in fishes (Gee, 1983) and altering depth (hydrostatic pressure) in sea snakes (*Pelamis platurus*; Graham *et al.*, 1987). In amphibians, gulping air at the surface and spitting are used to alter gaseous lift factor. Furthermore, lungs are very elastic and as a result lung volume can be adjusted rapidly. Air breathing also allows tadpoles to replace any gases lost to diffusion to the blood. Thus changes in specific gravity resulting either from substrate ingestion or its subsequent excretion can be compensated by adjusting gaseous lift factor to maintain buoyancy.

The overcompensation of buoyancy adjustment when tadpoles were feeding on detritus is puzzling at first, but may be explained simply in the following way. In a study by Seale and Wassersug (1979), tadpoles were found to regulate food intake as a function of biovolume rather than particle numbers. Although mechanisms involved in regulating feeding are not completely understood, their study showed that tadpoles can adjust pumping rate, buccal volume displacement and efficiency of entrapment so as to get the same biovolume of food regardless of differences in cell volume among various algae. So detection and regulation of ingested biovolume seems critical and essential to successful feeding. However, regulating intake by detection of biovolume alone will not provide a foolproof basis for buoyancy compensation. If a tadpole automatically associates a certain biovolume with a certain weight of ingesta

(based on some "mean ingesta density" somehow built into the tadpole's feeding control system) and adjusts its buoyancy on that basis to compensate for food intake, then ingestion of denser-than-average material should be undercompensated while ingestion of low-density material like detritus should be overcompensated. In other words, since detritus particles are less dense than silt or sand and therefore occupy a greater volume for a given weight, *R. septentrionalis* will exaggerate its buoyancy compensation for ingested detritus, if it measures food intake purely by volume and not by weight. There is also the possibility of a time lag between the increase in specific gravity and the increase in buoyancy index. If so, this period must be very short as results from the silt and sand treatments shows that the control of buoyancy on these substrates is precise.

There are several explanations as to why tadpoles consume substrate particles. Except for specialized species, larval anurans are opportunistic and indiscriminate feeders, consuming fauna and flora in similar proportion to that found in their environment (Farlowe, 1928; Jenssen, 1967; Nathan and James, 1972). Tadpoles ingest among other things: vascular plants, vascular plant-based detritus, free and attached algea, periphyton (a combination of filamentous green algae, cyanobacteria, diatoms, bacteria and desmids), particulate organic debris plus attached bacteria and meiofauna, substrate including mud and sand, various protozoans, fairy shrimp and other tadpoles (Jenssen, 1967; Nathan and James, 1972; Altig and Johnston, 1989; Kupferberg, 1997; Gee, unpub data).

Depending on feeding habits, different proportions of inorganic matter are ingested by different species. Benthic larvae feeding on the bottom of ponds and streams, such as *Bufo woodhousii* and *Rana pipiens* have more inorganic matter in their gut than those feeding in the water column, *e.g.*, *Gastrophryne*, *Hyla chrysoscelis* and *Rhinophrynus (*Altig and Kelly, 1974). Furthermore, Jenssen (1967) found that a "moderate portion" of the intestinal bulk of *Rana clamitans*, a benthic larvae, was composed of sand, decomposed higher plants and other debris. Garnier (1883) mentioned that intestines of *Rana septentrionalis* tadpoles were "often full of the common muddy matter found in all species of tadpoles". Thus benthic tadpoles ingest more indigestible matter than pelagic tadpoles, as they spend most of their time grazing on the substrate of ponds and stream. Differences in amount of inorganic matter can also be observed within a single species based on spatial distribution. Altig and Kelly (1974) sampled *Pseudacris triseriata* from two habitats of different productivity. Individuals sampled from the nonproductive habitat had more than twice the amount of inorganic matter than those sampled from the productive habitat. In the nonproductive habitat, *P. triseriata* were observed to spend more time feeding on the bottom, resulting in an increase in inorganic matter.

It seems that ingestion of substrate, particularily the inorganic particles, is important for normal growth and development of benthic species. Growth and development were recorded by Nathan and James (1972) for benthic tadpoles of *Bufo regularis* and *Bufo garmani* raised on either sustrate (ooze) only, lettuce only, or a combination of the two. Their results showed conclusively that a

continuous combination of substrate particles and plant material was necessary for normal growth and development, as it led to healthier, larger and more active tadpoles. When deprived of either substrate or plant material, tadpoles had difficulty maintaining themselves until metamorphosis. Mortality of tadpoles raised on substrate only was extremely high and those that survived showed minimal growth and did not metamorphose. Tadpoles raised on only lettuce became lethargic and very thin and moved erratically with apparent loss of balance; they also experienced a high mortality rate. In the latter group, addition of substrate reversed the tadpoles' condition. Other experiments comparing field substrate (containing live micro-organisms) with sterilized substrate and/or lettuce, showed that the substrate itself was essential for the tadpoles' normal growth rather than the micro-organisms contained within it. *Bufo regularis* could be raised successfully to metamorphosis in the absence of protozoa, but tadpoles which did not receive substrate metamorphosed at a much smaller size.

The nutritional role of the substrate, while clearly important, is not completely understood. Nathan and James (1972) hypothesised that sand particles could either stimulate peristalsis and hence breakdown of plant cells or serve as a type of grinding medium to macerate plant material. Altig *et al.* (1975) argued that substrate may contain protein fragments formed by bacterial degradation which may provide benthic tadpoles with a rich food source. Prior to stage 38, bottom-dwelling *Bufo* lack pepsin, which is necessary to break large protein molecules into smaller units. If smaller protein fragments acquired from the substrate could be digested by proteases other than pepsin, the nutritional

benefit to these larvae would be great. High protein diets are important as they result in rapid growth and development of larvae (Kupferberg, 1997). Whatever the reason(s) for the ingestion of substrate particles, their presence in the gut has a profound influence on the specific gravity of the tadpole.

2. Transit time of silt particles through the digestive tract and buoyancy response to increased weight from silt ingestion.

Rana septentrionalis at stages 27-28 ingested silt particles immediately on exposure to substrate, but 24 h were required before silt was excreted and compaction of particles in the gut occurred over an additional 24 h. As a result, specific gravity increased over 48 h. Concurrent with the increase in specific gravity was an increase in gaseous lift factor which offset the increase in weight. Thus buoyancy indices remained similar and no significant changes occurred while silt particles were ingested.

