

# Chapter 1

## Experimental Design and Testing: Hatching and Development in Brine Shrimp

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### Introduction to an Experimental Design

Experiments and observations in the elementary biology laboratory can be formulated in terms of scientific investigations rather than as demonstration and confirmation of principles. The laboratory experience modeled in this chapter, according to established scientific procedures, conforms to the concepts that biology, like all other sciences, should be taught and experienced as a “way of knowing.” Knowing and doing science — learning through experience — demands both active involvement and reflection by the student. There are nearly limitless opportunities to present laboratory science in this way: an example of it is the study of the brine shrimp, *Artemia salina*.

Outcomes of any such study in the sciences should:

1. Improve understanding of important biological questions and phenomena.
2. Develop the critical thinking/communicating skills of the students.
3. Be simple and inexpensive, allowing students a wide range of opportunities for designing and carrying out experience.
4. Meet all the criteria of humane and appropriate biological experimentation.
5. Enable the instructor to assess student development in certain aspects of scientific design and implementation.

*In addition, it would be desirable for the students that the experiment:*

6. Involve the use of some instrumentation and quantitative procedure and provide data for statistical analysis, if this seems appropriate to the instructor.
7. Provide the opportunity for interested and/or advance students to carry out further experiments with the system.

It is important that the design of the science experience/experiment involves students as active learners. This cannot be limited to students being busy doing procedures. Clearly, any experienced laboratory instructor realizes that doing science without reflection frequently results in very limited knowing and is not too far removed from rote learning.

Likewise, one needs to build into the laboratory design a number of activities that focus on the development of abilities that transcend the discipline but lead to the intellectual maturity of the

student. Inherent in the experimental component of this study, is the assumption that students should develop critical thinking skills and gain experiences in the communication of their hypotheses, experiments, data, and conclusion. If one reflects on the two abilities of communication and critical thinking, one can immediately identify multiple skills implied in each of these two broad abilities. For example, communication ability implies skill in reading, writing, listening, using quantification appropriately, and possibly effective computer usage.

Critical thinking abilities also can be broken open to reveal multiple skills. Some educators even perceive communication as a subset of critical thinking but for pedagogical reasons, communication and critical thinking will be identified as separate but interrelated abilities.

At Alverno College in 1984, a *Critical Thinking Network in Science* was created. Selected science faculty from across the United States met and identified their operational model for critical thinking. For further information on this network, contact L. Cromwell, Ph.D., Director of the Network, or L. Truchan, Ph.D., Liaison to the Science Network Team.

The more precise one can be in identifying the skills involved in developing an ability, the easier it is to design learning experiences and design laboratory needs to contribute to the development or reinforcement of one or more of these skills in a structured way. Assuming the goal of science teaching is to make students see that science is a “way of knowing,” how do laboratory experiences contribute to literacy in science?

How can one design experiences to enhance critical thinking or communication in a laboratory setting and still be confident that it is a “valid” laboratory experience? Or how do we consciously integrate content and process to assist student development? The student-designed laboratory is inherently well-suited to do both: to integrate content and process. What follows is an example of an Investigative Laboratory Design which can be readily adapted to other investigative laboratories. Ideally, over a semester or year, reinforcement of skill development and greater sophistication of the ability should also be introduced.

The text on the next few pages are divided into two columns to emphasize the teaching of communication and critical thinking in the laboratory. This format was selected to assist the reader to “see” both components of content and process. However, effective teachers need to integrate both columns in their teaching strategies. Our selection of *Artemia* sp. as the content area is, just that, an example of how both content knowledge and process can be integrated in an investigative laboratory design.

### Materials

*For each pair of students:*

Dissecting microscope (1)  
 Culture jars having a capacity of 500–1000 ml, aerated if possible (1–2)  
 Petri dishes, 15 × 100 mm, or smaller (5)  
 Pasteur pipet (5)  
 Marking pen or labels (1)  
 Brine shrimp cultures (2)

*For class use:*

Graduate cylinders and glassware  
 Balance  
 Supply of brine shrimp cultures  
 Distilled water to prepare media  
 Sodium chloride  
 Sodium bicarbonate  
 Silver nitrate  
 Sucrose  
 Potassium chloride  
 Sodium disulfate  
 Trehalose

## Student Directions for the Investigative Laboratory

*Note:* If you are distributing directions to the students, present all information into one column.

