MORPHOLOGICAL DEVELOPMENT OF UNIGLOMERULAR PROJECTION NEURONS IN THE OLFATORY LOBE OF THE MOTH, *MANDUCA SEXTA*

by

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I came to the Tolbert Lab in the Department of Neurobiology at the University of Arizona by adventure as a teacher enrolled in the General Biology Program for Secondary Science Teachers. It is my interest as an educator in behavior that guided me into this particular adventure. My studies in animal behavior, learning behavior, and educational psychology naturally led me to seek a better understanding of the structural mechanics of behavior at the neuronal level. I don’t even try to pretend that I understand the neural circuitry or biochemical pathways in a brain at anywhere near the extent of those who have dedicated years to those studies. My personal objective is to learn some of the basic principles of neuronal development and neurotransmission to increase my understanding of their roles in learning, behavior, and ultimately the interrelationships between organisms and their environments. My intent as a teacher is to increase my knowledge and experience in scientific research so that I can increase my effectiveness in science instruction. I am quite aware that a little knowledge can be very dangerous. So, I confess that I now have a much better understanding of how little I know about the adolescents I have been teaching for twenty years!

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ABSTRACT

The moth *Manduca sexta* has been a common model for the study of the insect olfactory systems. The neuronal architecture in the antennal lobes (ALs) of insects and in the olfactory lobes of vertebrates is similar in structure and development. In *Manduca*, as in other olfactory systems, sensory receptor neurons send axons into the AL where they form synapses with local interneurons (LNs) and projection neurons (PNs) within the structural units of glomeruli. Here, I present the morphological development of one type of interneuron, the uniglomerular projection neuron (uPN), in normal AL development and in AL development in the absence of olfactory receptor neurons (ORNs). Using fluorescent-dye labeling of uPNs and confocal microscopy, my results show that in the absence of ORNs, uPN dendritic arborization is uncharacteristic of that in normally developing ALs, reinforcing the concept that afferent input guides the development of architecture in sensory areas of the brain.
SECTION ONE

Morphological Development of Uniglomerular Projection Neurons
in the Olfactory Lobe of the Moth, Manduca sexta.
Introduction

In a variety of systems, input from the developing sensory apparatus affects the development of the target neurons in the central nervous system (CNS), initially through activity-independent mechanisms and later with refinement via activity (Oland and Tolbert, 1996; Key and St. John, 2002; Lopez-Mascaraque et al., 2002). This study takes advantage of the well-described moth olfactory pathway to explore the specific dependence of morphological development of projection neuron dendrites on interaction with receptor neurons.

The moth *Manduca sexta* has been used intensively in studies of olfactory system development because it shares the same basic neuronal architecture as found in other insects and vertebrates and provides relatively easy access to different neuron cell types (Boeckh and Tolbert, 1993; Malun et al., 1994; Oland and Tolbert, 1995, 1996; Sun et al., 1997). Olfactory receptor neurons (ORNs), whose cell bodies reside in the sensory epithelium of the antenna, send their axons to primary olfactory centers (antennal lobes) in the brain where they synapse with both local and projection neurons in roughly spheroidal compartments called glomeruli. The axonless local interneurons (LNs) arborize within many or most glomeruli. Projection neurons (PNs) typically arborize within one or a few glomeruli and project their axons to the calyces of the ipsilateral mushroom body and lateral horn in the protocerebrum (Diagram 1) (Homberg et al., 1988; Malun et al., 1994; Oland and Tolbert, 1993, 1995). Each glomerulus is almost
Diagram. 1. Schematic drawing (Malun, et al., 1994) of left half of Manduca brain in a frontal, semitransparent view shows the innervation pattern of a uniglomerular projection neuron (uPN). Each uPN has its cell body in the medial cell group of the antennal lobe (al) and forms a dendritic tuft in a single glomerulus. Its axon projects via the inner antennal-nerve tract (iact) into the calyces of the ipsilateral mushroom body and into the lateral horn (lh) of the protocerebrum (pc). The orientation of the brain in this figure is indicated by the axes which correspond to the position of the brain in the body: l, lateral; m, medial; d, dorsal; v, ventral. an, antennal nerve; g, glomerulus; ol, optic lobe; sog, suboesophageal ganglion.
completely surrounded by a multi-layered border of glial cells (Tolbert and Hildebrand, 1981).

Developmentally, the antennal lobes (AL) of the adult moth arise from rudimentary larval antennal lobes (Tolbert et al., 2004). In the nascent AL, the neuropil is composed almost entirely of the dendrites of LNs and the neuropil is surrounded by a shell of glial cells that separates the neuropil from the neuronal cell bodies that lie in the cortex around it. Glomerulus construction in the AL occurs during stages four to nine of metamorphic adult development, which proceeds over 18 defined developmental stages (Diagram 2) (Oland et al., 1996; Tolbert et al., 2004). Beginning at stage four, ORN axons reach the AL and begin to course through and around the glial layer to their target region (Diagram 3), thus forming a nerve layer around the existing neuropil. By early stage five, ORN axons have turned inward to form a continuous fringe between the glial border and the existing neuropil. The terminal branches of the receptor axons segregate into protoglomeruli during stage five. The earliest protoglomeruli will mature into the sexually dimorphic macrogglomerular complex in males and into the large female-specific glomeruli of females. A wave of protoglomerulus formation proceeds in a ventromedial direction during stage six. Glial cells begin to envelope each protoglomerulus about a day after formation, followed by the invasion of dendrites of local interneurons (Boeckh and Tolbert, 1993; Oland et al., 1996; Tolbert et al., 2004). A wave of synaptogenesis occurs between stages seven and twelve as ingrowing ORNs, LNs, PNs and glial cells complete the organization of the maturing glomeruli. The LNs and PNs develop tufted
Receptor neurons are born

AL neurons are born

Glia proliferate

Border glia are tenacin +

Receptor axons enter lobe

Protoglomeruli form

PN dendrites enter protoglomeruli

Glia develop border around developing glomeruli

LN and 5-HT neuron dendrites enter presumptive glomeruli

Wave of synaptogenesis occurs in developing glomeruli

Glomeruli stabilize

Dendrites of LNs become tufted

Synaptic transmission from receptor axons to AL neurons is present

| P | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | E |

Stage of Development

Diagram 2. Time line of glomerulus development in *Manduca sexta* (adapted from Oland and Tolbert, 1996).
Diagram 3. Schematic diagram (Malun et al., 1994) summarizes the morphological development of uPNs as they interface with antennal sensory axons (aa) and neuropil-associated glial cells (g) in the antennal lobe at consecutive stages (stages 4-7 of metamorphic development). Only the medial cell cluster of the antennal lobe is shown. pr, protoglomeruli.
arbors within the glomeruli. Synapses were found in electron micrographs of the developing glomeruli (Oland et al., 1990), but extracellular recordings from the antennal nerve showed no odor-induced responses until stage fifteen in males and stage sixteen in females (Sanes and Hildebrand, 1976) and even minimal spontaneous activity could not be detected until about stage seven, by which time the basic architecture of the glomeruli has nearly been completed. *In situ* intracellular recordings from antennal-lobe neurons revealed no responses to electrical stimulation of the antennal nerve prior to stage nine (Oland et al., 1996). Further refinement of glomeruli occurs until eclosion to the moth (Huetteroth and Schachtner, 2005).

The overall architecture of the moth antennal lobe (AL) is known to be dependent on receptor input for in its chronic absence, the glomeruli do not develop (Oland and Tolbert, 1987) and the LNs fail to develop glomerular tufts, instead arborizing diffusely across the neuropil (Oland et al., 1990). The loss of glomeruli and the loss of the underlying dendritic structure of LNs show that the presence of ORNs has a strong effect on the morphological development of AL neurons, but we have only suggestive preliminary evidence that PN dendritic structure is affected (Oland and Tolbert, 1987; Malun et al., 1994).

To study the effect of receptor axon deprivation on PNs in detail, I took advantage of previous work in the lab that had established the sequence of development of each of the major cellular elements in the antennal lobe (AL) (reviewed in Tolbert et al., 2004). I focused on the branching pattern of one class of PNs, the uniglomerular projection neurons (uPNs), because this class has been well described in the adult (Tolbert et al.,
1983) and because one of the cell body clusters of the AL consists entirely of this class, making them easy to identify for labeling purposes. The effect of receptor axon input is especially interesting for this population of neurons because they are the first of the different AL neuron classes to encounter receptor neuron axons (Malun et al., 1994) and thus have the potential to interact with the receptor neurons directly, either by cell surface molecular interactions or by synaptic interactions.

Morphological development of PNs has been studied in both vertebrates and invertebrates. In mice, axons of ORNs penetrate the olfactory bulb at the same time as mitral cells reorient and spread their dendrites into the OB (Blanchart et al., 2006). In opossums and in rats, mitral cell differentiation follows a sequence of overproduction of dendrites, selection of usually one primary apical dendrite, and retraction of supernumerary processes (Malun and Brunjes, 1996). In Drosophila, PN dendrites create a prototypic map which resembles the adult glomerular map prior to the arrival of ORN axons (Jefferis et al., 2004). The axons, however, also appear to set up a map, and the two maps come into register by mechanisms not yet well understood (Hummel and Zipursky, 2004). From a study of the development of the population of uPNs in Manduca, it is known that they extend processes into the neuropil at about the same time that the terminal branches of ORN axons form protoglomeruli, but nothing is known about whether they set up a PN map similar to that seen in Drosophila. uPNs are the first AL neurons to send dendritic processes into the protoglomeruli in normally developing lobes (Malun et al., 1994), and simultaneously glia extend processes and migrate towards the center of the neuropil to stabilize the protoglomeruli (Baumann et al., 1996; Oland
and Tolbert, 1996). Our understanding of the details of PN morphological development is limited however. In the Malun et al. (1994) study, which focused mainly on the spatial and temporal development of the PNs as a population, only a few uPNs were singly labeled, and the morphogenesis of the fine dendritic processes was difficult to discern in the preparations with many labeled PNs.