Tadpoles eat large quantities of food and clearance times are variable. In addition, not all food is assimilated as much of the material passes through the gut undigested. Clearance times of five anuran species (*Gastrophryne carolinensis*, *Acris gryllus*, *Bufo woodhousii*, *Rana catesbeiana*, *Rana heckscheri*) studied by Altig and McDearman (1975) ranged from 29 to 101 min and are the shortest reported in the literature for anurans. However, commercial food (rabbit pellets) was used as food in their study, and these may have been more digestible than food items eaten in the field accounting for the faster clearance rates. Clearance time could be a function of the relative length of the

gut compared to the length of the tadpole. However Altig and McDearman (1975) concluded that there was no such relationship in an analysis of the above five species. Other reported clearance times are longer and include 6.25 h for *Rana temporaria* and 3.75 h for *Bufo bufo* (Savage, 1962 as cited by Altig and McDearman, 1975) and 6 h for *Xenopus* and 2.75 - 6.25 h for *Rhacophorus cruciger* (Ueck, 1967 as cited by Altig and McDearman, 1975). Clearly, other factors including type of material ingested, developmental stage and temperature play a role in determining clearance time (Altig and McDearman, 1975). The clearance time I report herein for *Rana septentrionalis* (24 h) is that for ingested material containing mainly substrate particles. When ingested material is largely organic it may clear more rapidly than when it is largely inorganic particles. Temperature is also important since an increase of 10°C decreases the clearance time by about 2.5X while increasing percentage assimilation by 3.2X (Altig and McDearman, 1975). Clearance times reported by Altig and McDearman (1975) were obtained from tadpoles maintained at 22°C while the present results from *Rana septentrionalis* were from tadpoles at 13 ± 1°C. Although the intestine of *Rana septentrionalis* is quite long, the ratio of intestine length to total body length (3.81 ± 0.46) falls within ratios of other species (1.43 ± 0.14 to 8.08 ± 0.86) obtained by Altig and Kelly (1974). Gut lengths appear to be more related to diet than to clearance times, as carnivorous species such as *Gastrophryne* have a shorter gut relative to body length (1.56 ± 0.30) than herbivorous or partially herbivorous species such as *Rana pipiens* (5.10 ± 0.20).

Thus in larval anurans, the proportion of ingested inorganic matter as well as gut length are related to feeding habits.

Rapid ingestion and assimilation rates are important to meet nutritional demands of tadpoles, but rapid ingestion of inorganic substrate particles encountered by benthic larvae in the field results in an increase in specific gravity and the potential for a change in buoyancy. Although buoyancy levels were maintained over time, measures of specific gravity and gaseous lift factor showed considerable variation between 6 and 48 h, the time period in which distance travelled by silt as well as its degree of compaction was the greatest. This variation in these measures suggests that individual tadpoles ingest silt and/or react to its presence by increasing gaseous lift at different rates. Data on individual tadpoles over time are needed to determine the precision and speed of buoyancy adjustment. Nevertheless it is evident that compensation occurs (Fig. 15). There is a strong suggestion of a time lag in the response of buoyancy correction as a significant increase in specific gravity occurred at 6 h while a significant increase in gaseous lift factor did not appear until 12 h. *Rana septentrionalis* larvae are capable of correcting internal changes in specific gravity caused by an increase in tissue density due to substrate ingestion by altering gaseous lift factor to maintain buoyancy at a constant level. Gee and Waldick (1995) found a similar response but in this case to a change in the specific gravity caused by an increase in the density of the water. They exposed anuran larvae to an increase in water density using Percoll solution (density of 1.008 g/ml), which increased their buoyancy. Stages of species without lungs

inflated (*R. sylvatica* - stage 25e and *B. americanus* - stage 31) did not make any adjustment in specific gravity, but species with lungs inflated were able to compensate for the altered density of the medium and retain their original buoyancy. *Hyla* sp. (stage 26) and *Rana sylvatica* (stage 26) were able to compensate partially for their increased buoyancy; although gaseous lift decreased, the differences were not significant. However, later stages of *Rana sylvatica* (stages 27-28) were able to compensate completely for the added lift and maintain the original buoyancy level by reducing gaseous lift factor significantly. Thus regulation of gaseous lift factor by adjusting lung volume is most important in buoyancy control. This phenotypic plasticity of lung volume provides strong evidence of adaptiveness in larval anurans. Anuran larvae can detect changes in both their internal and external environments that alter their specific gravity and can respond by adjusting gaseous lift factor to maintain buoyancy at a particular level.

Similar responses have also been observed in snakes and fishes. The sea snake, *Pelamis platurus*, can regulate lung volume prior to diving as so to achieve near-neutral buoyancy at depth (Graham *et al.*, 1988). When swimming at depth, sea snakes use oxygen stored in their lungs without replacement with other gases, and as a result, lung volume and buoyancy are reduced. They correct for this by rising in the water column which reduces hydrostatic pressure and allows the lungs to expand, thus maintaining buoyancy at the original level. This buoyancy control allows sea snakes to maximize the length of the dive. Euryhaline fishes adjust swimbladder volume (and thus gaseous lift factor) in

response to variations in water density caused by changes in salinity (Gee, 1988: Gee and Holst, 1992). Although Pacific blue-eye (*Pseudomugil signifer*) first adjust hydrodynamic lift to compensate for a change in buoyancy, alteration of swimbladder volume is the primary mechanism to adjust and maintain buoyancy. Pacific blue-eye can maintain buoyancy over a range of salinity extending from 0 to 60‰ by reducing swimbladder volume up to about 50 h% (Gee, 1988). The same mechanism is used by sticklebacks (*Culaea inconstans* and *Pungitius pungitius*). Gee and Holst (1992) found that sticklebacks do not alter tissue density to compensate for the decrease in weight encountered in water of high salinity level but rather decrease gaseous lift factor significantly so that buoyancy index remains constant. Maintenance of an optimal buoyancy is critical in minimizing energy expenditure in locomotion and maintaining vertical position in the water column.

Behavioural responses to predators

1. Experiment I : vertical distribution, activity and buoyancy index.

In the presence of a predator, small *P. triseriata maculata* (test 1; stages 28-31) were less active and distributed lower in the water column than those in the absence of predators. This change in behaviour was accompanied by a reduction in buoyancy index. However larger larvae of *P. triseriata maculata* (test 2; stages 37-40) were not affected by the presence of predators in any of the above ways. Test 1 differed from test 2 in that the latter reached a higher

temperature and predators were not fed *P. triseriata maculata* on day 3. However, it appears unlikely that these differences influenced the results as there were no significant interactions between either activity or distribution and time (day of experiment) that would indicate changes in activity or vertical distribution with time in the experiment.