### Systems To Be Studied

*Artemia salina* “eggs” are seeded on the surface of an appropriate fluid (sea water, artificial sea water, sodium chloride solution, etc.), stored in small petri dishes, plastic cups, or jars. In about 1–5 days depending on temperature, the eggs hatch and *nauplii* emerge.

#### *Communication/Critical Thinking Abilities*

2. Read the notes on the biology of *Artemia salina* taken from the scientific literature (Appendix A). From Appendix C, select one of the qualities of an experienced critical thinker that you want to develop before continuing this laboratory.
3. Working as a collaborative team, divide the *Artemia* notes into four sections. Select one section and write out your paraphrase of this section so that your peers will understand it. Share your translation with your team. While each of you are presenting your section, recall what qualities are needed to be a critical listener (Appendix D).

#### *Laboratory Techniques Experimental Design*

1. Observe the structure and activity of the *nauplii* using a dissecting microscope. Familiarize yourself with this “new” biological system. Use Appendix B, Figures 1.1A to 1.1C to help you.
4. Observe again the activity of the *nauplii*. Attempt to see as much detail as possible. Use the graphics provided to assist you in your view of these organisms (Appendix B, Figures 1.1A and 1.1B).

*Communication/Critical  
Thinking Abilities*

5. Scan the summary notes again and each group member should be responsible for data on one of the following four environmental factors:

- Osmotic pressure
- Ionic composition
- Temperature
- Oxygen supply

Remember, your role is to become the *expert* regarding this factor. Assist your group to design an experiment that will give you insight into egg hatching and *nauplii* emergence.

6. In your collaborative team, take turns *thinking aloud* (Appendix E) and state what you know about each factor. Be clear as to what your common knowledge base is and how you can use this to experiment on your *new* system. Be aware that when one team member is *thinking aloud*, the other team members take the role of being *critical listeners* (Appendix D).

7. Working at researchable questions:

- Do you think osmotic pressure alone is a determinant of hatching success and survival?
- Would you use a non-electrolyte such as sucrose or trehalose as your choice?
- State *your* questions regarding osmotic pressure.
- What could you learn by comparing hatching/survival rates in different solutions?
- Why select various osmotic solutions?
- Share your question regarding osmotic solutions.
- What questions could you ask about temperature in regard to hatching and survival?
- What if more oxygen, air, or nitrogen were bubbled in?
- What are your questions about oxygen needs?

*Laboratory Techniques  
Experimental Design*

8. To help you design your experiment, read through the following summary statements:

- Osmotic pressure of hatching medium may range from 1/2 to several times the concentration of sea water.
- Ionic composition of the medium may be comparatively analyzed by recording hatching and survival in various solutions such as NaCl, KCl, NaHCO.
- Temperature in the range from 4°C to 35°C can be readily tested in the lab; this is the temperature range of many living organisms.
- Oxygen supply can be assured by bubbling oxygen through the medium.
- Brine shrimp are extremely sensitive to the toxic effects of carbon dioxide.

*Communication/Critical  
Thinking Abilities*

9. What is it that *you* really want to find out in your experiment?

12. Each team member should reflect on their research design. Think about the technique used and its relationship to the understanding of egg, hatching, and survival.

- What, if anything, would you modify if you wanted to further support your findings on factors affecting hatching and survival rates?
- What follow-up experiments need to be done to give you further insights on egg hatching and *nauplii* emergence?
- What are the critical thinking skills that you used during the experimental design or implementation? Give evidence.
- Which critical thinking skills(s) do you need to further develop to be more effective?

*Laboratory Techniques  
Experimental Design*

10. Design an experiment that will test the effects of environmental factors on hatching and survival.

- Identify the material that you need.
- Make a flow chart mapping your steps.

11. Do the Investigative Experiment.

- What is your role in your team?
- How are you gathering data?
- How will you convert *raw data*?
- Who will write up the team report?

