

FACTORS AFFECTING LABORATORY ACCLIMATIZATION OF FIFLD COLLECTED *LYMNAEA (BULLASTRA) CUMINGIANA* PFEIFFER (PULMONATA : LYMNAEIDAE)

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Abstract. *Lymnaea (Bullastra) cumingiana*, the newly discovered natural second intermediate host of *Echinostoma malayanum* in the Philippines, is a sensitive and delicate lymnaeid species which requires certain conditions for successful transport from the field and cultivation in the laboratory. Field collected specimens were found to be best transported in styrofoam containers lined with wet filter paper or containing natural substrate and vegetation instead of *Sphagnum* moss. The method is convenient and produces a survival rate of 73-86%. However, transport time is crucial and mortality increases the longer the snails are in transit. For optimal results in laboratory acclimatization, snails are best raised in wide-mouthed containers providing a large exposed water surface area. Adequate aeration is advised but vigorous bubbling of the water should be avoided. Water should be replaced with filtered dechlorinated water every 2 to 3 days, depending on water quality. A combination of fresh lettuce leaves and a few flakes of fish food was found to be ideal. Lastly, population density was the most significant factor affecting survival and so overcrowding should be avoided.

INTRODUCTION

Lymnaea (Bullastra) cumingiana Pfeiffer has recently been shown to be a species of potential medical and public health importance in the Philippines where it serves as the natural second intermediate host of *Echinostoma malayanum* Leiper (Monzon and Kitikoon, 1989). It is expected that more studies on this lymnaeid species will be carried out in the future and so it is necessary to investigate the factors which affect its survival and successful cultivation in the laboratory. This preliminary study was therefore conducted to determine optimal conditions for acclimatization prior to laboratory cultivation.

MATERIALS AND METHODS

Lymnaea (Bullastra) cumingiana adult specimens were collected by hand from the field (Echague, Isabela and San Pablo, Laguna) on four separate occasions from January to March,

1989. The snails were carefully washed and sorted prior to packaging and transport.

Three methods of packaging were employed. Styrofoam containers (22.5 cm × 22.5 cm × 8.5 cm) were either lined with wet filter paper, packed with moist *Sphagnum* moss or filled with the substrate and accompanying natural vegetation of the habitat in which the snails were found. A random number of clean healthy snails were placed in each of the 3 types of containers. Total travelling time was noted. Upon reaching Manila, the snails were brought to the Department of Parasitology, UP College of Public Health and were immediately transferred to clean dechlorinated water to determine the percentage survival from each of the 3 packaging methods.

The adjustment or acclimatization of surviving snails to varying laboratory conditions prior to maintenance and culture was then investigated. The following variables were manipulated : (1) type of container : with large surface area (half gallon ice cream plastic containers with height = 12

cm and diameter = 16 cm) or with small surface area (wide-mouthed glass bottles with height = 17 cm and diameter = 6.5 cm), (2) water aeration : with or without, (3) frequency of water replacement : daily or every 2 to 3 days, (4) type of food : lettuce only, fish food (Tetra Min) only or lettuce and fish food combined, and (5) snail density ("crowding effect") : one, five or ten specimens per container.

This was done by dividing the surviving snails among the 72 treatment groups that result when all the variable factors are combined as shown in Fig 1. Temperature was maintained at 25-30°C and the volume of water per container was 300 ml. The survival of the snails was monitored for one week. Three trials were performed. Egg masses laid during the acclimatization period were separated and used for subsequent experiments on cultivation.

Data were coded and entered into an Apple-compatible personal computer. The ABSTAT statistical package (Anderson-Bell) was used to perform multiple regression analysis, using dummy variables for the qualitative factors (all except for snail density) (Kleinbaum and Kupper, 1978), in order to determine which factors were significant to survival during the acclimatization phase.

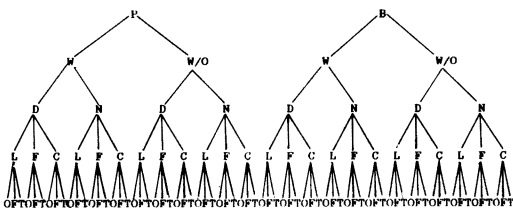


Fig 1—Experimental set-up for laboratory acclimatization experiments with field collected *Lymnaea (Bullastra) cumingiana*.