Small *P. triseriata maculata* larvae benefit from lowering their activity in the presence of a predator by minimizing detection and the risk of being eaten. "Sit and wait" predators such as dragonfly larvae and giant water bugs depend on prey movement for detection (Skelly, 1997). The prey's motion or pressure waves generated by movement are detected by odonates, either visually or by mechanosensory hairs; this elicits a strike from the predator, as shown with *Rana sylvatica* (Skelly, 1994; 1997). Active *R. sylvatica* were consumed 4 times more frequently than those that were anaesthetized with tricaine methanesulphonate, showing that activity is associated with risk of predation. Thus by reducing their activity, tadpoles become less conspicuous to predators. The tests with *P. triseriata maculata* show that vertical distribution and buoyancy are linked to activity.

Pseudacris triseriata is known to reduce its activity in the presence of a predator (Skelly, 1995; 1997). In a laboratory setting, tadpoles (stage 25) in the presence of caged *Anax* were >50% less active than those in the control group without predators (Skelly, 1995; 1997). *Pseudacris crucifer* (stage 25) also reduced its activity in the presence of caged *Anax* but to a lesser extent than *P. triseriata* (Skelly, 1995; 1997). This difference is most likely a reflection of

differences in mode of life of the two, as *P. crucifer* is less active and most common in permanent ponds while *P. triseriata* is more active and occurs in temporary ponds (see below). Other active species known to reduce their activity in the presence of predators include: *Bufo americanus* (Skelly and Werner, 1990), *Hyla versicolor* (Skelly, 1992; Lawler, 1989), *Bufo woodhousei* and *Hyla andersonii* (Lawler, 1989).

Possibly, the ability to detect predator presence is dependent on stage of development. In test 2, late larval stages were more active and remained higher in the water column than early larval stages in test 1. Those in test 2 were just above neutral buoyancy while those in test 1 were at negative buoyancy. These results indicate either an ontogenetic shift in behaviour and possibly habitat that may make *P. triseriata maculata* larvae less vulnerable and thus less responsive to predators in late larval stages or that they have grown sufficiently large to be invulnerable to predators, or both. Similar differences in response to the presence of predators between small and large larvae were also observed in *H. versicolor*. Skelly (1992) found that although early (stages 25-26) and late (stages 36-38) larvae of *H. versicolor* reduced activity in the presence of *Ambystoma tigrinum tigrinum*, the later stages appeared less responsive to predators than earlier stages. In addition to ontogenetic shifts in behavioural responses to predators, active species show different reactions depending on the species of predators used in the experiment. Lawler (1989) found that *B. woodhousei* reduced its activity in the presence of both *Notophthalmus viridescens* (newt) and *Enneacanthus obeses* (fish) but the reduction in activity

was about 25% greater with fish. On the other hand, *H. andersonii* reduced its activity in the presence of newts but not with fish.

With experiments on *Rana septentrionalis* (stages 26-27: test 3 and 28-29; test 4) there was a significant interaction between predation and time in both activity and vertical distribution in test 3 but not in test 4. This indicates that in test 3 there were significant differences between experimental (predator present) and control (predator absent) treatments on one or more days. Results (Figs. 16 and 17) showed that the greatest differences occurred on day 1 where activity was less and vertical distribution was lower in the predator-exposed larvae than in the control group. An examination of variances relative to means in both figures suggests that differences on remaining days are not significant. Partial water changes were made on days 2, 3, and 4 in tests 3 and 4 to remove mouldy food and increase the amount of dissolved oxygen in both tests. It is unlikely these changes would affect one test by diluting the concentration of chemical stimuli released by predators and not affect the other. Similarly it is unlikely that there is a stage-dependent response to predators as the stages in both tests (stages 26-27 test 3; stages 28-29 test 4) were very similar. Interaction could be due to either acclimation to the presence of predator as previous studies have shown that prey exposed to chemical cues of predators without any follow-up attack can result in habituation (Magurran and Girling, 1986; Jackson and Semlitsch, 1993) or to differences in predators used. In test 3 and 4 interspecific differences in either chemical cues released by predators or in their foraging behaviour may elicit different responses in *R. septentrionalis* larvae. *Rana*

septentrionalis remained inactive and benthic at negative buoyancy.

Pseudacris triseriata maculata in field populations is reported to be an active species (Skelly, 1995; 1997), while I have observed *R. septentrionalis* to be relatively inactive. Active species are common in temporary ponds where natural selection favours a mode of life with active, almost continuous feeding promoting fast growth such that metamorphosis is reached prior to the pond's drying. This strategy works in temporary ponds as predators are few, and tadpoles can rely on flexible defence mechanisms such as temporary immobility or refuge to increase survival (Lawler, 1989). On the other hand, more permanent ponds have more and larger predators. In these ponds, natural selection promotes a more inactive mode of life for tadpoles as it minimizes chances of encountering a predator, and as a result growth rate is slower. Since the baseline level of activity is different between species of these two habitats, differences in the magnitude of behavioural changes in the presence of a predator may be real. Similar results to those obtained with *R. septentrionalis* were found by Lawler (1989). *Hyla crucifer* is a benthic and inactive species. It, too, responded less to predators than the other 3 species studied by Lawler (1989) which were active. As an inactive species, *H. crucifer* showed less flexibility in its behaviour than active species. This inactivity appears to be selected for in many permanent pond-dwelling species of tadpoles (*e.g.*, *H. crucifer* and *R. septentrionalis*) as this increases their chances of survival by reducing the risk of encountering a predator. In contrast to my experiment, Lawler (1989) provided larvae with natural refugia composed of sand and gravel

topped with water-logged leaves and pine needles. *Hyla crucifer* decreased slightly its activity in the presence of a predator but did not change its microhabitat and remained at the bottom of the tank.

There was no effect of predators on activity, distribution or buoyancy of *R. sylvatica* (stages 28-31; test 5). This species remained inactive in midwater at near neutral buoyancy.

In my experiments, significant responses to predators were not strong. I suspect that experimental conditions may have unintentionally reduced the strength of the potential response of larvae to the presence of predators. Improvements to the experimental design could include among others: regulation of temperature and oxygen, greater number and diversity of predators, comparison between field- and laboratory-hatched larvae (as in tests 1, 2 and 5 larvae were hatched in the laboratory while in tests 3 and 4 they were hatched in the field) and the addition of refugia. During the summer of 1996 (from June 18 to August 22), dissolved oxygen levels in the field ranged from 5.4 to 8.4 ppm on-shore while off-shore readings of 6.3 to 7.8 ppm (at the surface) and 2.2 to 6.4 ppm (at the bottom) were recorded. Most *R. septentrionalis* were caught near shore where the levels of dissolved oxygen were high. All behavioural experiments were conducted without an air stone. Although tadpoles in the experiment appeared not to be affected by low levels of oxygen, it may have played a role as larvae can respond to oxygen stress by altering vertical distribution (*i.e.*, spend more time floating at the surface) or by increased bobbing (*i.e.*, increased frequency of air breaths at the surface, followed

frequently by the release of air bubbles on descent (Wassersug and Seibert, 1975)). These behaviours were observed in the "frequency of air breathing" experiment. In experiment I, dissolved oxygen within the water decreased to as little as 3.5 ppm (except for test 1) and although partial water changes helped to restore some of the oxygen, the level experienced by the tadpoles could have stressed some larvae. Wassersug and Seibert (1975) found that bobbing increased with hypoxia. The threshold for increased bobbing is between 2.5 to 4.5 ppm for the species studied by Wassersug and Seibert (1975) with the level for *P. triseriata maculata* being 4 ppm.