Development of PN morphology in principle could be a process intrinsic to the cell or could be modulated by a variety of factors including interaction with sensory neurons. A previous study in Manduca concluded, for example, that glomerulus formation occurs even when Na⁺-dependent spontaneous activity is blocked during the period of receptor axons ingrowth to the AL (Oland et al., 1996). A few uPNs were labeled in preparations in which the number of glial cells was greatly reduced (Baumann et al., 1996); these cells continued to have tufted arbors although they had a greater than normal lateral spread, suggesting that uPN arborization could be mostly cell autonomous. The very small number of dye-filled PNs available from preparations developing in the chronic absence of ORN input also suggested that the dendritic tree was larger than usual but still had a tufted arbor reminiscent in its basic architecture of the arbor found in PNs in normal ALs. Nevertheless, the number of dye-filled cells in these experimental conditions is too small to make real comparisons possible.

This study was thus designed to provide a more detailed analysis of uPN development in normal animals, and to use these data as the basis for comparison of different stages of uPN development in the absence of ORN input. If uPN dendritic arborization is dependent upon interaction with ORNs, then comparison between the two
groups will provide clues as to when and with what effect on morphology interaction with olfactory receptor neurons becomes a guiding factor for uPN development.
Materials and Methods

Animals and Basic Preparation

Female Manduca sexta (Lepidoptera: Sphingidae) were reared in the departmental insect rearing facility as described previously (Baumann and Oland, 1996). Pupae were staged by viewing structures visible under the pupal cuticle using fiber optic illumination (Oland and Tolbert, 1987).

Dissection and preparation of brains proceeded as follows (as described in Malun, et al., 1994; Oland and Tolbert, 1998). Pupae were anesthetized by cooling in ice for 15-20 minutes, and brains were dissected out of the head. Brains were immersed in phosphate-buffered saline (PBS) and perineural sheaths were removed. The brains were then immersed into a fixative solution containing 4% paraformaldehyde and 0.15% glutaraldehyde in 0.1M phosphate buffer, pH 7.4 and stored at 4°C for at least 12 hours prior to labeling projection neurons with fluorescent dye.

To produce ALs developing in the chronic absence of olfactory receptor neuron input (deantennated), the antennal anlagen in stage-one pupae were removed, leaving only input from the labial pit organ, whose axons terminate in a single glomerular complex in the ventral antennal lobe (Kent et al., 1987). The antennal anlagen were removed by anesthetizing stage-one pupae as described above, then cutting a small opening in the cuticle just above the location where the antennae have their origin. Antenna anlage was scraped away with fine forceps, and the cuticle opening then sealed with melted wax (as described in Malun et al., 1994). Pupae were returned to the rearing chamber to continue development until stages six to seven. Brains of deantennated pupae
were dissected and prepared using the same methods as used for normal pupae as described above.

**Dye Labeling, Microscopy, and Image Analysis**

The fluorescent dyes DiI C16 (3) and DiI C18 (3) (Molecular Probes, Eugene, OR) are lipophilic dyes that diffuse along cell membranes. Projection neurons were labeled with DiI using a modification of the technique developed in previous studies of *Manduca* (Malun *et al*., 1994; Baumann *et al*., 1996; Oland *et al*., 1996). Cell bodies of uPNs in the medial cluster (MC) of the antennal lobe are visible under a dissecting microscope when brains are illuminated from below with fiber-optic illumination through a dark-field filter. Dissected brains that had been immersed in fixative solution for at least 24 hours were pinned with fine insect pins through the optic lobes onto Petri dishes coated with Sylgard. Excess fixative solution was wicked away from brains with absorbent tissue. DiI was then applied to cell bodies in the MC by inserting the tip of a micropipette (pulled on a Kopff micropipette puller) coated with DiI dissolved in a drop of 70% ethanol into the MC. The brains were placed back into the fixative solution and kept in the dark (under aluminum foil) at room temperature for at least seven days to allow the DiI to diffuse throughout neuronal membranes, with the exceptions of early-stage-five and deantennated brains in which incubation times of five days provided specimens with better dye fills. DiI C16 (3) diffused through cell membranes of normal brains well, but good labeling was much more difficult to achieve in deantennated brains for reasons we don’t understand. Cell bodies in MCs of deantennated brains labeled with DiI C18 (3) using the same technique described above (with the exception that 90%
ethanol was used instead of 70% ethanol) resulted in better labeling of neurons in deantennated brains.

To prepare brains for viewing on a laser-scanning confocal microscope, the following procedures were completed with the brains covered with aluminum foil (except when necessary for processing) to prevent excitation and subsequent bleaching of DiI by room light. Brains were immersed in PBS for 2 x 10 min to remove fixative solution and then embedded into 7% low-melting-point agarose blocks. Agarose blocks were cut with razor blades to align brains in a frontal plane for sectioning, then glued with cyanoacrylate glue to a metal plate, which in turn was held by a magnet glued to the bottom of a plastic dish containing PBS. Brains were Vibratome sectioned into 100-μm-thick sections and the sections placed into fresh PBS. Sections were rinsed in Tris buffered saline (TBS) for 10 minutes, then in 0.05 M Tris buffer 2 x 10 minutes. Glial nuclei were labeled by immersing the sections in a solution of Syto 13 (Molecular Probes, Eugene, OR), diluted 1 L Syto 13 to 3000 L 0.05M Tris buffer. The sections were cleared in 50% glycerol in water for 15 min followed by 80% glycerol in water for 15 min, and the sections were mounted onto microscope slides in 80% glycerol in water.

Labeled sections were viewed with a laser-scanning confocal microscope, either a Nikon PC2000 using PCI software (Compix Inc., Cranberry Township, PA), or a Zeiss 510 Meta using LSM software, both equipped with argon, and green and red He Ne lasers and appropriate filters for excitation of DiI and Syto 13 dyes. For AL orientation and identification, 10X and 20X images of prepared sections were saved. Serial optical sections were collected through the depth of the Vibratome sections at 0.8 μm intervals.
with a 60X objective and saved as three-dimensional stacks. Confocal image stacks were scanned to select sequences providing the best optical clarity of uPN dendrites. Selected stacks were imported into Corel Draw or Adobe Photoshop, where annotations were added. Adobe Photoshop was used to adjust image hue, intensity and contrast, and to flip or rotate images to maintain uniform orientation of AL images.
Results

Because PN dendrites begin to enter protoglomeruli during stage four, and most of the glomerular components are in place in the AL by the end of stage seven, pupae in stages five (subdivided into early-, mid- and late-stage five), six and seven of metamorphic development were selected to study the morphological development of arborizations of projection neurons as they invade glomerular sites in the antennal lobe (Valverde et al, 1992; Malun et al, 1994; Oland and Tolbert, 1998).

Brains from 399 Manduca pupae, stages five through seven, were dissected and prepared as previously described. Of these, 118 brains showed enough DiI labeling of MCs to collect image stacks with a confocal microscope. Many of these image stacks either had multiple neurons filled, had additional labeling of neurons that had somata in the AC or LC, or dye labeling that was inadequate for analyses. Thirty-two antennal lobes provided the data discussed in this paper. Although general morphology of uPNs within the developing antennal lobes (AL) could be readily seen in brains with multiple neurons filled, only 15 ALs had labeling both strong enough and well-defined enough for identifying the morphological characteristics of individual uPNs, from main neurites through branching of higher order dendritic arbors.

In the figures that follow, all micrographs of individual ALs are at 60X magnification. Insets are either 10X or 20X magnification of the brain and have a white arrow pointing to the lobe shown in the larger figure. All 60X micrographs are stacks of optical sections approximately 0.8 m per section, and each is labeled with number of
sections per stack. Some micrographs of individual lobes have been rotated or flipped to keep medial-lateral orientation consistent in all figures.

**Position of the Medial Cluster of Neuronal Cell Bodies**

The medial cluster in the adult AL is positioned dorsally, medially and somewhat posteriorly with respect to the AL neuropil. Its adult position, however, develops gradually. At early stage five, the MC has not developed much and is a small structure that does not protrude much from the surface of the AL. As the lobe itself moves upward during development, it also rotates slightly, moving the MC more posteriorly and slightly ventrally. At stage six, cell bodies have increased in size and the MC is prominent. By stage seven, however, the MC becomes positioned in the space between the protocerebrum and the AL and is more difficult to see. In deantennated ALs, no ORN axonal nerve layer develops, but the neuropil grows some as the AL neurons develop, with the net effect being that the MC is less prominent as a structure protruding from the surface of the AL.

**Normal Development**

**Early Stage Five**

Twenty-six antennal lobes of early-stage-five pupae had enough dye labeling for imaging on the confocal, of which only five showed well-defined neurites or arborizations. In early-stage-five, as the terminal branches of the receptor axons begin to segregate into protoglomeruli (Malun *et al.*, 1994; Oland and Tolbert, 1995), uPNs belonging to the medial cell group (MC) in the dorso-medial region of the lobe begin to send thick main neurites around the coarse neuropil and beneath the surrounding glial
border. The primary neurites of some uPNs from the medial cell group immediately turn and begin to arborize in the dorso-medial region of the lobe just beneath the glial border (Fig. 1, 2). Fig. 3 shows multiple uPNs of the MC arborizing in more dorsal regions of the neuropil. Homberg et al. (1988) classified these uPNs with somata in the MC as type Pla neurons. In some of the preparations, possibly closer to mid-stage-five, as more uPNs enter the lobe, some are seen that extend their primary neurites into deeper regions of the neuropil and further from the glia border surrounding the neuropil (Fig. 3, 4). When the developing arbors of simple uPNs at this stage were seen, they were sparsely branching, and their arbors were not fully oriented toward the glial border around the neuropil.

**Mid Stage Five**

Eight mid-stage-five ALs had dye labeling adequate for confocal imaging, of which four showed enough detail for analyses of uPN morphological development. By mid-stage-five, uPNs with longer primary neurites have reached more distal regions (towards the equator) of the lobe (Fig. 5, 6). Glia begin to migrate inward into the neuropil, around the apices of protoglomeruli. In this stage and in later stages, bundles of four to eight uPN primary neurites can be seen that travel through the coarse neuropil towards the more distal protoglomerular areas (Fig. 7, 8). uPNs from the MC arborize in the peripheral regions of the lobe and at least one specimen (Fig. 9), suggested that some PNs from the MC may innervate more than one protoglomerulus. Fascicles of uPN processes are tightly bundled as they enter the lobe through the glial border from the MC in the primary neurite tract (Fig. 10). In these mid-stage-five lobes, uPNs do not begin to
branch until they arrive near the glial border and begin to branch beneath the ORN axon layer. The ORN axons at this time are forming protoglomeruli; these appear in the figures (Fig. 6) as dark regions just under the glial border, but actually are filled with the axons of ORNs (Malun, et al., 1994; Oland and Tolbert, 1987). The protoglomerular zones between the uPN dendritic tufts and the glial border at the apices of the protoglomeruli have a few filopodia of uPNs extending towards the glial borders, whereas most of the developing arbor appears as a dense fringe of processes further from the glial border. Along the fringes of these uPN arborizations, numerous growth cones can be seen (Fig. 7, 8).