Variables :

- 1) container type : wide mouthed plastic container (P) vs narrow mouthed glass bottle (B).
- 2) water aeration : with (W) vs without (W/O).
- 3) water replacement : daily (D) vs every 2-3 days (N).
- 4) food : lettuce (L) vs fish food (F) vs combination (C).
- 5) snail density : one (O) vs five (F) vs ten (T) per container.

RESULTS

Survival rates during transport from the field to the laboratory using different packing materials are summarized in Table 1. Containers lined with wet filter paper and those containing natural substrate and vegetation gave significantly higher average survival rates (79.1% and 76.9%, respectively) than containers packed with *Sphagnum* moss (64.7%) ($X^2 = 73.7, p < 0.01$). Survival was also significantly higher among snails collected from San Pablo, Laguna (84.9% and 77.6%) than those from Echague, Isabela (67.7% and 69.6%) ($X^2 = 82.8, p < 0.01$).

Table 2 summarizes the average percentage survival rates of the snails among the 72 treatment groups, based on 3 trials. If each factor is considered separately without consideration of its interaction with other factors, the data roughly suggest that survival is higher in the plastic containers (70.4%) than in the glass bottles (65.5%). It is also slightly higher in the absence of aeration (68.9%) than with aeration (66.9%). Daily water replacement (69.3%) is slightly better than changing the water every 2 to 3 days (66.6%). A combination of lettuce and fish food (71.1%) appears to be the best among the 3 food options followed by lettuce only (70.7%) and fish food alone (61.9%). Lastly, snail density seems to be a very important factor. Snails raised in isolation have the highest survival rates (98.1%); survival rates drop drastically as density increases : 64.3% for groups of 5 and 41.4% for groups of 10 per container. These results are summarized in Table 3.

To determine which factors significantly affect percentage survival rates, multiple linear regression analysis was performed and results are shown in Table 4. The estimated regression equation is $Y = 4.90738 X_1 - 1.94446 X_2 + 2.68519 X_3 + 9.16663 X_4 + 0.41664 X_5 - 6.22615 X_6 + 91.9099$ where Y is the percentage survival rate, X_1 is for the type of container used (1 = plastic container, 0 = glass bottle), X_2 is for aeration (1 = with, 0 = without), X_3 is for frequency of water replacement (1 = daily, 0 = every 2-3 days), X_4 and X_5 are for type of food (1, 0 = lettuce only, 0, 1 = fish food only and 1, 1 = lettuce and fish food combined) and X_6 is for density of snails (1, 5 or 10). The F-test value of 48.0207 implies that the overall regression equation is significant ($p < 0.0001$).

Table 1

Survival rates of snails transported from the field to the laboratory using different packing materials.

Packing material	Trial				Total (average)
	1*	2*	3**	4**	
Filter paper	73.4% (292/398)	74.9% (299/399)	85.6% (712/832)	75.5% (290/384)	79.1% (1,593/2,013)
<i>Sphagnum</i> moss	62.0% (241/389)	61.2% (260/425)	not done	82.2% (120/146)	64.7% (621/960)
Natural*** substrate	not done	73.8% (245/332)	81.5% (145/178)	78.5% (95/121)	76.9% (485/631)
Total (Average)	67.7% (533/787)	69.6% (804/1,156)	84.9% (857/1,010)	77.6% (505/651)	74.9% (2,699/3,604)

* collected from Echague, Isabela; 9-10 hrs from Manila.

** collected from San Pablo, Laguna; 2-3 hrs from Manila.

*** at Echague, Isabela ; mud, fresh rice seedlings and decaying rice stalks; at San Pablo, Laguna : sand and small rocks, *Lemna* (duckweed), *Eichhornia crassipes* (water hyacinth), *Pistia stratiotes* (water lettuce), *Egeria (Elodea) densa* (waterweed) and various lilies.

meaning that all independent variables considered together can explain a significant amount of the variation observed in the dependent variable. However, r^2 is only equal to $(0.761303)^2 = 0.58$. This means that 42% of the variation in percentage survival rates can not be explained by the equation above (Kleinbaum and Kupper, 1978).