Responses expressed by tadpoles to predators appear to be innate; however the degree of expression of a behavioural response appears to be linked to rearing conditions and the history of exposure to predators (Lawler, 1989; Semlitsch and Reyer, 1992; Bridges and Gutzke, 1997). In some experiments, naïve tadpoles, which had never been exposed to predators, decreased their activity when placed in the presence of predators (Lawler, 1989). Naïve *Hyla chrysoscelis* larvae spent twice as much time in refugia in response to predators as those raised in the presence of the predators (Bridges and Gutzke, 1997). In natural habitats, vegetation plays an extremely important role in providing cover from predators. Bridges and Gutzke (1997) examined only the use of refugia as an anti-predator response in *H. chrysoscelis*. In the presence of predators (fish and crayfish), *H. chrysoscelis* spent significantly more time in the refugium than the control group, and, within the predator treatment, the most vulnerable stages (hatchlings) used refugia significantly more often than the

other stages. Watkins (1997) showed that *R. septentrionalis* has a strong preference for vegetation over open areas in field and laboratory observations. The presence of vegetation could permit a greater diversity of responses to predators, but within a limited range in terms of activity, vertical distribution and buoyancy. Thus, the absence of vegetation in the present experiments may have masked the true magnitude of the response to predators. In this regard, observations made on *H. crucifer*, also an inactive species, may be more conclusive (Lawler, 1989). Although *H. crucifer* did not change its choice of microhabitat, it reduced its activity in the presence of black-banded sunfish *Enneacanthus obesus* when refugia were present. There could be an interaction between predators and vegetation which determines activity, vertical distribution and ultimately buoyancy.

2. Experiment II : frequency of air breathing.

In test 1 with small *P. triseriata maculata*, there were no significant differences in the frequency of air breaths between tadpoles in the presence or absence of predators. Observations of air breaths in remaining tests are excluded from discussion because the hypothesis could not be tested. The hypothesis was that in the presence of predators there would be a decrease in activity, vertical distribution and buoyancy and that this would be accompanied by a reduction in frequency of air breaths. Since the above behaviours were not observed in tests 2 to 5, further analysis is unnecessary.

The decrease in activity, vertical distribution and buoyancy in test 1 was

not accompanied by a reduction in frequency of air breaths. As a result, tadpoles in the predator treatments were not prevented from going to the surface. Thus the differences in activity and vertical distribution reflect the ability of the tadpoles to modify their behaviour to avoid predation. Tadpoles went to the surface, inspiring the appropriate amount of air necessary to maintain lung volume at an optimal buoyancy (more negative in the presence of predators than in their absence). With small *P. triseriata maculata*, air breaths were few and were taken at random over time, suggesting that they have not evolved group synchrony in air breathing, a mechanism to reduce contact with predators as observed with the mudminnow *Umbra limi* by Gee (1980). This in fact makes sense, because *P. triseriata maculata* is common in temporary ponds which have few predators. But such an adaptation of a group synchronizing its breathing and reducing its exposure to predation may occur in anuran species of permanent ponds. This needs further study.

Relationship between buoyancy and mode of life

Differences in buoyancy among species and within species during development can reflect particular aspects of the mode of life of either a species or a particular stage(s) of development of a given species. The range of variation in buoyancy index levels expressed by larvae from several species (Fig. 20) is considerable and can be related to mode of life. Species can be divided into two groups, those that inflate their lungs in early larval stages (group 1) and those that delay inflation to metamorphosis (group 2). Species in group 1

occupy one of two buoyancy zones, either zone A or B, or alternate between these two zones. Species in zone A are near neutral buoyancy (BI > -0.004) and are active, swimming in midwater. Species in buoyancy zone B are negatively buoyant (BI is -0.004 to -0.026), inactive and benthic with intermittent trips to the surface for air breaths. Gee and Waldick (1995) arbitrarily identified this switch in behaviour to be at a buoyancy index of -0.004, the level used in Fig. 20 to delimit the two zones. Thus as buoyancy decreases from zone A to B, there is a shift in vertical distribution from pelagic to benthic as well as a shift in activity from active to inactive in group 1 species.

Buoyant species in zone A are more common in temporary ponds while the less buoyant species in zone B are common in permanent ponds, as well as streams and rivers. In addition, species in zone A possess more flexible defense adaptations as in temporary ponds they encounter fewer and smaller predators while species in zone B, in more permanent bodies of water, encounter more and larger predators and as a result have more fixed defense mechanisms.

Species in group 2 occupy either buoyancy zones B or C. Larvae in zone B differ from species which inflate their lungs (group 1) as they are benthic and active and found in temporary ponds and streams. Those in zone C are highly negatively buoyant (BI < -0.026), benthic and inactive. These species are highly specialized torrent-adapted tadpoles whose extreme negative buoyancy is achieved not only by delayed lung inflation to metamorphosis but by a body water content that is among the lowest of all species examined by Gee (unpub. data). However, those species in group 2, with buoyancy levels in zone B but

with lungs deflated, attain their relatively high buoyancy levels by having the highest body water content of all species examined by Gee (unpub. data). For group 2 species, the decrease in buoyancy index from zone B to C is not related to a shift in vertical distribution, as species in both zones are benthic, but rather it is related to a shift in activity as species in zone B are benthic and active while those in zone C are suctorial species and thus are benthic and inactive. Group 2 zone B species are in temporary ponds and streams while those in group 2 zone C are in the headwaters of permanent rainforest streams but with no fishes and few predatory invertebrates.

Rana septentrionalis inflates its lungs at the start of larval development and all stages of development occur in buoyancy zone B. Negative buoyancy is adaptive to its mode of life and in addition to topics already discussed in previous sections, this level of buoyancy would aid in: holding position if water currents were encountered during periods of water level fluctuations, holding position just above the substrate for feeding, concealment from predators and burrowing into the substrate for successful overwintering, all of which help to increase survivorship.