Late Stage Five

Eight ALs of late-stage-five pupae were labeled and imaged, four of which could be used for uPN morphological comparisons. By late stage-five, many of the uPNs of the MC have reached their protoglomerular sites (Fig. 11a) and are sending dense fringes of fine processes into the protoglomeruli (Fig. 11b). Filopodia extend towards the glial border at the apex of each protoglomerulus. Along the sides of the protoglomeruli and at the apices, a few fine dendritic processes extend along but not among glial processes (Fig. 12a, 12b) that are forming a border around the developing protoglomeruli, suggesting that the dendritic filopodia are confined to the glomerular neuropil by the glial cells. The protoglomeruli in late-stage-five ALs are more elongated (elliptical) than in mid-stage-five ALs, and the dark region at the apices that is filled with axons of ORNs (zone between uPN arbors and glial border) is larger. Growth cones can be seen close to the glial border (Fig. 12c, 13). While it is difficult to establish the actual numbers of MC
uPNs that enter each glomerulus given that the goal was to label individual uPNs, from many preparations with multiple uPNs labeled, the stout processes of from four to seven uPNs were typically seen entering a glomerulus (Fig. 14).

**Stage Six**

Figures 15-23 show the characteristics of MC uPNs in stage-six of development. Sixteen ALs of stage-six brains had dye labeling of MC neurons, nine of which could be used for analyses. By stage-six, as LN dendrites enter the developing glomeruli and the glomeruli begin to stabilize, the primary neurites of several uPNs have reached and entered the base of each developing glomerulus (Fig. 15). A pattern of dendritic branching is repeatedly seen in uPNs from stage-six on, where the primary neurite branches at the base of the developing glomerulus into a few intermediate processes, and at the distal ends, these intermediate processes branch into dense, tufted arbors, with a fringe of filopodia or very fine branches extending toward the apex of the developing glomerulus (Fig. 15, 17-18, 21-22). There seems to be noticeably fewer growth cones at the distal dendritic tips of uPNs. By mid-stage-six, glia have almost completely ensheathed the developing glomeruli.

Fig. 19 shows an image from a preparation in which several anterior cluster (AC) neurons were labeled. Like the neurons belonging to the MC, AC neurons are uPNs (Selchow, 1998). Their dendritic morphology very closely resembles that of the MC uPNs with several thick (up to 4-5 m in diameter) branches entering the base of a glomerulus, dividing into several orders of still rather thick branches, and finally forming a dense tuft of branches in the apex of their glomerulus.
Stage Seven

Twelve ALs of stage-seven pupae had dye labeling of MCs in which morphological features of uPNs could be distinguished, in seven of which the finer detail of dendritic arbors and filopodia could be seen. By stage seven, as glomeruli have stabilized, uPN dendritic arbors have completely filled the well-defined glomeruli (Fig. 24-30), except for the most apical part where fascicles of ORN axons are entering from the nerve layer that lies just outside the glomerular layer. There are two characteristic forms of uPNs that are repeatedly seen: (1) a more common, densely tufted form (Fig. 24-29b); and (2) a loosely branching form (Fig. 30). Some of the distal branches are still tipped with growth cones (Fig. 26, 27), but visually fewer than at stages 5 and 6.

uPNs in Deafferented Antennal Lobes

In only three ALs (stage-seven) were uPNs labeled in deantennated animals, one having multiple fills. In the chronic absence of ORNs, the AL neuropil is smaller, there are no glomeruli, and glial cell bodies remain in a thick rind around the neuropil (Hildebrand et al., 1979; Oland and Tolbert, 1987). uPNs in these few examples (Fig. 31-32) had very thick neurites; in one case large branches radiated in different directions, looking completely unlike normal uPNs in their fundamental branching structure as well as the territory they serve. Neurites in the fine-textured neuropil of the lobe lack the characteristic tufting. Their primary neurites were 8-10 μm in diameter. In the preparation with multiple fills (Fig. 33a-33c), regardless of whether the neurons were uPNs, LNs or multiglomerular PNs, dendritic organization was clearly disrupted. Branches of these neurons turned in multiple directions, sometimes turned away from the
glial border of the lobe, and had no directional orientation towards the glial border as in normal stage-seven animals. Nor was there evidence of any clustering of dendritic processes from several neurons into groups, suggesting that in the absence of ORN axons, AL neurons do not position themselves at precise positions in the developing neuropil.

Figure 34 provides a summary of the changes in uPN morphology during the early and middles stages of ORN axon ingrowth and glomerulus development, and differences in uPN morphology in deantennated ALs. uPNs were extracted in Adobe Photoshop and rotated for uniform orientation for comparison.
Figure 1. An early-stage-five antennal lobe (AL) (52 optical sections) showing the characteristic thick main neurite (arrowheads) of a uPN and typical branching pattern near the glial border of the antennal lobe neuropil. In this and all subsequent figures, glial cell nuclei are green.

Figure 2. An early-stage-five AL (31 optical sections) showing the first arriving uPNs turn and begin branching towards the glial border. As in Fig. 1, uPNs have thick processes (arrows) and seem to be growing near the edge of the neuropil. Arrowhead points to the MC primary neurite tract.
Figure 3. An early-stage-five AL (nine optical sections) showing that the general pattern of uPN growth from the medial cell cluster (MC) seems to progress around the developing neuropil, and just beneath the glial border.

Figure 4a. An early-stage-five AL (13 optical sections) showing some filopodia (white arrows) of uPNs may be extending into the glial border. The uPN is sparsely branching and not yet oriented perpendicular to the glial border, as shown below in figure 4b.
Figure 5. A mid-stage-five AL (39 optical sections) shows up to four uPNs (arrowheads) branching in a very tight protoglomerular area (dotted outline). The white arrow points to another uPN arborization growing laterally and ventrally from its primary neurite.

Figure 6. A different stack of the same AL as in Fig. 5 above (62 optical sections) shows a uPN branching beginning beneath the ORN axon layer. This uPN’s arbor is relatively wide, but it was not possible to determine whether its arbor subtends one or two axonal protoglomeruli. Dotted line shows territory of ORN axons in protoglomerulus.
Figure 7. This mid-stage-five AL (84 optical sections) shows that uPNs originating from the medial cluster arborize in the dorsal regions of the antennal lobe neuropil, perhaps slightly more ventrally in the lateral half of the neuropil. Numerous growth cones (arrows) are visible along the apical fringe of the arbors.

Figure 8. This mid-stage-five AL (62 optical sections) shows the filopodia growing towards the glial border. Filopodia that look like they are extending into the glial border are deeper in section’s stacks and do not actually extend into the glial border.
Figure 9. This mid-stage-five AL (50 optical sections) shows uPNs branching in three distinct protoglomeruli (*). One has a very large neurite (upper left, arrowhead) with tufted distal arborization. Another uPN has an arbor whose branches (arrows) span greater than the width of one protoglomerulus.

Figure 10. Fascicles of uPN processes (arrow) travel tightly banded together as they enter the neuropil of this mid-stage-five AL (21 optical sections).
Figure 11. This late-stage-five AL (20 optical sections) shows dense fringes of uPN filopodia (arrows) extending into protoglomeruli (rough boundaries indicated by dashed lines).

Figure 12a. In this late-stage-five AL (25 optical sections), the lower left protoglomerular territory invaded by at least four to five uPNs (arrow). Some filopodia (arrowheads) extend to the developing glial border and grow along the curvature of the border or along glial processes. Overlap of dendritic processes with glia at asterisks is a result of stacking optical sections.
Figure 12b. Sixteen optical sections of the same lobe in Fig. 12a above shows the glia border forming around the protoglomeruli. Growth cones (arrows) are visible and some filopodia extend close to the glia border.

Figure 12c. In another region (16 optical sections) of the same AL as in Fig. 12a and 12b above, filopodia extend into the protoglomerulus. The upper left inset is an enlargement (two optical sections) of same filopodium (arrows) growing just inside the glial border.
Figure 13. A stack of sixteen optical sections from a late-stage-five AL (Fig 13) shows a filopodium (arrow) growing very near a glial process.

Figure 14. This late-stage-five lobe (16 optical sections) shows a very large number of uPNs entering into a protoglomerulus (rough boundary shown by dashed line).
Figure 15. This micrograph of an early-stage-six AL (41 optical sections) shows tight fasciculation of primary neurites at the base of a protoglomerulus (arrow).

Figure 16. Dendritic processes closely tract the glia border (arrow) in this early-stage-six lobe (15 optical sections). Upper left inset is a magnification of the region described.
Figure 17a. This mid-stage six brain (52 optical sections) shows a PIa uPN morphology. Fewer growth cones are visible than in earlier stages. See extracted uPN in Figure 17b below.

Figure 17b

Figure 18. This micrograph of a stage-six lobe (13 optical sections) shows both ORNs (large arrow) and uPNs (arrowhead). ORNs have relatively simple terminal arbors with only a few branches. The uPNs show densely branched distal arbor. Small arrows show direction of branching.
Figure 19. Fortuitous labeling of anterior cluster (*) uPNs (arrows) at stage-six (19 optical sections). They have branching patterns similar to uPNs originating from the medial cluster. ORN axons (arrowhead) enter the apex and branches enter the base of the developing glomerulus at bottom left. Note change in orientation. Figures 19-21 show horizontal sections through the AL.