Based on the regression coefficients, it can be concluded that the plastic containers with larger surface area are better than the glass bottles, no aeration is better than aeration, daily water replacement is better than changing water every 2-3 days, a combination of lettuce and fish food is better than either lettuce or fish food alone and lower snail density is better than high snail density in increasing the percentage survival rate.

However, the t-values associated with each regression coefficient determine whether the factor significantly affects survival rates. Comparison of these values with the tabulated critical values reveals that only type of container ($p < 0.05$),

type of food ($p < 0.005$) and snail density ($p < 0.0005$) significantly affect the survival of the snails.

DISCUSSION

Cleaning of snails prior to their transport from field to laboratory is essential since field collected specimens are normally contaminated with mud, various macro- and microorganisms and fermented organic matter which can initiate putrefaction or fermentation in the water. Thus, snails must be washed thoroughly. Before transferring into shipping containers, they must also be sorted so that only healthy undamaged individuals are selected. Weak or dead specimens must be discarded (Kitikoon, 1981).

Lymnaea (Bullastra) cumingiana snails should be handled carefully during the cleaning and sorting process because of their delicate shells and the

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Table 2

Average percentage survival rates of *Lymnaea (Bullastra) cumingiana* among the 72 treatment groups based on 3 trials.

			Plastic containers		Glass bottles	
			with aeration	without aeration	with aeration	without aeration
Water replacement*, food** and density***						
D	L	1	100.0%	100.0%	100.0%	100.0%
D	L	5	60.0%	73.3%	73.3%	80.0%
D	L	10	40.0%	53.3%	50.0%	33.3%
D	F	1	100.0%	100.0%	100.0%	100.0%
D	F	5	46.7%	60.0%	53.3%	53.3%
D	F	10	33.3%	36.7%	36.7%	36.7%
D	C	1	86.7%	100.0%	100.0%	100.0%
D	C	5	66.7%	86.7%	80.0%	66.7%
D	C	10	43.3%	60.0%	43.3%	40.0%
N	L	1	100.0%	100.0%	100.0%	100.0%
N	L	5	73.3%	73.3%	53.3%	53.3%
N	L	10	50.0%	60.0%	30.0%	40.0%
N	F	1	93.3%	93.3%	100.0%	93.3%
N	F	5	53.3%	53.3%	53.3%	66.7%
N	F	10	30.0%	43.3%	30.0%	20.0%
N	C	1	100.0%	100.0%	93.3%	93.3%
N	C	5	60.0%	80.0%	66.7%	56.7%
N	C	10	63.3%	60.0%	46.7%	13.3%

* D = daily, N = non-daily or every 2-3 days

** L = lettuce only, F = fish food only, C = combination L/F

*** 1 = 1 snail, 5 = 5 snails, 10 = 10 snails per container

sensitive nature of the organism. Its broad extensive mantle usually covers the entire shell. Mucus secretion is greatly increased when the snails are disturbed and this may affect their survival when they are packed and transported together in large quantities.

Styrofoam containers lined with wet filter paper or containing the natural vegetation and substrate of the habitat were found to be satisfactory for transporting the snails from the field to the laboratory. Total immersion in water is not recommended because movement during travel also induces mucus formation which fouls up the water causing increased mortality.

Moist *Sphagnum* moss was suggested by Malek

(1962) to be an ideal shipping material because of its cushioning and water-retaining properties. However a significantly lower survival rate was obtained using this method. The snails seemed to be adversely affected by some extracts of the plant since increased mucus secretion and putrefaction of many snail bodies were observed.

Because of the bulky nature of mud, sand and other substrates, it is more advisable and practical to use wet filter paper-lined styrofoam containers in transporting these snails. Natural vegetation may be supplemented since they serve as both a source of food and protection.

Comparing the results of collections done at Isabela and Laguna (Table 1), it was evident that

Table 3

Average percentage survival rates of *Lymnaea (Bullastra) cumingiana* according to individual factors under investigation.