By no means are the results and ideas presented here complete, but they provide information that could be added to enhance existing methods of classifying and describing an organism's mode of life. Altig and Johnston (1989) have described ecomorphological guilds for anuran larvae. Using their guide, *Rana septentrionalis* is classified as a benthic species of type 2 (profundal) within the lotic species (section II) but this guide does not incorporate information

about buoyancy levels and related variables. Such information would assist in providing a more detailed and complete description of an organism's guild.

LITERATURE CITED

Alexander, R. McN. 1966. Physical aspects of swimbladder function. Biol. Rev. **41**:141-176.

Alexander, R. McN. 1990. Size, speed and buoyancy adaptations in aquatic animals. Amer. Zool. **30**:189-196.

Alford, R.A., and R.N. Harris. 1988. Effects of larval growth history on anuran metamorphosis. Amer. Nat. **131**:91-106.

Altig, R., and G.F. Johnston. 1989. Guilds of anuran larvae: relationships among developmental modes, morphologies, and habitats. Herpetological Monographs **3**:81-109.

Altig, R., and J.P. Kelly. 1974. Indices of feeding in anuran tadpoles as indicated by gut characteristics. Herpetologica **30**:200-203.

Altig, R., J.P. Kelly, M. Wells, and J. Phillips. 1975. Digestive enzymes of seven species of anuran tadpoles. Herpetologica **31**:104-108.

Altig, R., and W. McDearman. 1975. Percent assimilation and clearance times of five anuran tadpoles. Herpetologica **31**:67-69.

Babbitt, K.J., and G.W. Tanner. 1997. Effects of cover and predator identity on predation of *Hyla squirella* tadpoles. J. Herp. **31(1)**:128-130.

Beaver, B.J., and J.H. Gee. 1988. Role of water current and related variables in determining buoyancy in the sticklebacks *Culaea inconstans* and *Pungitius pungitius*. Can. J. Zool. **66**:2006-2014.

Beiswenger, R.E. 1977. Diel patterns of aggregative behavior in tadpoles of *Bufo americanus*, in relation to light and temperature. Ecology **58**:98-108.

Bidigare, R.R., and D.C. Biggs. 1980. The role of sulfate exclusion in buoyancy maintenance by siphonophores and other oceanic gelatinous zooplankton. Comp. Biochem. Physiol. **66A**:467-471.

Bridges, C.M., and W.H.N. Gutzke. 1997. Effects of environmental history, sibship, and age on predator-avoidance responses of tadpoles. Can. J. Zool. **75**:87-93.

Brodie, E.D. Jr., and D.R. Formanowicz Jr. 1983. Prey size preference of predators: differential vulnerability of larval anurans. Herpetologica **39(1)**:67-75.

Brown, P.S., B. Barry, and S.C. Brown. 1988. Changes in absolute and proportional water content during growth and metamorphosis of *Rana sylvatica*. Comp. Biochem. Physiol. **91A**:189-194.

Bruce, R.C., C.K. Beachy, P.G. Lenzo, S.P. Pronych, and R.J. Wassersug. 1994. Effects of lung reduction on rheotactic performance in amphibian larvae. J. Exp. Zool. **268**:377-380.

Caldwell, J.P., J.H. Thorp, and T.O. Jervey. 1980. Predator-prey relationships among larval dragonflies, salamanders, and frogs. Oecologia **46**:285-289.

Calef, G.W. 1973. Natural mortality of tadpoles in a population of *Rana aurora*. Ecology **54(4)**:741-758.

Campeny, R., and A. Casinos. 1989. Densities and buoyancy in tadpoles of midwife toad, *Alytes obstetricans*. Zool. Anz. **223 1/2**:6-12.

Childress, J.J., and M. Nygaard. 1974. Chemical composition and buoyancy of midwater crustaceans as function of depth of occurrence off southern California. Mar. Biol. **27**:225-238.

Conant, R., and J.T. Collins. 1991. A field guide to reptiles and amphibians of eastern and central North America. Houghton Mifflin Co., New York. 450 p.

Courtois, D., R. Leclair Jr., S. Lacasse, and P. Magnan. 1995. Habitats préférentiels d'amphibiens ranidés dans des lacs oligotrophes du Bouclier laurentien, Québec. Can. J. Zool. **73**:1744-1753.

Crump, M.L., 1984. Ontogenetic changes in vulnerability to predation in tadpoles of *Hyla pseudopuma*. Herpetologica **40(3)**:265-271.

Denton, E.J. 1974. On buoyancy and the lives of modern and fossil cephalopods. Proc. Roy. Soc. B **85**:273-299.

Denton, E.J., F.R.S., J.B.Gilpin-Brown, and T.I. Shaw. 1969. A buoyancy mechanism found in cranchid squid. Proc. Roy. Soc. Lond. B. **174**:271-279.

Duellman, W.E., and L. Trueb. 1994. Biology of amphibians. Johns Hopkins University Press. London. 670 p.

Farlowe, V. 1928. Algae of ponds as determined by an examination of the intestinal contents of tadpoles. Biol. Bull. **55**:443-448.

Feder, M.E. 1981. Effect of body size, trophic state, time of day, and experimental stress on oxygen consumption of anuran larvae: an experimental assessment and evaluation of the literature. Comp. Biochem. Physiol. **70A**:497-508.

Feder, M.E., and R.J. Wassersug. 1984. Aerial versus aquatic oxygen consumption in larvae of the clawed frog, *Xenopus laevis*. J. Exp. Biol. **108**:231-245.

Folsom, T.C., and N.C. Collins. 1984. The diet and foraging behavior of the larval dragonfly *Anax junius* (Aeshnidae), with an assessment of the role of refuges and prey activity. Oikos **42**:105-113.

Garnier, J.H. 1883. The mink or hoosier frog. Amer. Nat. **17**:945-954.

Gee, J.H. 1968. Adjustment of buoyancy by longnose dace (*Rhinichthys cataractae*) in relation to velocity of water. J. Fish. Res. Board Can. **25(7)**:1485-1496.

Gee, J.H. 1976. Buoyancy and aerial respiration: factors influencing the evolution of reduced swim-bladder volume of some Central American catfishes (Trichomycteridae, Callichthyidae, Loricariidae, Astroblepidae). Can. J. Zool. **54**:1030-1037.

Gee, J.H. 1977. Effects of size of fish, water temperature and water velocity on buoyancy alteration by fathead minnows, *Pimephales promelas*. Comp. Biochem. Physiol. **56A**:503-508.

Gee, J.H. 1980. Respiratory patterns and antipredator responses in the central mudminnow, *Umbra limi*, a continuous, facultative, air-breathing fish. Can. J. Zool. **58**:819-827.

Gee, J.H. 1983. Ecologic implications of buoyancy control in fish. pp.140-176. *In*: Fish Biomechanics. P.W. Webb and D. Weihs (eds). New York. Praeger.