Figure 20. A stage-six uPN (11 optical sections) with very tightly arborizing dendritic processes. A few processes extend beyond the main body of the distal arbor toward the glia border at the apex of the developing glomerulus. A few of these are tipped with growth cones (arrows). Processes from a lightly labeled neuron cross the 2nd order branches of the predominant neuron (arrowheads).
Figure 21. These stage-six cells (19 optical sections) have an intermediate region of their arbor in which the number of branches is greater, but the branches are still relatively thick, and an apical portion with many very fine branches that form a web of processes. These neurons’ branching patterns are very different from the one in Fig. 20 above and may be uPNs originating from the anterior cluster (AC).

Figure 22. Stage-six AL (35 optical sections) showing a portion of at least one uPN in the developing glomerulus (roughly outlined with dashed line). The arrows point to examples of growth cones.
Figure 23. Stage-six AL (46 optical sections) showing branching pattern of uPNs similar to those in Fig. 21 above. Notice that there are fewer growth cones (arrows) than in earlier stages.

Figure 24. A stage-seven AL (35 optical sections) with at least two uPNs arborizing in the glomerulus. Arborization is similar to that seen in Fig. 21 and 23 above.
Figure 25a. A stage-seven (26 optical sections) uPN has densely tufted arborization.

Figure 25b. Expanded view of dendritic arbor in Fig. 25a shows filopodia at glial border (arrows), some appearing to turn away from the border or travel along it, and some extending toward the glia border.

Figure 26. A stage-seven AL (10 optical sections) with multiple uPNs filled. The arbor in each glomerulus has dense fine branching at the apex of the glomerulus. The arrows point to examples of growth cones.
Figure 27. A stage-seven AL (one optical section) showing higher-order branching patterns of uPNs.

Figure 28a. A stage-seven AL (40 optical sections) has filopodia turning at edge of glial border (arrows) and either turning slightly, or at least not entering the border. Figures 28b and 28c below are 10 optical sections of same stack showing filopodia at glial borders (arrows).
Figure 29a. A deeper view of the same lobe in Fig. 28a above (43 optical sections) shows two glomeruli with dashed lines roughly outlining their borders. The lower left glomerulus has a very tight arborization of uPN processes, while the upper right glomerulus has a much wider arborization of uPN processes. The lower arbor is viewed in an oblique plane.

Figure 29b. Short stack of 20 optical sections of upper arbor in Fig. 29a shows finer branching of arbor than 20 optical sections of upper arbor in Fig. 30 below.

Figure 30. A stage-seven AL (20 optical sections) shows two very different uPN arborizations within these two glomeruli. Notice several small expansions (arrows) in the branches.
Figure 31. A stage-seven uPN in deantennated AL (98 optical sections). Primary neurite (arrow) is very thick and its branches radiate out in several directions. Low power view in inset shows glial cell bodies remain in a ring surrounding the neuropil.

Figure 32. A stage-seven uPN in deantennated AL (45 optical sections). Primary neurite (arrow) is very thick as is in Fig. 31 above.
Figure 33a. Stage-seven neurons in deantennated AL (30 optical sections). Multiple labeling of different types of neurons, including possible neurons from the lateral cluster, prevents interpretation regarding uPN morphology, but many of the fine branches do not show the typical, apically-oriented morphology of normal AL neurons.

Figure 33a

Figure 33b. Deeper view of same neurons in Figure 33a above (30 optical sections). Primary neurites are thicker than in normal animals and terminal branches are not apically-oriented. Many fine branches (arrows) are coming off of primary neurites in the coarse neuropil.

Figure 33b
Figure 33c. Multiple labeling of neurons in same lobe (medial-dorsal region) as in Fig. 33a and Fig. 33b above shows atypical morphology of neuronal dendritic tufts and no evidence of glomerular territory.
Figure 34. Normal uPN morphological development from early stage five to stage seven, and uPN development in the absence of ORNs at stages seven to eight. uPNs extracted in Adobe Photoshop from micrographs. Right uPN extraction of stage six includes at least two uPNs.
In this paper I have examined the development of the morphological features of uniglomerular projection neurons (uPNs) from the medial cluster of neurons in the antennal lobe (AL) of Manduca sexta during the early stages of antennal lobe development, stages early five to seven of metamorphic development. In addition, I have examined uPNs in ALs developing in the chronic absence of olfactory sensory neurons. Although labeling in deantennated antennal lobes provided very few specimens for analysis, the data from these preparations suggest that ORNs do affect uPN morphology and/or organization.

Various methods were attempted for the labeling of uPNs prior to those described in Methods. Labeling with Lucifer Yellow using microelectrodes was unsuccessful in these early stages. The impalements of these young neurites were too unstable to obtain complete dye fills. Pressure injection of Dextran Texas Red into the medial cell cluster (MC) provided too few labeled specimens for the number of animals and the time involved. Even using the methods described above, poor success was achieved using DiI in early stage-5 and deantennated animals, suggesting that phospholipid membranes of neurons at these stages or conditions may have slightly different chemistry than in normal, older neurons. Greater success was achieved in early-stage-5 and deantennated brains using DiI (18) than with DiI (16). DiI (16) provided greater success in labeling of normal brains from late stage five on. In light of the difficulties encountered with the
various techniques attempted other than DiI, it was decided to focus on use of DiI to study uPN development.

*Normal uPN Development*

From previous studies (Malun *et al.*, 1994), it was clear that uPNs of the MC begin adult metamorphic development with little or no dendritic arborization in the AL neuropil. As the terminal branches of the receptor axons begin to segregate into protoglomeruli during stage five, uPNs of the MC almost immediately begin to send dendrites into the protoglomeruli (Malun *et al.*, 1994). Meanwhile, ORN axons have entered the AL and have formed a nerve layer within and just under the glial border (Boeckh and Tolbert, 1993; Oland *et al.*, 1996). As uPN neurites enter the neuropil via the MC primary neurite tract and they first invade the dorso-medial region of the AL (early-stage-five), their processes typically grow close to the glial border that surrounds the neuropil. By mid stage five, longer primary neurites of uPNs travel in bundles, either around the neuropil just beneath the glia border, or more deeply through the coarse neuropil toward more distal target regions near the dorsal-ventral equator of the AL. uPNs from the MC arborize in the AL more dorsolaterally than dorsomedially, while the ventral and medial regions of the AL are known to be invaded by uPNs from the AC and the LC (Homberg *et al.*, 1988).

The current study shows that by early stage five, the primary neurites of some uPNs have turned outward toward the glial border and developing nerve layer and have begun to form higher order branches. As a group, their branches combine to form a fringe of processes just under the glial border and nerve layer.
In all cases except one, developing MC uPNs invaded a single developing glomerulus. In that one case a uPN can be seen that may be extending branches into more than one protoglomerulus, suggesting that some neurons of the MC may be multiglomerular. Another possibility is, as in opposum and rat mitral cells (Malun and Brunjes, 1996), some of the PNs begin with processes extending to more than one glomerulus, but prune their branches to leave each uPN arbor investing just one glomerular territory in the mature olfactory lobe. Numerous growth cones can be seen along the apical fringes of the dendritic arbors at mid stage five. By late stage five, as glia begin to migrate inward from the apices of protoglomeruli, uPNs of the MC already have tufted arbors extending from several branches off their primary neurites. Numerous growth cones are present and filopodia extending towards the apices of the protoglomeruli can be seen growing just beneath glia borders.

The borders of developing glomeruli are well defined by glial cells by stage 6 and uPNs have well-developed branching patterns that closely resemble those of mature uPNs of the MC. Intermediate branches stem from the primary neurite at the base of the developing glomerulus, and higher-order, tufted branching forms a dense arbor near its apex. Fewer growth cones are seen on the dendritic arbors of uPNs at stage six. The AL glomeruli are stabilized by stage seven (Bauman et al., 1996), and the uPN dendritic arbors of each glomerulus extend laterally to the glial borders of the glomeruli. Expansions on some uPN fine processes can be seen near the apices of the glomeruli, possibly where synapses with ORNs will occur.
Two different morphologies of MC uPNs are seen in these preparations, suggesting different subtypes of MC uPNs. One type of uPN has a tight, tufted higher-order arborization, (Fig. 28-29b). The other type of uPN morphology seen, though infrequently, has a loosely branching arbor with slightly longer intermediate branches (Fig. 30). Possibly a third subtype of uPN can be distinguished from the other two. This type occupies narrow, elongated glomeruli, has longer intermediate branches that grow more tightly together and more parallel to each other, and has an extremely dense higher-order branching pattern at the apex of the glomerulus (Fig. 25a). In all cases the uPN arbors fill the width of the glomerulus.

The small number of individually labeled cells, coupled with the difficulty of obtaining the same plane of orientation through glomeruli with labeled uPNs, and the fact that development across the lobe is not synchronous, makes it difficult to ascertain that these are truly different subtypes. In *Periplaneta* (Nishino et al., 2003) and in *Drosophila* (Jefferis et al., 2002, 2007; Marin et al., 2002; Ramaekers; et al., 2005) different dendritic morphologies of PNs correlated to their target areas in the protocerebrum. My study did not include analysis of the axonal arbors in the protocerebrum and thus I cannot use this as an additional criterion of subtype in *Manduca*.

*Role of Glial Cells in the Development of uPN Dendritic Arbors*

In principle, the glial cells that stabilize the protoglomeruli by enveloping them (Bauman et al., 1996) could constrain the ingrowing dendritic processes. Malun et al. (1994) found uPN processes extending into the glial border around protoglomeruli, and
electron microscopy revealed close apposition between uPN dendrites and neuropil glia. Analyses of single optical sections of stacks collected with the confocal microscope in this study showed uPN filopodia growing very close to the glial border, but never into the border. In several specimens uPN filopodia turned and extended just beneath the glial border or even turned back towards the center of the developing glomeruli. Whether filopodia actually came in contact with glial processes could not be determined with certainty since glial cells were labeled with a nucleic acid stain that only faintly reveals glial processes. Interestingly, however, Krull et al. (1994) found that in culture, tenascin-like molecules associated with glia that form distinct borders around glomeruli may be barriers to neurite growth in Manduca. In Drosophila, N-cadherin, though not expressed on glia, is a cell adhesion molecule expressed in all PN classes and was found to be essential for PNs to restrict their dendrites to single glomeruli (Zhu and Luo, 2004). It is likely that the expression of several cell molecular components, some of which are expressed by glial cells, help to delineate and restrict uPN dendritic arbors within Manduca glomeruli.