Factor investigated	Variations	Average % survival
1. Type of container	plastic (larger surface area)	70.4%
	glass bottles (smaller surface area)	65.5%
2. Aeration	with	66.9%
	without	68.9%
3. Frequency of water replacement	daily	69.3%
	every 2-3 days	66.6%
4. Type of food	lettuce only	70.7%
	fish food only	61.9%
	combination	71.1%
5. Snail density (number per container)	one snail	98.1%
	five snails	64.3%
	ten snails	41.4%

travelling time is a crucial factor. Transit time from Echague, Isabela was 9-10 hours but only 2-3 hours from San Pablo, Laguna. Survival rates and transport time seem to be inversely related. Gradual dessication of the medium, increased collisions among the specimens and accumulation of mucus and waste products may contribute to the rise in mortality. For longer trips, it may be necessary to change the wet filter paper beddings periodically.

In the laboratory, a statistically significantly higher survival rate occurred among snails raised in the plastic containers ($p < 0.05$). The wider mouth of the plastic containers gives a higher air-exposed surface area (201.1 cm²) than the glass bottles (33.2 cm²). The wider surface area provided by the plastic containers may be an essential requirement of these snails which have been observed to frequently float upside down on the water surface to feed or breathe atmospheric oxygen through their pneumostome. The amount of dissolved oxygen in the water may also be affected by the amount of water surface area exposed to the outside air.

Survival was also significantly affected by the type of food provided. Lettuce combined with fish food or lettuce alone were superior to fish food alone. Plant material seems to be the preferred food of this species which inhabits ricefields and freshwater lakes abundant in vegetation, both microscopic and macroscopic. The fish food may supply additional and more complex organic nutrients. However, fish food alone did not seem to be satisfactory since the snails did not consume it completely and putrefaction of leftovers eventually fouled up the water leading to increased mortality.

Snail density was the most significant factor identified in this experiment. Snails raised in isolation had the highest survival rates. Mortality was higher in containers with 5 specimens and highest in those with 10 specimens per container.

In working with *Bulinus foskalii* cultures, Wright (1960) discovered that isolated individuals also grew faster, reached a greater final length and produced more than twice as many living young as any of the other experimental groups. It was

Table 4

Multiple linear regression analysis on factors affecting the survival of *Lymnaea (Bullastra) cumingiana* in the laboratory based on 3 trials (216 valid cases).

ANOVA Table				
Source of variance	df	Sum of squares	Mean squares	F-test
Regression	6	1.192 E + 05	19875.9	48.0207
Residuals	209	86505.9	413.904	
Total	215	2.051 E + 05		
Independent variable		Regression coefficient		t-value
1 Container type*		4.90738		1.77254
2 Aeration		- 1.94446		- 0.70234
3 Frequency water replacement		2.68519		0.96989
4 Food 1**		9.16663		2.70340
5 Food 2		0.41664		0.12288
6 Number (snail density)***		- 6.22615		- 16.55980

* = $p < 0.05$

** = $p < 0.005$

*** = $p < 0.0005$

Dependent variable : Percentage survival rate

Multiple correlation coefficient (r) = 0.761303

Estimated constant term = 91.9099

suggested that increased density led to overcrowding which in turn produced competition for food, increased number of collisions and chemical pollution. Growth and survival of *B. forskalii* colonies were found to improve upon the use of activated charcoal which presumably removed the excretory products of the snails.

Since it is impractical, space consuming and expensive to raise snails individually, it is suggested that a reasonable number be raised together in one container and that subculturing be practiced when the snail population rises.

The slight increases in survival rates associated with the absence of aeration and daily replacement of water were not statistically significant. It was observed that water movement induced mucus secretion in these snails. The bubbling of water may irritate the snails and cause them to produce copious quantities of mucus which pol-

lute the water and impede absorption of oxygen through the water surface. In spite of this drawback, aeration is still recommended since oxidation of metabolic waste products and oxygenation of the water increases chances of survival. As to the frequency of water change, it would also be more economical to change water every 2 to 3 days instead of daily, depending of the condition of the water.

There may be other important factors influencing the survival of *Lymnaea (Bullastra) cumingiana* colonies during laboratory acclimatization such as light, special food supplementation, addition of soil to the culture, etc which were not tackled in this study. This merits further investigation.

It was observed that acclimatized individuals eventually survived for only a few weeks, oviposited along the sides of the container and then

died. Investigations on the optimal factors in rearing the juveniles arising from egg masses laid should be undertaken to develop an efficient method of cultivating this species in large numbers.

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