### Suggestions for Further Studies by Students

1. The salt secreting organs of *Artemia* sp. are the neck gland (*nauplii*) and the special metepipodite sections of the appendages. This has been established by ingenious techniques developed by Croghan (1958). The animals are rinsed in distilled water (several changes for 1–2 hours), then immersed briefly (from 2 to 5 minutes) in 0.001 M AgNO<sub>3</sub>. Silver ions precipitate with chloride ions in the cuticle over the salt-secreting cells. The presence of AgCl is then revealed by 1–2 minute exposure to photographic developer (e.g., D-76) or by longer exposure to sunlight, with formation of a black precipitate in the secreting tissue. Students might investigate effects of ionic differences in the medium, or of inhibitors of salt transport, on the degree of blackening of the salt secreting organs.
2. The salt secreting tissues operate by active transport of sodium ions (into the body through the gut, and out through the neck gland or gills) by the Na,K-ATPase system (sodium pump) present throughout the range of eukaryotic animals. The sodium pump can be blocked by the plant product *ouabain*. In many animals, active Na transport is accompanied by mediated Cl transport; this is frequently blocked by pharmacological agents such as *furosemide*. Can *Artemia* survive in the presence of, for example, 0.0001 M *ouabain*? 0.0001 M *furosemide*? Are the effects of these agents dependent on the composition of the external medium? For further information see Ewing et al. (1974).

*Ouabain* (*Strophanthin-G*; F.W. 729) is obtained from the seeds of plants (e.g., *Strophanthus gratus*) and is readily available commercially because of its widespread use in biological laboratories as a highly specific blocker of Na,K-ATPase.

*Furosemide* (F.W. 331) is a synthetic compound, developed by pharmacologists as an effective diuretic for use in heart conditions, for example. It is termed a “loop diuretic” because its effects are very specific in blocking the coupled transport of chloride and sodium ions in the kidneys' loope of Henle.

Both these agents can be purchased from chemical supply companies (e.g., Sigma Chemical Company, P.O. Box 14508, St. Louis MO 63178.)

3. Students who are interested in environmental problems can test the effects of relevant pollutants on *Artemia* sp. Heavy metals are toxic, as are many organic compounds (a simple example might be very dilute solutions of formaldehyde.) A special case is the acute sensitivity of brine shrimp to silver ions, perhaps related to their effect on the salt secreting tissues. For further information see Sorgeloos et al. (1978).

### Additional Notes on Experimental Procedures for Teachers or Students

#### Solutions for Culturing Brine Shrimp

The range of solutions for culturing is wide and may include sea water, instant ocean, or solutions of various solutes. Typical sea water tonicity is about 1000 osmol/liter. Solutions of about the same tonicity may be made with common solutes as follows:

Solutes	Molecular weight equivalent to seawater/g/100 ml HOH	Osmol/mol	Concentration*
NaCl	58.2	2	2.9
NaSO	268.1	3	8.9
NaHCO	84.0	2	4.2
KCl	74.0	2	3.7
Sucrose	342.3	1	34.2
Trehalose	378.3	1	37.8

\*This is approximate, depending on the degree of dissociation of the electrolyte.

### Methods for Culture and Preservation

Dried eggs may be purchased from biological supply companies (e.g., Carolina Biological Supply Co.) or from a local pet shop or tropical fish store. The “eggs” (cysts) are seeded on the surface of sea water or other appropriate solution in the range from 1 to 2.5% sodium chloride. Suitable containers are petri dishes, plastic cups, or jars. These cysts will hatch in 24–48 hours at 25°C; the length of time is shortened if the temperature is 30°C and will emerge more slowly at lower temperatures.

For rearing the brine shrimp to maturity and to establish a continuing culture, the density of the animals should not be too great since they are exceedingly sensitive to lack of oxygen or a high accumulation of carbon dioxide. They can be maintained in a jar containing 500–1000 ml of the culture medium; they thrive best if air is bubbled continuously through the medium. Evaporation is compensated for by replacing lost volume with water, which can also serve as a vehicle for added food. This added food can be yeast; the dried yeast sold in supermarkets is suitable. Make a dilute suspension of yeast in water and add to the culture; algae scraped from a freshwater aquarium can be a substitute for the yeast. It is important to limit the food supply so that the water does not become contaminated. Feeding once or twice a week is adequate depending on culture conditions. Mature *Artemia* sp. are beautiful, active animals; they can serve as a fine system for a wide variety of physiological and behavioral studies.