The glial envelopes also could form a physical barrier that keeps uPN dendrites restricted to the territory of a single glomerulus. In Xenopus laevis tadpoles, neither glial cells nor glial processes form glomerular borders in the olfactory bulb, and glomerular architecture is solely determined by the tufted terminal branches of ORN axons and mitral/tufted cells (Nezlin et al., 2003). In Drosophila, Jefferis et al. (2003) and Oland et al. (2008) did not find significant glial process invasion of the developing AL neuropil prior to PN dendritic patterning, limiting the possible role of glia in affecting...
development of typically tufted uPN arbors. In *Manduca*, however, the glial processes
are present at the relevant time. My limited results suggest that even as the glial
envelope is forming, and thus unlikely to form a surrounding physical barrier, uPN
dendritic processes still do not cross glial processes to grow toward the neuropil of
adjacent developing glomeruli. This suggests that the uPNs are responding either to a
molecular barrier, as described above, or to the ORNs, which serve as an attractive target.

**uPN Morphology in Deantennated Antennal Lobes**

Only two specimens had DiI labeling in which individual uPN neurites could be
discerned. In both of these, the primary neurites enter the neuropil and grow towards its
periphery in much the same pattern as normal uPNs. However, in both specimens, the
primary and intermediate branches are very thick in comparison to normal uPNs, and the
thicker-than-normal secondary branches radiate in various directions, not uniformly
oriented towards what would be the region of roughly a single glomerulus in normal ALs.
These secondary branches still grow, however, towards the periphery of the neuropil.
One specimen, in which multiple neurons were labeled, had what looked like
intermediate branches of uPNs growing very near the glial border surrounding the
neuropil. The dendritic tufts were not as finely branched as in normal uPNs, and
processes radiated in various directions off of the thicker neurites and showed no
evidence of glomerular territories. In addition, the multiple fills revealed disorganized
dendrites, especially at the periphery where some branches turned back toward the center
of the neuropil. Malun *et al.* (1994) observed uPNs in deantennated lobes to have the
basic arbor of normal uPNs, but with dendritic tufts less distinct and more sparsely
branched than in normal lobes, suggesting that there is only fine tuning of uPN dendritic branches by ORNs. Similarly, in previous studies (Hayashi and Hildebrand, 1990; Oland and Hayashi, 1993), several types of projection neurons grown in vitro could be distinguished by their distinctive morphology, which included a small, branched arborization near the cell body and a long, mostly unbranched process presumed to be the axon. Taken together, these results suggest that since ORN axons are not present in deantennated preparations, and since neuropil glia remain in a rind surrounding the neuropil in these preparations, uPNs have a cell-autonomous program guiding development at least up to the branching of the primary neurite into intermediate branches, and that neuropil glia and ORNs provide signals for subsequent dendritic refinement.

As previously mentioned, in the absence of ORNs, glia do not migrate into the neuropil and glomeruli fail to form, and local interneurons (LNs) fail to form glomerular dendritic tufts (Oland and Tolbert, 1987). Yet, when Na⁺-dependent activity is blocked in ORNs with tetrodotoxin (TTX), the architecture of the AL glomeruli developed as in normal animals (Oland et al., 1996). When the medial cluster (MC), containing somata of uPNs, was removed prior to AL development, the architecture of the AL glomeruli also developed as in normal animals (Oland and Tolbert, 1998), at least at a gross level. The role that LNs play in glomerulus formation in Manduca needs further study, but as their growth into the protoglomeruli follows that of the uPNs by about a day, they could only be involved in the fine-tuning of the AL glomerular architecture. Thus the presence of ORNs, but not their activity, and the clustering of their terminal branches into,
perhaps, an attractive target, seems to set up the basic protoglomerular template, as previously suggested by Oland et al. (1998) and Rossler, et al (1999). With the data collected here, I cannot resolve more specific effects of ORNs on particular aspects of dendritic development.

Comparison with PN Development in Other Systems

The development of the AL in Manduca seems most similar in process to that in mice (Blanchart et al., 2006), opposums and rats (Malun and Brunjes, 1996) where mitral cells enter the protoglomeruli almost immediately after the ORN axons turn into the neuropil from the nerve layer. Furthermore, in both mice and in Manduca, there is a wave of development across the bulb/lobe in which the most advanced development is found in the regions that the ingrowing ORN axons first encounter; as the axons penetrate further into the bulb/lobe, these regions begin to develop (Malun et al., 1994, Blanchert et al., 2006). In both mice (Blanchert et al., 2006) and Manduca (Malun et al., 1994; this study), the PNs grow first more or less tangentially to the developing nerve layer and then turn radially toward the nerve layer and begin to form their tufted arbors. Also like in mice, where axons of the mitral cells have extended into the lateral olfactory tract to higher order centers before they build their glomerular arbors (Blanchert et al., 2006), in Manduca, the MC uPN axons projecting to the protocerebrum are also present before the adult dendritic arbors form (Malun et al., 1994). In mice (Jimenez et al., 2000) and in Manduca (Malun et al., 1994; this study), PNs show some intrinsic basic branching patterns, but the higher-order branching pattern and possibly the orientation of dendritic
tufts seems to be extrinsically determined. In contrast, *Drosophila* (Jefferis *et al*., 2004) PNs create a prototypic glomerular map prior to the arrival of ORN axons.

**Considerations for Continuation of Study**

Although the success rate of MC uPN labeling was low with the various methods attempted for the time and resources invested, the knowledge acquired about the anatomical, and to some degree, the physiological changes that occur during the early development of the *Manduca* antennal lobes will be useful for future studies of these cells. Knowledge of the size and location of the medial cell group as it changes during AL development with respect to orientation of the brain as a whole is paramount to preparing brains for successful dye fills. Different fluorescent dyes must be used at various stages of development, as do the amounts applied. Optimal incubation times also change with developmental stages.

With this background experience in hand, and using some of the newer technologies developed for 3-D reconstructions, further research into the different morphologies of uPNs would include obtaining more individually labeled specimens at the various stages, paying attention to their axonal arbors in the protocerebrum, using electron microscopy to establish the sequence of synaptogenesis between (1) ORNS and uPNs and (2) uPNs and LNs, and finally, completing a much more detailed study of individually filled uPNs that have developed in the chronic absence of ORN axons.
References


SECTION TWO

General Biology Program for Teachers:

Problem Based Learning (PBL) Units
Master’s Program in General Biology

Major goals of the Master’s Program in General Biology for teachers are: (1) to involve teachers in a research experience so that they can take that experience into their classrooms to improve instruction in scientific research methodology, (2) to develop problem-based learning (PBL) or inquiry-based science units that engage students in the scientific processes, and (3) update science teachers on recent developments and technologies in biology.

Educational research abounds with data that student learning and performance increases with hands-on learning. In science education, Inquiry Learning (IL) and Problem Based Learning (PBL) have been shown to increase higher order thinking skills and concept comprehension in students (American Association for the Advancement of Science, 1993; National Research Council, 1996). Student comprehension of concepts and development of skills is increased when students practice science rather than just learn about the science that researchers do. Therefore, much of the focus of the General Biology Program for Teachers is to have teachers develop PBL or IL units to use in their classrooms.

During the coursework of the program, I developed three PBL units for use in my own 7th and 8th grade science classes. The first unit presented, Effects of Antennal Removal in Manduca sexta, is based on The Manduca Project, and is designed to expose students in my 7th grade classes to the field of Neurobiology. I use it as an introduction to a unit on the human nervous system. The next unit presented, Transmissible Diseases,
was designed as a backwards approach to introduce 7th graders to cell biology and basic genetics. It uses news headlines of the most recent outbreaks to capture student interest. The third unit, *Water in Arizona*, was designed for my 8th grade classes to not only educate students on the issues of water quantity and quality, but also to engage students in more rigorous literature research using internet resources.

Each PBL unit described below has all student handouts and articles included in the appendices, *Effects of Antennal Removal in Manduca sexta* (Appendix A), *Transmissible Diseases* (Appendix B), and *Water in Arizona* (Appendix C).
Effects of Antennal Removal in *Manduca sexta* PBL

**Goals**

1. Students will become familiar with dilutions and concentrations measured in parts per million (ppm).
2. Students will gain experience in surgical (dissection) techniques using humane methods to reduce amount of injury to test animals.
3. Students will gain an understanding of the functions of the antennae.
4. Students will improve their skills in science methodology.

**Student Objectives.** The student will be able to:

1. Identify the following anatomical structures in *M. sexta* pupae: eyes, legs, antennae, proboscis, wings, head, thorax and abdomen.
2. Determine sex of pupae.
3. Surgically remove parts of antennae in pupae.
4. Calculate and mix different concentrations of plant leaf extract in parts per million (ppm).
5. Collect and analyze behavioral data on adult *M. sexta*.
6. Evaluate experimental methods and make changes to improve experimental design.
7. Design an experiment that further tests the functions of antennae in *M. sexta*. 
Materials List (for each group)

1. Moth box with moon lamp, daylight lamp, and timer (see procedures for construction in *The Manduca Project*). Necessary for development and observation of pupae and adults.

2. Materials for Surgeries or Dissections:
   a. Dissecting microscope or magnifying lens
   b. Dissection scissors, forceps (tweezers) and scalpel (Exacto knives with sharp, pointed blades work better)
   c. Metal chemistry spatula (or flat metal blade)
   d. Candle and matches
   e. Crushed ice and plastic tub
   f. Stage 1 *Manduca sexta* pupae (at least two per student)

3. Materials for Testing Sensory Ability of Antenna:
   a. Tomato plants
   b. Mortar and pestle
   c. Graduated cylinder
   d. 4 beakers or cups
   e. Masking tape
   f. Refrigerator
   g. Filter paper (or stiff, porous fiber art paper)
   h. Clay
Prior to Unit

Check materials list and determine teams. Moth boxes should be made prior to these activities. Check The Manduca Project CD or website (http://insected.arizona.edu/Manduca/) for instructions on building a rearing box. Make copies of student handouts (Appendix A).