Gee, J.H. 1987. Ecological and evolutionary implications of phenotypic plasticity of swim-bladder volume and lift in stream environments, pp.150-154. *In*: Community and evolutionary ecology of North American stream fishes. W.J. Matthews and D.C. Heins (eds). Univ. Of Oklahoma Press. Norman.

Gee, J.H. 1988. Pacific blue-eye *Pseudomugil signifer* Kner (Pisces: Melanotaeniidae) maintains buoyancy in varying salinities by altering swimbladder volume. J. Exp. Mar. Biol. Ecol. **120**:97-104.

Gee, J.H., and P.A. Gee. 1976. Alteration of buoyancy by some Central American stream fishes, and a comparison with North American species. Can. J. Zool. **54**-386-391.

Gee, J.H., and P.A. Gee. 1991. Reactions of gobioid fishes to hypoxia: buoyancy control and aquatic surface respiration. Copeia **1991**: 17-28.

Gee, J.H., and P.A. Gee. 1995. Aquatic surface respiration, buoyancy control and the evolution of air-breathing in gobies (Gobiidae: Pisces). J. Exp. Biol. **198**:79-89.

Gee, J.H., and J.B. Graham, 1978. Respiratory and hydrostatic functions of the intestine of the catfishes *Hoplosternum thoracatum* and *Brochis splendens*. J. Exp. Biol. **74**:1-16.

Gee, J.H., and H.M. Holst. 1992. Buoyancy regulation by the sticklebacks *Culaea inconstans* and *Pungitius pungitius* in response to different salinities and water densities. Can. J. Zool. **70**:1590-1594.

Gee, J.H., and R.C. Waldick. 1995. Ontogenetic buoyancy changes and hydrostatic control in larval anurans. Copeia **1995(4)**:861-870.

Gosner, K.L.1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica **16**:183-190.

Graham, J.B., J.H. Gee, J. Motta, and I. Rubinoff. 1987. Subsurface buoyancy regulation by the sea snake *Pelamis platurus*. Physiol. Zool. **60(2)**:251-261.

Gromko, M.H., F.S. Mason, and S.J. Smith-Gill. 1973. Analysis of the crowding effect in *Rana pipiens* tadpoles. J. Exp. Zool. **186**:63-72.

Hedeen, S.E. 1971. Growth of the tadpoles of the mink frog, *Rana septentrionalis*. Herpetologica **27**:160-165.

Hedeen, S.E. 1972a. Food and feeding behavior of the mink frog, *Rana septentrionalis* Baird, in Minnesota. Amer. Midl. Nat. **88(2)**:291-300.

Hedeen, S.E. 1972b. Escape behavior and causes of death of the mink frog, *Rana septentrionalis*. Herpetologica **28(3)**:261-262.

Hedeen, S.E. 1986. The southern geographic limit of the mink frog, *Rana septentrionalis*. Copeia **1986(1)**:239-244.

Heller, J.H., M.S. Heller, S. Springer, and E. Clark. 1957. Squalene content of various shark livers. Nature **179**:919-920.

Hews, D.K., and A.R. B'austein. 1985. An investigation of the alarm response in *Bufo boreas* and *Rana cascadae* tadpoles. Behav. Neural Biol. **43**:47-57.

Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs **54(2)**:187-211.

Jackson, M.E., and R.D. Semlitsch. 1993. Paedomorphosis in the salamander *Ambystoma talpoideum*: effects of a fish predator. Ecology **74**:342-350.

Jenssen, T.A. 1967. Food habits of the green frog, *Rana clamitans*, before and during metamorphosis. Copeia **1967(1)**:214-218.

Kupferberg, S.J. 1997. The role of larval diet in anuran metamorphosis. Amer. Zool. **37**:146-159.

Lawler, S.P. 1989. Behavioural responses to predators and predation risk in four species of larval anurans. Anim. Behav. **38**:1039-1047.

Licht, L.E. 1967. Growth inhibition in crowded tadpoles: intraspecific and interspecific effects. Ecology **48**:736-745.

Machniak, K., and J.H. Gee. 1975. Adjustment of buoyancy by tadpole madtom, *Noturus gyrinus*, and black bullhead, *Ictalurus melas*, in response to a change in water velocity. J. Fish. Res. Board Can. **32**:303-307.

Magnuson, J.J. 1970. Hydrostatic equilibrium of *Euthynnus affinis*, a pelagic teleost without a gas bladder. Copeia **1970**:56-85.

Magurran, A.E., and S.L. Girling. 1986. Predator recognition and response habituation in shoaling minnows. Anim. Behav. **34**:510-518

Moore, J.A. 1949. Patterns of evolution in the genus *Rana*. *In*: Genetics. Paleontology, and Evolution. G.L. Jepsen, E. Mayr and G.G. Simpson (eds.). Princeton University Press, Princeton. New Jersey. pp. 315-338.

Moore, J.A. 1952. An analytical study of the geographic distribution of *Rana septentrionalis*. Amer. Nat. **86**:5-22.

Morris, M.J., G. Gust, and J.J. Torres. 1985. Propulsion efficiency and cost of transport for copepods: a hydromechanical model of crustacean swimming. Mar. Biol. **86**:283-295.

Nathan, J.M., and V.G. James. 1972. The role of protozoa in the nutrition of tadpoles. Copeia **1972(4)**:669-679.

Neave, N.M., C.L. Dilworth, J.G. Eales, and R.L. Saunders. 1966. Adjustment of buoyancy in Atlantic salmon parr in relation to changing water velocity. J. Fish. Res. Board Can. **23(10)**:1617-1620.

O'Dor, R.K. 1988. The forces acting on swimming squid. J. Exp. Biol. **137**:421-442.

Petranka, J.W., L.B. Kats, and A. Sih. 1987. Predator-prey interactions among fish and larval amphibians: use of chemical cues to detect predatory fish. Anim. Behav. **35**:420-425.

Pinder, L.J., and J.G. Eales. 1969. Seasonal buoyancy changes in Atlantic salmon (*Salmo salar*) parr and smolt. J. Fish. Res. Board Can. **26**:2093-2100.

Preston, W.B. 1982. The amphibians and reptiles of Manitoba. Manitoba Museum of Man and Nature. Winnipeg. 128 pp.

Rappaport, R.Jr. 1954. The uptake of water during development of amphibian tissues. J. Exp. Zool. **127**:27-52.

Rozas, L.P., and W.E. Odum, 1988. Occupation of submerged aquatic vegetation by fishes: testing the roles of food and refuge. Oecologia **77**:101-106.