Animals can be preserved at different stages of development. They are transferred by pipet to a suitable container such as a small petri dish or concavity slide. Bouin's fixative is slowly added to the medium. The medium-Bouin's fixative mixture is drawn off and replaced with fresh Bouin's. For permanent preparations, standard procedures of dehydration and mounting may be used.

Bouin's solution is made up in the following proportions: 15 ml aqueous saturated picric acid + 5 ml formalin + 1 ml glacial acetic acid.

## Blocking Agents

The two agents previously mentioned are *ouabain* (*Strophanthin-G*; F.W. 729) and *furosemide* (F.W. 331). Please refer to item 2 of Suggestions for Further Work for an explanation of the mode of action of these two blocking agents.

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## APPENDIX A

### *Suggested Reading*

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## APPENDIX B

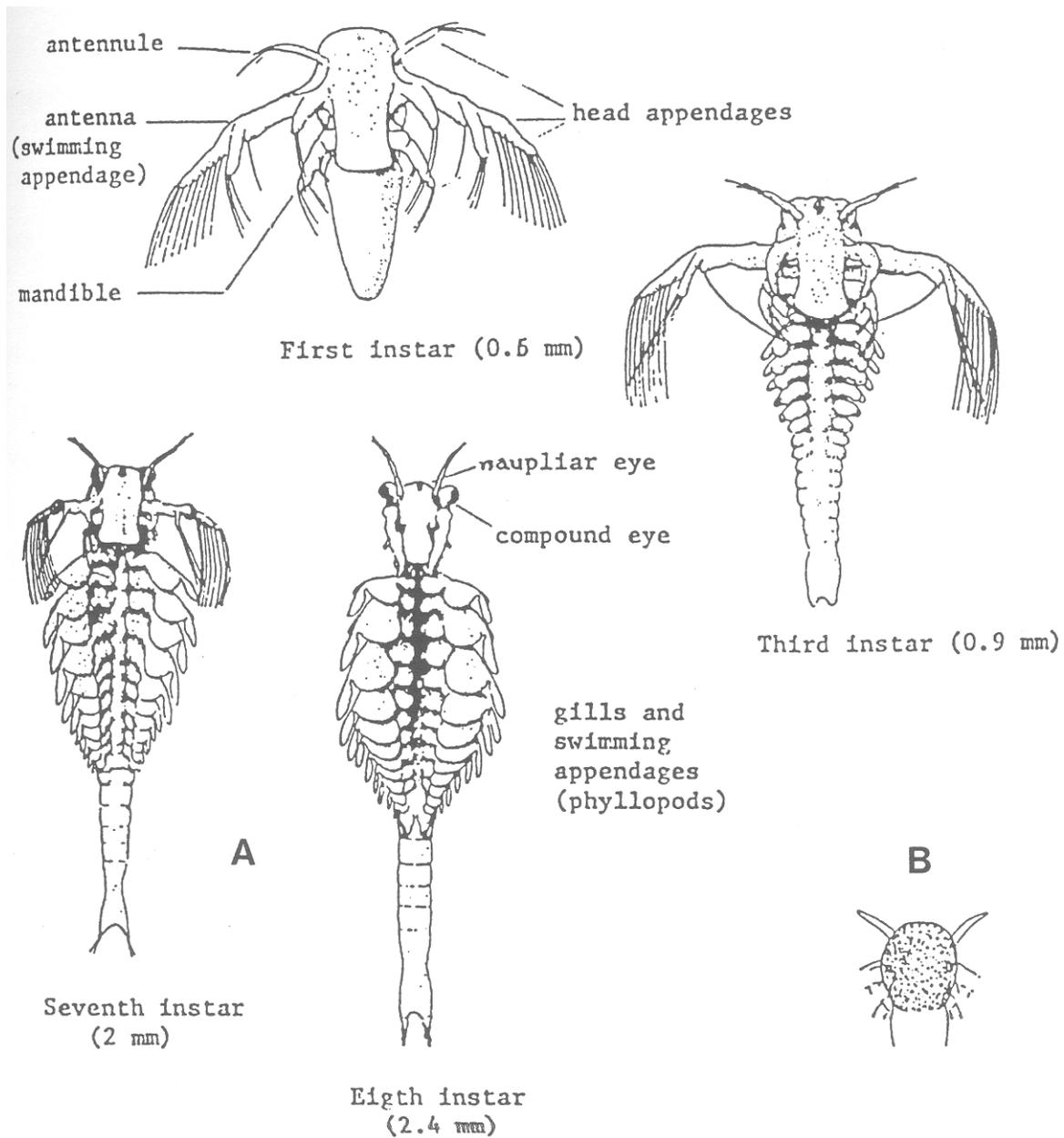
*Notes on the Biology of Artemia salina*