Day 1

Pass out the handout titled Basic Anatomy of the Manduca Pupa and Adult. As a class, complete Activity 1 (15 minutes). Identify and label the following parts on the diagrams of both the pupae and the adult: eyes, legs, antennae, proboscis, wings, head, thorax and abdomen. Have students complete Activity 2 (15 minutes) individually. Students fill in characteristics that are similar and characteristics that are different between the pupa and the adult. Then, have students discuss and share their observations with the class for Activity 3 (15 minutes). Pass out the handout titled What Do The Antennae Do? for the students to work on for homework.

Day 2

Go over the homework assignment What Do The Antennae Do? with the students in class. Make sure all students have the correct definitions or responses for questions 1, 2 and 5. Ask for some examples of how the students would test for olfaction ability.
Then explain to the students that they are going to do a couple of experiments on olfaction.

Pass out the handout titled *Effects of Partial Deantennation on Olfaction in Manduca sexta*. Read through the overview, student objectives and experimental design with the class (10 minutes). Next, divide the students into their teams (2-4 as materials and space allow) and have the teams work on *Activity 1* (20 minutes). While teams are working on *Activity 1*, pass out the handout titled *Deantennation Procedures*. The teams should complete *Activity 2* (20 minutes) by the end of the class period.

*Day 3*

Briefly review procedures for deantennation with the class (5 minutes). Then have students work in their teams to complete their surgeries. When the teams have completed their surgeries, have them place their pupae into their moth boxes. This will require most of the remaining class time. Then pass out the handout titled *Tomato Leaf Extract Procedures*. Students need to read through these procedures for homework, and be able to work in their teams the next day to prepare the different concentrations.

*Day 4*

Go through the procedures for making different concentrations of tomato leaf extracts with the class and make sure that the students did the calculations correctly.

*Procedure 2*: 10% means 10 parts per 100 total mixture (10 + 90 = 100 total).

\[
1 \text{ mL/10 mL} = 100,000 \text{ ppm.}
\]

*Procedure 3*: Add 100 mL water to dilute 1 mL of 100,000 ppm solution to 1000 ppm solution.
**Procedure 4:** Add 100 mL water to 1 mL 1000 ppm solution to make 10 ppm solution.

**Procedure 5:** Add 10 mL water to 1 mL 10ppm solution to make 1 ppm solution.

Have the teams mix and label their solutions. The solutions need to be covered and refrigerated until they are used. Other containers are more suitable for storage if they are available (plastic ware with seals).

**Day 5**

Pass out the handout titled *Artificial Tomato Plant Procedures* and the accompanying *Artificial Tomato Leaf* template. Have the teams construct their artificial tomato plants and place them into their boxes without the leaves (45 minutes). Next, pass out the handout titled *Testing Moth Antennae*. Individual students need to read through this handout and come up with methods for measuring and recording test data (remaining class time and homework).

**Day 6**

The class must make a decision on whether all the teams will use the same methods for measuring and recording test data, or whether the teams will use different methods to see which methods work the best. Allow the teams 15 minutes to work on *Activity 1*. Allow the class the rest of the period to work on *Activity 2*. *This may have to be done quickly if moths are beginning to emerge from their pupae. These procedures should be written and ready to use by the time the moths are flying.*
Day 2

When the moths begin to fly around about the 2nd to 3rd day after eclosion, the students will need to test their concentrations within one class period. Have students do their experiments and collect their data.

Day After Experiments

Pass out the handout titled Effects of Partial Deantennation Results. Have students discuss Activity 1 in their teams (10 minutes). Have all the teams discuss Activity 2 in class (30 minutes). Explain and assign Activity 3 for homework (10 minutes).

2nd Day After Experiments

Have each student complete Activity 4 of the handout Effects of Partial Deantennation Results for a final evaluation.
Transmissible Diseases PBL

Focus

Students take part in a mock pet giveaway and get to choose which pets they want. Some of the exotic pets are carriers of a disease. As students share their pets with each other, the disease is spread to different students. Once students learn how pathogens can be transmitted by animals, students role play different positions on a CDC Investigative Team to track down the cause and origin of a new viral epidemic. These activities will give the students a good understanding of how diseases spread, and provide the interest to discover what viruses are, how they reproduce, and why they make us sick. Using viruses as the topic for inquiry, students can then explore genetics and cells in future units.

Audience and Time

These activities are designed to introduce middle school students to the effects of the interactions of different organisms and their roles as pathogens, carriers and hosts. It is designed for students that have had no background in biology. The unit will require four to five 55 minute periods, or three block periods.

Major Concepts

Interactions among different organisms occur everywhere in nature, and the relationships can be quite complex and far-reaching. Most organisms cannot survive independent of others, and many times the benefits to one organism are to the detriment of another. Diseases are an excellent example of these relationships. Pathogens require hosts to reproduce, resulting in damage or death to the host’s cells. Some pathogens need
another organism to transfer them from one host to another. Many times, the carrier is not harmed by the pathogen. Finally, the immune systems of hosts are constantly active seeking out, identifying and destroying pathogens. In an ongoing battle between host cells and pathogens, genetic change is much more the rule than the exception.

Objectives

The student will be able to:

1. Define the terms: pathogen, host, and transmissible disease,
2. Describe the relationships between pathogens and hosts in two diseases that have had recent (within the last year) outbreaks in the United States.
3. Write up a recommendation for regulation of the pet trade.
4. As the member of a CDC Investigative Team, write up a report summarizing you and your team’s conclusions and recommendations for a recent outbreak of a disease.

Arizona State Standards Addressed:

Standard 1 Science as Inquiry

1SC-E1. Identify a question, formulate a hypothesis, control and manipulate variables, devise experiments, predict outcomes, compare and analyze results, and defend conclusions.

PO 3 Analyze results of an experiment

PO 4 Defend conclusions drawn from the analysis

1SC-E4. Identify and refine questions from previous investigations.

PO1 Analyze the results of previous investigations
PO2 Refine a hypothesis from a previous investigation

1SC-E6. Analyze scientific reports from magazines, television and other media.

PO 1 Evaluate information for accuracy, logic, bias and impact

Standard 3 Personal and Social Perspectives in Science and Technology

3SC-E1. Recognize how scientific knowledge, thinking processes and skills are used in a great variety of careers.

PO 1 Explain how scientific knowledge, thinking processes and skills are used to solve problems in a variety of careers

3SC-E2. Develop and use a systematic approach to analyze the risks associated with natural and biological hazards.

PO 1 Analyze the risk factors associated with natural and biological hazards

3SC-E3. Identify a specific need and propose a solution or product that addresses this need, taking into consideration various factors.

PO 1 Design a solution or product that addresses a need and considers the factors of an environmental or human problem

Standard 4 Life Science

4SC-E2. Compare and contrast the basic structures, components and functions of various cells.

PO 1 Analyze the basic structures, components and functions of various cells

PO 2 Differentiate among types of various cells

4SC-E5. Describe changes or constancy in groups of organisms over geologic time.

PO 1 Describe organism adaptations or constancy over geologic time
PO 2 Identify environmental factors that may determine adaptations or constancy of an organism over geologic time

4SC-E6. Describe the role of genes in heredity.

PO 1 Explain the basic principles of heredity and genetics

PO 3 Describe information carried in a gene

4SC-E7. Explain and model the interaction and interdependence of living and non-living components within ecosystems, including the adaptations of plants and animals to their environment.

PO 1 Explain the role of living/non-living components in an ecosystem

Prerequisite Knowledge

This series of activities requires no prior knowledge of disease, and is a lead into the study of transmissible diseases, how they affect organisms and cells, and ultimately into a unit on cells and genetics.

Introduction/Teacher Background Information

Recent stories in the news and media have included the outbreaks of severe acute respiratory syndrome (SARS) and monkeypox. Both of these diseases are viral diseases with vertebrate hosts. Palm civets and badgers in the pet markets in southern China are believed to be the original hosts of the coronavirus that causes SARS. The first cases of SARS occurred in China in February, 2003. In less than six months, SARS spread to 27 countries and infected over 7000 people. The virus is thought to have mutated, and jumped the species barrier into humans. It is spread by droplets of saliva from person to
person. It is not thought to be airborne or waterborne, and quarantine methods have seemed to stop the spread of the epidemic.

Monkeypox is a virus in the pox-family and is very similar to cowpox. It is not a new disease, having been discovered in 1959, and several outbreaks have occurred in Africa over the years. The original hosts were four species of African squirrels. However, it has shown up in monkeys, Gambian rats, prairie dogs, and humans. The first cases to have ever occurred in the United States appeared in early May, 2003. The cases were quickly tracked to prairie dogs from a distributor in Texas. It is believed that the virus was spread to prairie dogs from a shipment of infected Gambian rats.

Both of these diseases bring up issues dealing with the pet trade (animal hosts), a mobile society (transporting infected hosts worldwide), and transmissible diseases (illnesses caused by infectious agents, pathogens, that can be spread from one host to another). With the rapidity that viruses and bacteria can mutate into new forms, transporting and mixing of host species may further increase the rate at which new diseases evolve leading to major worldwide epidemics.

Materials and Preparation

All student handouts and articles are included in Appendix B. You will need to prepare the following materials before conducting these activities:

Activity 1: IMACON.COM

- Copy and laminate a set of the “Pet Cards”. You will need at least one card per student
- Can of “Pam” or other spray cooking oil.
✓ Bottle or can of paprika.
✓ Cleared-off table top or counter
✓ Copies of student handouts.
✓ Copies of the following articles:


_Cute but wild: The perilous lure of exotic pets_ by Mark Derr (2003).

**Activity 2: CDC Investigative Team** [adapted from CDC Trainee Web Quest, created by Time Lawrence, Chris Watford, Valeria Guzman and Lori Bowser (http://www.davidson.k12.nc.us/chandra/biohazard) and from CDC Field Training Course Summer 2003, Secondary Biology Lab Curricula (BIOC 633), by Lisa Elfring.]
✓ Copies of student handouts for CDC Investigative Team
✓ Copies of the following articles:

_The origins of a viral killer_ by Zara Herkovita (2003).

_Houston sees 1st West Nile cases of 2003_ by Todd Ackerman (2003).

_Encephalitis joins west nile as threat_ by Daniel Yee (2003).

_Out of China: SARS virus’ genome hints at independent evolution_ by Ben Harder (2003).