Saunders, R.L. 1965. Adjustment of buoyancy in young Atlantic salmon and brook trout by changes in swimbladder volume. J. Fish. Res. Board Can. **22**:335-352.

Schuster, S. 1989. The volume of air within the swimbladder and breathing cavities of the anabantoid fish *Colisa lalia* (Perciformes, Belontiidae). J. Exp. Biol. **144**: 185-198.

Sclafani. M.C., T. Taggart, and K. R. Thompson. 1993. Condition, buoyancy and the distribution of larval fish: implications for vertical migration and retention. J. Plank. Res. **15**:413-435.

Seale, D.B., and R.J. Wassersug. 1979. Suspension feeding dynamics of anuran larvae related to their functional morphology. Oecologia **39**:259-272.

Semlitsch, R.D., and H.-U. Reyer. 1992. Modification of anti-predator behaviour in tadpoles by environmental conditioning. J. Anim. Ecol. **61**:353-360.

Skelly, D.K. 1992. Field evidence for a cost of behavioral antipredator response in a larval amphibian. Ecology **73(2)**:704-708.

Skelly, D.K. 1994. Activity level and the susceptibility of anuran larvae to predation. Anim. Behav. **47**:465-468.

Skelly, D.K. 1995. A behavioral trade-off and its consequences for the distribution of *Pseudacris* treefrog larvae. Ecology **76(1)**:150-164.

Skelly, D.K. 1997. Tadpole communities: pond permanence and predation are powerful forces shaping the structure of tadpole communities. Amer. Sci. **85(1)**:36-45.

Skelly, D.K., and E.E. Werner. 1990. Behavioral and life-historical responses of larval american toads to an odonate predator. Ecology **71(6)**:2313-2322.

Stauffer, H.P., and R.D. Semlitsch. 1993. Effects of visual, chemical and tactile cues of fish on the behavioural responses of tadpoles. Anim. Behav. **46**:355-354.

Taylor, M.A. 1993. Stomach stones for feeding or buoyancy? The occurrence and function of gastroliths in marine tetrapods. Phil. Trans. R. Soc. Lond. B. **341(1296)**:163-175.

Travis, J., W.H. Keen, and J. Juilianna. 1985. The role of relative body size in a predator-prey relationship between dragonfly naiads and larval anurans. Oikos **45**:59-65.

Visser, P.M., B. W. Ibelings, and L. Mur. 1995. Autumnal sedimentation of Microcystis spp. as a result of an increase in carbohydrate ballast at reduced temperature. J. Plank. Res. **17**:919-933.

Wassersug, R.J., and M.E. Feder. 1983. The effects of aquatic oxygen concentration, body size and respiratory behaviour on the stamina of obligate aquatic (*Bufo americanus*) and facultative air-breathing (*Xenopus leavis* and *Rana berlandieri)* anuran larvae. J. Exp. Biol. **105**:173-190.

Wassersug, R.J., and E.A. Seibert. 1975. Behavioral responses of amphibian larvae to variation in dissolved oxygen. Copeia **1975**:86-103.

Watkins, E. 1997. Habitat selection and antipredator responses of larval mink frogs, *Rana septentrionalis*. BSc. Honors thesis, Department of Zoology, University of Manitoba, Winnipeg.

Wilbur, H.M. and J.P. Collins. 1973. Ecological aspects of amphibian metamorphosis. Science **182**:1305-1314.

Wilson, D.J., and H. Lefcort. 1993. The effect of predator diet on the alarm response of red-legged frog, *Rana aurora*, tadpoles. Anim. Behav. **46**:1017-1019.

APPENDICES

Appendix A: Repeated measures ANOVA on activity (control and predator are done separately) to see if there is a difference between the two halves of the week, before and after predators are exchanged. Only tanks without mortality are used in the analysis. "Type" compares the first part of the week to the second part.

		Treatment					
Test	Factor	Control			Predator		
		df	F	p	df	F	p
1	Time	2,28	1.51	ns	2,32	0.81	ns
	Type	1,14	0.05	ns	1,16	0.94	ns
	Interaction	2,	8.04	0.005	2,	0.51	ns
2	Time	2,36	1.99	ns	2,36	0.15	ns
	Type	1,18	0.60	ns	1,18	0.91	ns
	Interaction	2,	1.32	ns	2,	0.097	ns
3	Time	2,28	2.43	ns	2,36	1.85	ns
	Type	1,14	0.36	ns	1,18	3.08	ns
	Interaction	2,	4.27	0.038	2,	1.94	ns
4	Time	2,32	2.99	ns	2,32	0.80	ns
	Type	1,16	0.47	ns	1,16	0.00	ns
	Interaction	2,	3.00	ns	2,	0.83	ns
5	Time	2,28	0.11	ns	2,36	0.88	ns
	Type	1,14	0.21	ns	1,18	7.95	0.011
	Interaction	2,	14.84	<0.001	2,	4.48	0.027

"ns" for p values > 0.05

Appendix B: Repeated measures ANOVA on distribution score (control and predator are done separately) to see if there is a difference between the two halves of the week, before and after predators are exchanged. Only tanks without mortality are used in the analysis. "Type" compares the first part of the week to the second part.

		Treatment					
Test	Factor	Control			Predator		
		df	F	p	df	F	p
1	Time	2,28	0.93	ns	2,32	3.71	0.035
	Type	1,14	0.62	ns	1,16	2.35	ns
	Interaction	2,	1.22	ns	2,	0.17	ns
2	Time	2,36	15.19	<0.001	2,36	17.39	<0.001
	Type	1,18	4.03	ns	1,18	2.60	ns
	Interaction	2,	5.12	0.018	2,	0.097	ns
3	Time	2,28	3.14	ns	2,36	0.02	ns
	Type	1,14	3.33	ns	1,18	2.16	ns
	Interaction	2,	3.39	ns	2,	0.63	ns
4	Time	2,32	1.56	ns	2,32	0.00	ns
	Type	1,16	1.07	ns	1,16	0.60	ns
	Interaction	2,	0.567	ns	2,	0.21	ns
5	Time	2,28	8.62	0.001	2,36	11.40	<0.001
	Type	1,14	0.08	ns	1,18	0.05	ns
	Interaction	2,	4.19	0.039	2,	23.58	<0.001

"ns" for p values > 0.05

Appendix C: Means (± SD) of the average number of active tadpoles/tank/day. Only tanks without tadpole mortality were included in the analyses. Control - predators absent; experimental - predators present. Tests and species as per Table 8.