Brine shrimp of the genus *Artemia* are members of the fairly primitive crustacean sub-class Branchipoda, order Anostraca. They are found world-wide in saline lakes and ponds, and have truly remarkable mechanisms for surviving under harsh and variable environmental conditions. Survival depends in part on the formation of extremely resistant “eggs” (cysts). These “eggs” are, in fact, embryos arrested at the gastrula stage, and encased in chitinous shells. The eggs will not hatch unless they have first dried out to an extremely low water content (capacity of 5%). In the dehydrated state, metabolism is completely arrested; the embryos are in a state of cryptobiosis which is sometimes called “suspended animation.” After this dehydration, exposure to water activates the *Artemia* sp. egg. In a few hours or days, larval *nauplii* emerge and swim actively by rhythmic movements of the head appendages (Figure 1.1A). For a few days, the nauplius subsists on yolk; then begins to feed and develop through a series of molts and successive instars (Figure 1.1B) to the reproductive stage at the 12th or 13th instar.

Males recognize females by vision. After copulation and fertilization of the eggs, the females produce the first batch of eggs by an ovoviviparous method; the first instar nauplii develop in an escape from the female's brood sac. The female's next batch of eggs is in the arrested condition, and must be dehydrated before hatching can occur. In some races or species of *Artemia*, reproduction can be parthenogenic; such a species is found in China.

The most striking physiological capacity of *Artemia* is its ability to tolerate extremes of environmental salinity. Among the members of the order Anostraca, this ability is unique to *Artemia*, as all other anostracans live in fresh water. *Artemia* can survive and even thrive in media ranging from 1/2 the tonicity of sea water (about 1.5% NaCl) to concentrated brines in which salts actually crystallize out; this would be approximately at a 15% NaCl concentration. Several physiological mechanisms serve as the basis of *Artemia's* toleration of dehydration and osmotic stress:

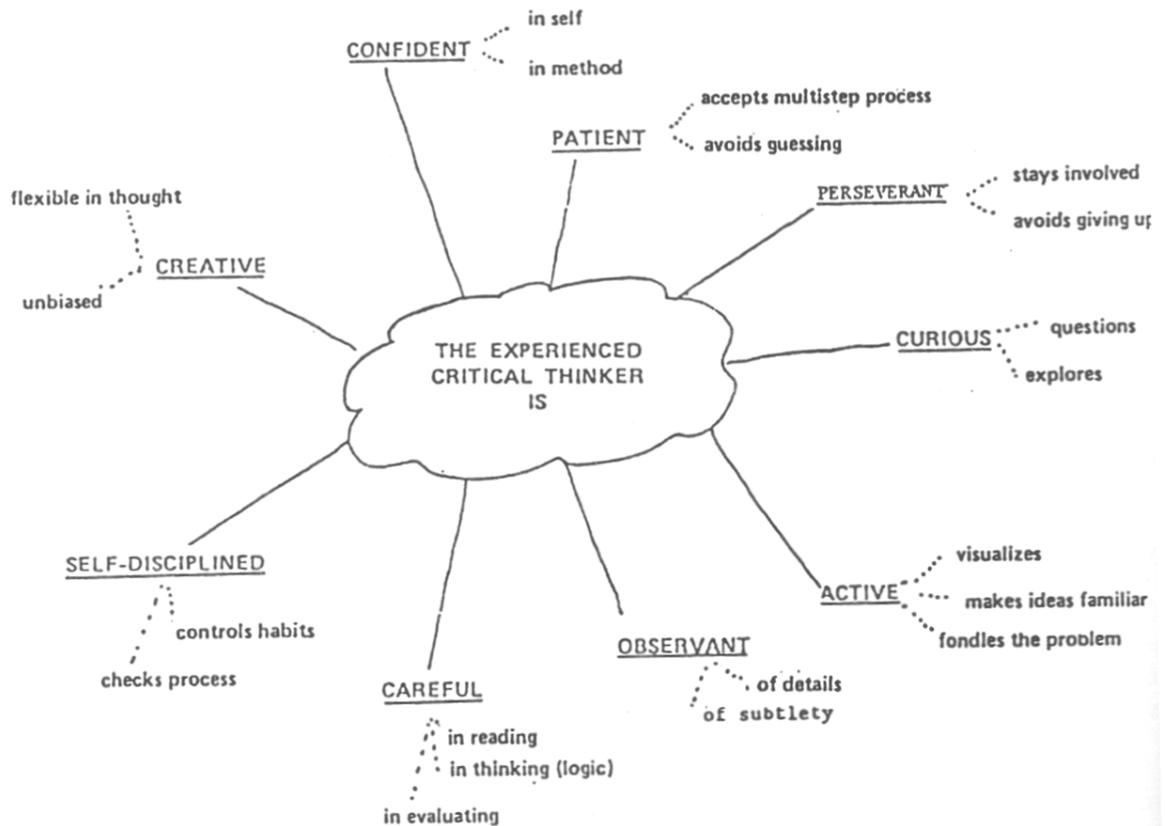
1. In the “egg” (cyst), an extremely high concentration of glycerol and trehalose protects delicate proteins and subcellular structures from disruption by loss of water.
2. The *nauplius* drinks the medium; it takes in water with sodium ions and chloride ions through the gut, and conserves the water while excreting excess sodium ions and chloride ions through the specialized neck organ (Figure 1.1C). Sodium transport is effected by the Na,K-ATPase in the neck organ. Synthesis of this membrane system begins explosively within a few hours of hatching.
3. As the animal matures, it develops gills with specialized parts (metepipodites) that take over the function of the *nauplius's* head organs which disappear during the developing process. These specialized organs have an extremely effective Na,K-ATPase transport system. Drinking of the medium can occur through both the mouth and anus.
4. The chitin covering the “egg” and body surface at all stages is highly impermeable, except at the sites of ion intake and excretion. *Artemia salina* has other physiological mechanisms which assist its survival in a wide range of harsh environments. For example, *Artemia* has hemoglobin in the blood (hemolymph), serving as an oxygen transport function closely parallel to the transport function of hemoglobin in vertebrates. If reared under conditions of partial oxygen deficiency, brine shrimps maintain increased levels of hemoglobin in the blood.
5. Brine shrimp have complex behavior. Feeding is by filtration and is very effective. Brine shrimp react to light; they are positively phototrophic in dim light and negatively phototropic in bright light. Their reaction to light varies with environmental conditions. The compound eyes have color sensitivity and visual recognition is important in reproduction. They would be a suitable organism for chronobiological studies.



**Figure 1.1.** Structure of *Artemia* sp. at different developmental stages. *A*: Ventral views; brine shrimp typically swim with ventral side uppermost. *B*: Dorsal view of neck organ of nauplius. Modified from H. Heath (The external development of certain phyllopods. *Journal of Morphology*, 38:453–483, 1924).

APPENDIX C  
*Qualities of the Experienced Critical Thinker*

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**Consequences of Being an Experienced Critical Thinker**

- Confident
- In self, in own reasoning abilities, is willing to try and feels can succeed in process, willing to use reasoning approach to problems.
  - Doesn't fear being mistaken or criticized.
  - Uses mistakes and criticism as learning experiences.