_Worries fill town as W. Nile threatens_ by Carla McClain (2003).
Procedures

1. There is no prior knowledge necessary for you to review with the students for this unit. Begin this unit with Activity 1: IMACON.COM PET STORE. Prior to class lightly spray the backs of the cards of the following animals with “Pam” and lightly dust with paprika: black-tailed prairie dog, suricate, key deer, giant Gambian pouched rat, sugar glider, hedgehog, and civets. Handle these cards carefully and don’t let them touch each other or any other cards (the oil and paprika will smear onto whatever they touch). Lay all cards out onto the tabletop with the pictures facing up. Separate the rest of the cards into the following stacks, dogs, cats and others. Note: Glo-germ can be used with a UV light instead of paprika – so students don’t see the powder until under they hold their hands under UV light.

2. Day 1. Begin the class by telling students to pretend that you have just started a new online pet store business called IMACON.COM PET STORE. For the opening promotional, you are holding an online drawing for visitors to your web page, and the students in the class are some of the lucky winners who were drawn. Each winner gets to select a free pet from your store, but prizes are limited to the overstocked animals (cards) you have available. Pass out to each student a copy of “Activity 1: IMACON.COM PET STORE. Let them know that some of the cards, which represent their animals, have been dusted with paprika to represent a strong tick and flea powder. So, as they pick up
their cards (animals) do not put their hands anywhere near their faces or on their clothes until the activity is over and they have washed their hands.

3. Tell students that you have dogs, cats and a few other unusual pets. Ask students to write down in Part I of their handout three pets they would like to have and to write a short paragraph of why they would like to own their top priority. Allow students five minutes to complete Part I. Then have students raise their hands if they had a dog on their list. To each of these students, hand out one of the dog cards. Repeat this step for cats. For the remaining students, have them come up to the table and pick out a card from those that remain (they must choose before they pick up the card).

4. Once every student has a card, allow the students five minutes to share their animal (card) and its information with their friends. After students return to their seats, have each student press their fingers and thumbs onto the white space in Part II, checking for any tick and flea powder (paprika).

5. Ask all students that had paprika on their paper to raise their hands. Say “Congratulations, you have just been infected with a very harmful pathogen that is carried by one or more of the animals distributed by IMACON.COM. You will be given five minutes to answer the questions in Part III of your handout.”

6. Have students go back to the groups of friends they originally shared their animals with and have them discuss where the disease came from and what the disease may be. Allow five minutes for the groups to discuss. Have
students return to their seats and summarize their group’s discussion in Part IV. Allow three minutes for students to summarize.

7. Have someone from each group summarize for the class what they think the disease may be and what animal(s) it originally came from. Allow 10 minutes for discussion.

8. Pass out to each student a copy of each of the following articles:


For homework, students are to read both articles and answer the questions in Part V on their handout. They should be ready to discuss the articles in the next class.

9. Answers to questions in Part V

a. A coronavirus causes SARS, and the host animals are believed to be palm civets and badgers in southern China pet markets.

b. A virus in the pox family causes monkeypox and the known host animals are Gambian rats and prairie dogs.

c. Monkeypox reached the United States in a shipment of Gambian rats destined for distribution in pet stores. It spread from the Gambian rats into prairie dogs.

d. Answers will vary.

e. Replies will vary.
5. Day 2. Begin the class with a discussion of the homework questions. minutes for discussion.

6. Divide the class into groups of five students per group and assign a commander for each group. Pass out the CDC Investigative Team Packets to each team commander. Have the team commanders read the Introduction for CDC Investigators, and assign a role and corresponding information sheet to each member. Allow 20 minutes for this step.

7. Have students read the article in the packet titled Mysterious Outbreak Sickens Chinatown Congregation. Allow the teams 15 minutes to read and discuss the article. Pass out to each team a copy of each of the following articles:

   The origins of a viral killer by Zara Herkovita (2003).
   Houston sees 1st West Nile cases of 2003 by Todd Ackerman (2003).
   Encephalitis joins west nile as threat by Daniel Yee (2003).
   Out of China: SARS virus’ genome hints at independent evolution by Ben Harder (2003).
   Worries fill town as W. Nile threatens by Carla McClain (2003).

Have teams split up the articles among members and allow the rest of the class period for individuals to do their research. Team members will summarize their articles and share the information with the team. Inform teams that they must have their research completed by the next class period.

13. Day 3. Begin the class with a class discussion of the articles passed out during the last class. Explain the homework assignment to the class. Each
student must write up a report that justifies the student’s conclusions about this disease based on the student’s own research and the team’s research and discussions. Reports should also include a plan of action to bring the disease under control. Allow 15 minutes for discussion.

14. Split the class into their teams and allow the teams 20 minutes to review the findings of the student investigators.

8. Pass out to each team commander a packet containing instructions and patient interviews. Allow the teams the remaining class period to finalize their discussions and conclusions, or to do further research.

9. Day 4. Begin the class with a discussion of each team’s conclusions. Allow 15 minutes for discussion. Collect homework (reports).

10. Pass out the following article:

*Out of China: SARS virus’ genome hints at independent evolution* by Ben Harder (2003).

Have students read the article. Allow 20 minutes. Have the class discuss the following questions (these are questions that are going to be addressed in the next unit):

a. What is a virus?

b. How do viruses reproduce?

c. How and why do viruses make us sick?

d. What is a genome?

e. What is a mutation?
f. What is recombination?

_Potential Extensions_

- Have each student research a specific disease.
- Have students write a predictive scenario where a disease such as monkeypox either mutates or crosses into another host species.

_Student Masters_

**Activity 1: IMACON.COM PET STORE**

Pet Card Masters may be made on 3”x5” index cards with names (and pictures off internet sources) of several different breeds of cats and dogs. Some cards should include exotic animals imported as pets, and definitely include the gamian rat, and prairie dog.

**Activity 2: CDC Investigative Team**

Packet 1 for Day 1:

- Introduction for Trainees
- Mysterious Outbreak Sickens Chinatown Congregation
- Commander, Day One
- Commander/Safety Information
- Disease Specialist
- Disease Information Sheet
- Insect Specialist
- Insect Information Sheet
- Animal Disease Specialist
Animal Information Sheet

Food/Water-borne Disease Specialist

Food/Water-borne Disease Information Sheet

Packet 2 for Day 2:

Commander, Day Two

Patient Interview: The Cho Family

Patient Interview: Mr. Chen

Patient Interview: The Tsing Family

Patient Interview: Margaret Otis

Patient Interview: Rose Ng
Water in Arizona PBL

Arizona State Science Standards Addressed:

Strand 1: Inquiry Process

Concept 1 (PO 1-3), Concept 2 (PO 1-5), Concept 3 (PO 1-7) and Concept 4 (PO 1-5).

Strand 2: History and Nature of Science

Concept 1 (PO 1-4) and Concept 2 (PO 1-3)

Strand 3: Science in Personal and Social Perspectives

Concept 1 (PO 1-3) and Concept 2 (PO 1-4)

Strand 4: Life Science

Concept 3 (PO 1-6)

Student Objectives.

1. Water as a Transporter (students are to explore the question “how has water been used as a transporter historically, geographically, mechanically and chemically?”). This addresses Strand 2 (History and Nature of Science) and Strand 3 (Science in Personal and Social Perspectives).

   Assessment: Group presentation of a significant historical use of water for transportation purposes by mankind.

2. Water Use (students explore the various ways in which water is used within their daily routines and within their community). This addresses Strand 1 (Inquiry Process) and Strand 2 (Science in Personal and Social Perspectives) and Strand 3 (Science in Personal and Social Perspectives).
Assessment: Students are to summarize quantitative and qualitative data collected on water use. Review and Test.

3. *Quantity of Water* (students explore the question “how much water is available?”) This addresses Strand 1 (Inquiry Process), Strand 2 (History and Nature of Science) and Strand 3 (Science in Personal and Social Perspectives). Students will learn how to use Geographic Information Systems (GIS) software and Global Positioning Systems (GPS) for collecting and analyzing data.
Assessment: Group presentation of water resources globally, regionally and locally. Review and Test.

4. *Quality of Water* (students explore the questions “how clean is our water and how clean does water need to be?”). This addresses Strand 1 (Inquiry Process), Strand 2 (History and Nature of Science), Strand 3 (Personal and Social Perspectives) and Strand 4 (Life Science).
Assessment: Lab report on water quality testing, Review and Test.

5. *Water in the Future* (students research and develop predictions for future water quantity and quality, and future needs). This addresses Strand 1 (Inquiry Process), Strand 2 (History and Nature of Science), Strand 3 (Personal and Social Perspectives) and Strand 4 (Life Science).
Assessment: Group presentations of societal management and use of water resources in the year 2100, speculating on new technologies.
Teacher Resources and Background Information

The following documents that are included (Appendix C) contain information that is covered in this unit, student handouts, articles, and websites where additional information may be found. Students should be given a list of the websites prior to the start of this unit to aid them in their research. It is recommended that the teacher review the documents and the websites prior to starting this unit to become familiar with the information and to become updated on current research.

Water Use and Availability Key

Newspaper Article Excerpts

Clean Water Act & EPA

Biological Contaminants

Water Use Websites

Section 1: Water As A Transporter

Student Objectives. The student will be able to:

1. Describe three historical events that relied on water as a means of transporting people or goods.

2. Identify three different geographic locations and describe how and when water is used as a means of transportation with respect to the limitations of water resources within each region.

3. Describe two ways in which water is used to transport materials mechanically.

4. Describe one way in which water is used to transport materials chemically.
Arizona State Science Standards Addressed: Grade 7, S1 (all concepts), S2C1(PO1-PO4), S2C1(PO1-PO3), S3C1(PO1).

Day 1: Class discussion on uses of water as a transporter.

Make sure each student has notebook paper and a writing instrument to write down information from class discussion. Inform students not to write anything down until told to do so. 5 minutes.

Ask students the first question: “What major events do you know of in mankind’s history when the use of water to transport people or goods was a major factor in shaping the future of civilization?” Write this question on the board and have students write the question on their papers. Call on students as they come up with examples and write their examples on the board beneath the question. Students should not write down any responses to the question yet. 15 minutes.