Test	Predation	Rep.	Time					Mean
		n	day 1	day 2	day 3	day 4	day 5	
Test 1	Control	8	0.88 ± 0.6	0.84 ± 0.4	0.44 ± 0.4	0.84 ± 0.3	1.00 ± 0.5	0.80
	Experimental	9	0.39 ± 0.4	0.25 ± 0.3	0.22 ± 0.2	0.31 ± 0.4	0.11 ± 0.2	0.26
	Difference		0.49	0.59	0.22	0.54	0.89	
	Mean		0.63	0.55	0.33	0.57	0.56	
Test 2	Control	10	1.35 ± 0.5	1.45 ± 0.5	1.30 ± 0.5	0.95 ± 0.7	1.05 ± 0.7	1.22
	Experimental	10	1.30 ± 0.8	1.38 ± 0.4	1.20 ± 0.5	1.13 ± 0.3	1.10 ± 0.4	1.22
	Difference		0.05	0.08	0.10	-0.18	-0.05	
	Mean		1.33	1.41	1.25	1.04	1.08	
Test 3	Control	8	1.31 ± 0.6	1.31 ± 0.3	1.25 ± 0.3	1 72 ± 0.3	0.88 ± 0.7	1.29
	Experimental	10	0.65 ± 0.4	1.13 ± 0.5	1.20 ± 0.4	1.38 ± 0.6	1.25 ± 0.9	1.12
	Difference		0.66	0.19	0.05	0.34	-0.38	
	Mean		0.98	1 22	1.23	1.55	1.06	
Test 4	Control	9	1.39 ± 0.9	1.28 ± 0.4	1.53 ± 0.6	1.64 ± 0.6	1.00 ± 0.5	1.37
	Experimental	9	1.33 ± 1.0	1.19 ± 0.3	1.53 ± 0.6	1.19 ± 0.5	1.17 ± 0.7	1.29
	Difference		0.06	0.08	0.00	0.44	-0.17	
	Mean		1.36	1.24	1.53	1.42	1.08	
Test 5	Control	8	1.00 ± 0.6	0.38 ± 0.3	0.44 ± 0.4	0.88 ± 0.8	0.88 ± 0.2	0.71
	Experimental	10	0.60 ± 0.5	0.15 ± 0.2	0.38 ± 0.3	0.75 ± 0.7	0.85 ± 0.3	0.55
	Difference		0.40	0.23	0.06	0.13	0.03	
	Mean		0.80	0.26	0.41	0.81	0.86	

Appendix D: **Means (± SD) of the average distribution score (score reflects height in tank - see p. 40) of tadpoles/tank/day. Only tanks without tadpole mortality were included in the analyses. Control represent tadpoles with predators absent and experimental tadpoles with predators present. Tests and species as per Table 8.**

Tests	Predation	Rep.	Time					Mean
		n	day 1	day 2	day 3	day 4	day 5	
Test 1	Control	8	3.44 ± 0.8	3.41 ± 0.7	4.00 ± 0.5	3.66 ± 0.7	3.69 ± 0.6	3.63
	Experimental	9	3.11 ± 0.3	3.00 ± 0.0	3.31 ± 0.6	3.11 ± 0.2	3.67 ± 0.7	3.24
	Difference		0.33	0.41	0.66	0.55	0.02	
	Mean		3.27	3.20	3.64	3.38	3.68	
Test 2	Control	10	5.55 ± 1.4	5.25 ± 0.8	7.58 ± 1.4	6.08 ± 1.8	7.65 ± 1.1	6.42
	Experimental	10	5.85 ± 1.4	4.73 ± 1.0	6.65 ± 1.7	5.35 ± 1.5	7.85 ±1.1	6.09
	Difference		-0.30	0.53	0.93	0.73	-0.20	
	Mean		5.70	4.99	7.11	5.71	7.75	
Test 3	Control	8	4.31 ± 0.8	4.13 ± 0.7	3.75 ± 0.6	4.38 ± 0.5	3.31 ± 0.6	3.98
	Experimental	10	3.45 ± 0.4	3.70 ± 0.7	3.83 ± 0.7	3.75 ± 0.8	3.95 ± 0.8	3.74
	Difference		0.86	0.43	-0.08	0.63	-0.64	
	Mean		3.88	3.91	3.79	4.06	3.63	
Test 4	Control	9	4.11 ± 1.0	3.83 ± 0.8	4.50 ± 1.3	3.92 ± 0.7	4.28 ± 0.9	4.13
	Experimental	9	3.89 ± 1.0	3.86 ± 0.6	4.08 ± 0.8	4.22 ± 1.0	4.06 ± 1.2	4.02
	Difference		0.22	-0.03	0.42	-0.31	0.22	
	Mean		4.00	3.85	4.29	4.07	4.17	
Test 5	Control	8	7.50 ± 1.6	4.97 ± 1.0	5.94 ± 1.4	5.34 ± 1.2	6.56 ± 1.4	6.06
	Experimental	10	7.45 ± 1.3	4.85 ± 1.2	4.80 ± 1.8	4.75 ± 1.1	6.85 ± 1.5	5.74
	Difference		0.05	0.12	1.14	0.59	-0.29	
	Mean		7.48	4.91	5.37	5.05	6.71	

Appendix E: Changes in buoyancy-related variables for the 5 tests to assess behavioural responses to predators. Mean values (n = 12) of (specific gravity-1), gaseous lift factor and buoyancy index for each test are given with 95% confidence limits on the means.

Tests	Predation	Variables		
		Gaseous lift factor	Specific gravity-1	Buoyancy index
Pseudacris Stages 28-31 Test 1	Control	0.01153 ± 0.00282	0.01850 ± 0.00099	-0.00697 ± 0.00309
	Experimental	0.00542 ± 0.00221	0.01704 ± 0.00082	-0.01162 ± 0.00183
Pseudacris Stages 37-40 Test 2	Control	0.02589 ± 0.00626	0.02342 ± 0.00107	0.00248 ± 0.00596
	Experimental	0.02579 ± 0.01178	0.02308 ± 0.00099	0.00270 ± 0.01192
R. septentrionalis Stages 26-27 Test 3	Control	0.01685 ± 0.01125	0.02133 ± 0.00124	-0.00448 ± 0.01120
	Experimental	0.01823 ± 0.00766	0.02113 ± 0.00061	-0.00289 ± 0.00755
R. septentrionalis Stages 28-29 Test 4	Control	0.01591 ± 0.00374	0.02304 ± 0.00127	-0.00714 ± 0.00335
	Experimental	0.01307 ± 0.00444	0.02379 ± 0.00100	-0.01072 ± 0.00461
R. sylvatica Stages 28-31 Test 5	Control	0.01617 ± 0.00234	0.01808 ± 0.00112	-0.00192 ± 0.00277
	Experimental	0.02131 ± 0.00960	0.01825 ± 0.00091	0.00306 ± 0.00951