- |                  |   |
|------------------|---|
| Patient          | <ul style="list-style-type: none"> <li>• With self — paces work to avoid error.</li> <li>• With process — willing to use reasoning approach in multistage setting — uses step-by-step</li> </ul>  |
| Perseverant      | <ul style="list-style-type: none"> <li>• approach in complex situations — avoids premature guessing.</li> <li>• Avoids “giving up” on self or process.</li> <li>• Stays actively involved in thinking process.</li> <li>• Withholds from drawing conclusions from incomplete thought.</li> <li>• When catches mistakes, restarts process.</li> </ul>  |
| Curious          | <ul style="list-style-type: none"> <li>• Asks questions (of self, of problem, of others).</li> <li>• Explores a problem or situation.</li> <li>• Begins with best known areas, proceeds to least.</li> </ul>  |
| Active           | <ul style="list-style-type: none"> <li>• Organizes data and observations.</li> <li>• Fondles data and observations — restates in own words, re-orders, re-organizes.</li> <li>• Uses mental aids to thinking — visualizing the problem, relating abstract ideas to concrete items, making ideas and problem familiar by restating in own words or relating to past experience.</li> <li>• Uses physical aids to thinking — drawings, diagrams, graphs, physical models, pointing, talking to self, idea maps, hands.</li> </ul> |
| Observant        | <ul style="list-style-type: none"> <li>• Checks on understanding of unfamiliar words, concepts, or procedures before drawing conclusions.</li> <li>• Uses context of problem or situation to sort out relevant from irrelevant information.</li> <li>• Doesn't omit units in numerical work.</li> <li>• Draws upon available resources and her prior knowledge and experience to make sense of a situation or idea.</li> <li>• Searches for patterns, similarities, differences, correlations.</li> </ul>                       |
| Careful          | <ul style="list-style-type: none"> <li>• Checks reading/listening to ensure accuracy.</li> <li>• Checks calculations and logical processes for accuracy and consistency.</li> <li>• Checks conclusions for reasonableness by using approximation methods and comparing with prior knowledge.</li> <li>• Uses context of problem or situation to select formulas and procedures.</li> <li>• Accounts for all data whether used in an analysis or not.</li> </ul>   |
| Self-disciplined | <ul style="list-style-type: none"> <li>• Controls self-defeating habits in reasoning.</li> <li>• Willingly checks his or her own process.</li> <li>• Articulates assumptions used.</li> <li>• Can work within a time frame.</li> </ul>  |
| Creative         | <ul style="list-style-type: none"> <li>• Uses intuition and hunches but does not rely on them.</li> <li>• Generates many ideas for testing, not just preconceived approaches.</li> </ul>  |

APPENDIX D  
*Role of the Listener in the Thinking Aloud Process*

As a Listener you have two important objectives:

- To help the Thinker to practice and refine her or his critical thinking skills (here in the context of solving problems).
- To use the experience of evaluating another's thinking process to learn to evaluate your own.

To do this effectively you should:

- Concentrate on the Thinker's process, not your own.
- Check for consistency and accuracy at *every* step, not just overall.
- Work *with* the Thinker, not ahead of her or him.
- Help the Thinker to go at a pace that allows for accuracy (not too fast).
- Point out the existence of errors or inconsistencies, but do *not* correct them (let the Thinker work).
- Help the Thinker to recheck her or his process.
- Demand “thinking aloud” — get the Thinker to explain herself or himself.

APPENDIX E  
*Criteria Checklist for Critical Thinking Aloud*

Here is a checklist of some sources and types of errors you might find in the process of thinking aloud. (There is some unavoidable overlap.) Read the checklist and discuss any items that are unclear. Then, as you solve problems in later exercises, be careful not to make these errors. Moreover, if you recognize some particular error to which you are habitually prone, take extra pains to guard against it. Feel free to talk with your instructor about your progress or problems with this technique.

In addition, when you are listening to another student think aloud in solving a problem, watch her approach for errors of the type listed below.

**A: Accuracy in Reading**

1. Thinker reads the material without concentrating strongly on its meaning. She was not careful about whether she understood it fully. She read sections without realizing that her understanding was vague. She did not constantly ask herself: "Do I understand that completely?" This showed up in errors she made later.
2. Thinker read the material too rapidly, at the expense of full comprehension, or did not spend enough time rereading a difficult section to clarify its meaning completely.
3. Thinker missed one or more words (or misread one or more words or facts or ideas) because the material was not read carefully enough.

**B: Accuracy in Thinking**

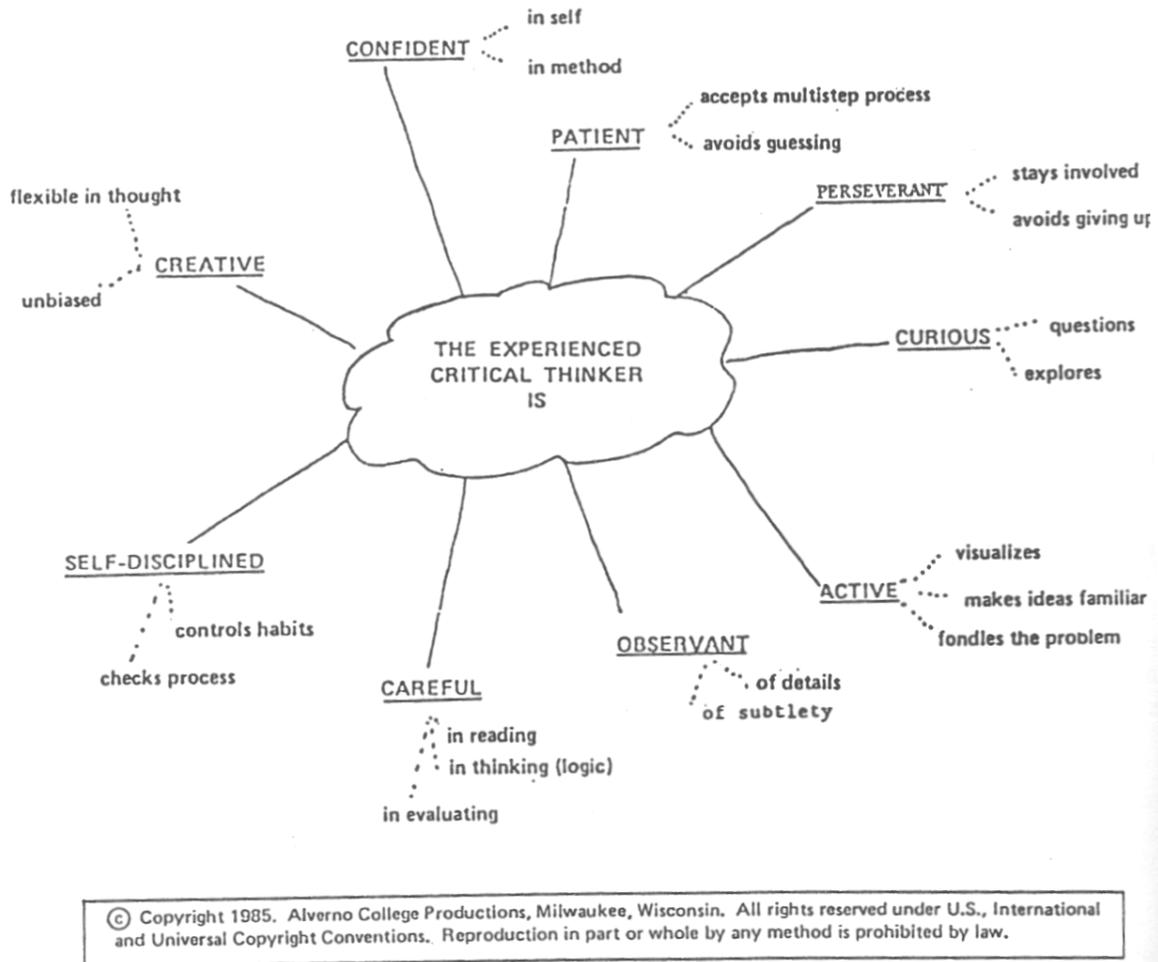
4. Thinker was not sufficiently careful in performing some operation (such as counting letters) or observing some fact (such as which of several figures is the tallest).
5. Thinker was not *consistent* in the way he interpreted words or performed operations.
6. Thinker was uncertain about the correctness of some answer or conclusion, or was uncertain about whether a formula or procedure she used to solve the problem was really appropriate, but did not check it.
7. Thinker worked too rapidly, which produced errors.
8. Thinker was inaccurate in visualizing a description or a relationship described in the text.
9. Thinker drew a conclusion in the middle of the problem without sufficient thought.

**C: Problem Breakdown and Analysis – Activeness in Problem Solving**

10. Thinker did not break a complex problem into parts. He did not begin with a part of the problem that he could handle in order to get a foothold. He did not proceed from one small step to the next small step, being extremely accurate with each one. He did not use the parts of

APPENDIX C  
*Qualities of the Experienced Critical Thinker*

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- Confident
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