When students run out of examples, ask students which of the events listed on the board had the most impact on future civilizations, and why? When students have completed giving responses to this question, have students prioritize the three most significant events by voting in the following manner: each student should hold up three fingers event he/she believes to be the most significant, two fingers for the next most significant event, and one finger for the third most significant event. Count all fingers that students hold up for each event, and write down the votes next to each event on the board. 25 minutes.

Have students write down on their handouts the three events in order of significance as the class voted. With each event, ask students to give the date that the
event occurred, the geographic location(s) where it occurred, and what new technology or invention at the time allowed for the occurrence of the event. Students may not know all of these answers. Information not known, as well as further information about the three events and their significance, is to be assigned as homework for students to research. Each student will need to contribute information to the knowledge base of the class the next day. 5 minutes.

*Day 2:* Group discussions on uses of water as a transporter.

Pass out to each student the handout titled *Water as a Transporter.*

Split the class into groups of four to five students each. Tell the groups to discuss the historical events that they researched, and to fill in first section of the handout, *Historical Use of Water as a Transporter.* They should come to an agreement on the most significant impacts on civilization and the technologies that allowed for the event to occur. 20 minutes.

At the end of the group discussion time, have members of the different groups volunteer their group’s information. Complete filling in the information of the three historical events on the board. If there are differences in opinions on the different events, have students give their reasons for their responses. It is acceptable for students to have different opinions on the significance of the events, but they should be able to support their views with facts. 20 minutes.

Assign students the following homework. On a piece of notebook paper, each student is to write a paragraph listing the similarities between the three historical events.
Follow that paragraph with the differences between the three events. The third paragraph should compare a current use of water as a transporter to its historical use. This last paragraph should include the similarities as well as any changes in the technology used. 10 minutes.

*Day 3: Uses of water geographically*

Collect the students homework assignment comparing and contrasting water use as a transporter. 5 minutes.

Next, call on students to state the current uses that they used as examples in their homework assignment. List some of the examples on the board, including the different technologies used. 5 minutes.

Have students individually complete the section titled *Uses of Water Geographically* on their *Water as a Transporter* handout. You may need to give an example to clarify what the expectations are on this section. Example: Geographic Location - Panama Canal in Central America; Limitations - time of travel for ships to go around South America between the Pacific and Atlantic Oceans; Technologies or Adaptations – canal and locks bridging the two oceans with a shipping passage across Central America. 20 minutes.

Ask students what the difference is between mechanical transport and chemical transport with respect to water. Guide students towards a general understanding of the two concepts with the following questions. What is mechanical movement? What makes objects move? What is a chemical? What is a chemical solution? What is a chemical reaction? Summarize the discussion by explaining that what you mean by mechanical
transport is that the force of moving water can move objects, or that other forces like moving air can move objects across the surface of water. Chemical transport involves the interactions between molecules of water and other molecules, so that molecules dissolved in water are carried with water molecules. Allow no more than 20 minutes for this discussion. 10 minutes.

Class Demonstration: in a clear plastic tub, pour water down an inclined board covered with sand (mechanical transport); dissolve sugar, salt or food coloring into beaker or glass of water and pour down inclined board (chemical transport). 5 minutes.

Assign students the last two sections of the Water as a Transporter handout as homework. Each student should list and explain two examples of mechanical use of water and one example of chemical use of water in transporting objects or substances. 5 minutes.

Day 4: Compare and contrast (group presentations)

Split the class into groups of four to five students each. Provide each group with a large piece of butcher paper or poster board, and markers or crayons. The groups will have only 15 minutes to prepare a poster presentation to the class that includes the following: one historical event of the use of water as a transporter and one current use; a comparison of the two uses and the technologies involved; an example of mechanical transport; an example of chemical transport; and a brief summary of the significance of water’s use as a transporter on human civilization. Allow each group no more than five minutes to present their information. Total time: 50 minutes.
Section 2: Water Use and Availability

Student Objectives. The student will be able to:

1. Identify and quantify personal water use.
2. Estimate quantity of home (domestic) water use.
3. Identify and estimate water use in the community.
4. Define the terms: domestic, agriculture, industry, mining, thermoelectric, acre-foot, and cubic kilometers.
5. Define and/or illustrate diagrams of: aquifer, groundwater, aquifer recharge, aquifer withdrawal, water table, surface water, and watershed.
6. Describe how geographic location and landform effects watersheds.
7. Research water use and availability for local region and for home state.
8. Research water use and availability nationally and globally.

Arizona State Science Standards Addressed: Grade 7, S1 (all concepts), S2 (all concepts), and S3C2(PO1-4).

Day 1: Personal water use

Ask the class the following question, and write the question on the board: “How much water do you use in a day?” Allow students a couple of minutes to think about their answers before calling on some for their replies. Next, have students brainstorm all of the ways in which they use water during a day as individuals. Write the different water uses on the board. 10 minutes.
Next, have the students estimate how much water (in gallons or parts of gallons) that different things require (drinks, baths or showers, cooking, etc.). 10 minutes.

Pass out the handout titled *Water Use*. Have students fill in their predictions for their own personal water use in a 24-hour period. Next show the students the water use log on page three of their handout. Explain how to fill in the log, so that each student can record and tally his/her own daily use of water. Impress upon students that they are to only record what they use, not what other members of the family use. 10 minutes.

Have students research the national averages for water use for the following categories: washing and rinsing dishes in the sink, electric dishwashers, washing machines, bathtubs, showers, toilets, cooking, and watering lawns or washing cars (gallons per hour for water hoses). Five minutes prior to end of class, pass out the handout titled *Average Daily Water Consumption*, and tell students to use the national averages for calculating their daily personal water use for homework. 20 minutes.

*Day 2: Personal and domestic water use*

Have students call off their totals for their daily water use and write their figures on the board. Have students complete their results on their handouts. Then have students calculate the class average for annual individual water use. 15 minutes.

Next, read over the section of the handout for domestic water use. Define domestic use and review the national averages for domestic water use with the students. Explain that for homework, they will need to talk with different members of their family to get an accurate estimate on weekly water use, monthly water use and yearly water use. They will need to consider items not listed in the chart that their family may have. Some
families have a swimming pool, or pets that need to be watered. Some families may have large lawns, fruit trees or gardens. Impress upon students that the more complete they are with gathering data for their family’s water use, the more accurate the overall class averages will be. 15 minutes.

Let the class brainstorm other uses of water in their community other than home (domestic) use. Ask students where they think the most water is used. Ask them where the water comes from. Have students start the research at the bottom of page two of their handout, to find local, state and national averages of water use. 20 minutes.

Day 3: Water use and availability

Pass out pieces of notebook paper, or have students use spiral bound notebooks to keep notes for the rest of the unit on water use and availability. Have students call off their totals for family domestic use of water and list the totals on the board. Then have the class calculate the average annual domestic use per person (within a family), and multiply that average by the population of their community. Have them record the average annual domestic use of water for their community. 25 minutes.

Next, ask students for information they found through their research on water use and availability of water. List different figures the students came up with on the board under the three different categories: local, state, and national. Discuss how the state and national averages compare to the students’ data. Then ask students what units of measurement are the best for comparing water use and water availability, gallons, acre-feet, millions of acre feet (MAF), or cubic kilometers? 5 minutes.
Finally, ask students where their water comes from? Prompt them with questions leading to the water cycle, surface water and groundwater. What is the major source of water for their community, surface water or groundwater? Review the water cycle with the students and have them draw a diagram of the water cycle in their notebooks. Include groundwater, water table, and aquifer recharge in the diagram. 20 minutes.

Day 4: Water use and availability

Pass out the following maps of Arizona (maps can be downloaded and copied from the Arizona Geographic Alliance or from the USGS): Arizona’s Landforms and Rivers, and Arizona’s Cities, with Compass Rose. Pass out crayons or markers for students to color with. On the Arizona’s Landforms and Rivers map, have students color in the rivers and lakes in blue. Then have them add the cities using the Arizona Cities, with Compass Rose map. Finally, have them color the mountain ranges in green, and the spaces between mountains in a lighter color. 30 minutes.

When students have finished coloring in their maps, ask them where most of Arizona’s water is located? Point out that the source of most of Arizona’s water is in the northern half of the state. Ask them where most of the cities are located? Are they near major water sources? 10 minutes.

Ask the students what information they found on water use and water availability in their community (San Pedro River Basin used in this unit) or in the state. List figures given by students on the board. Then pass out the handout titled Water Availability and review information they will need to obtain. 10 minutes.
Day 5: Water use and availability

Begin class by listing data obtained by students on water use and water availability on the board. Ask students why there may be different estimates from various sources? Finally list the figures that you expect the students to learn about water use and water availability. See Water Availability Key. 35 minutes.

Hold a class discussion on the importance of water for agriculture and industry. Which continents have the greatest human populations? Which have the greatest need for water? Is there enough fresh water for everyone’s needs? The world’s human population reached over 6 billion in 2000. If the trend in population growth continues as expected, the population will double by 2020. Is there enough fresh water available and is it distributed adequately to sustain a population of 12 billion people? Homework: What does sustainable mean with respect to water resources? 15 minutes.

Day 6: Review: water use and availability

Pass out the handout Review: Water Use and Availability. Have students fill in review using their notes. 35 minutes.

Go over review to make sure students have all information correct. 15 minutes.

Day 7: Test: water use and availability

Evaluate students using the handout Test: Water Use and Availability. 50 minutes.
Section 3: Water Quality

Student Objectives. The student will be able to:

1. List three chemical pollutants of water, identify their sources, explain their mode of movement into water supplies, and list the health problems associated with each.
2. List three biological contaminants of water, identify their sources, explain their mode of movement into water supplies, and list the health problems associated with each.
4. Explain the role of the Environmental Protection Agency with respect to water quality.

Arizona State Science Standards Addressed: Grade 7, S1 (all concepts), S2 (all concepts), S3 (all concepts), and S4C3(PO2-6).

Day 1: Water quality

Pass out the handout titled Water Quality. Have students answer the questions (survey) on page one of the handout. 10 minutes.

Next, pass out the excerpts from articles (see Excerpts from Newspaper Articles document downloaded from Water Filters home page) dealing with water quality problems in different regions of the United States. Choose articles so that a variety of common pollutants are covered. Each student should get a different article. Each student should read his/her article and fill in the information for the article on page two of the handout. Point out to students that the handout has room for information for three
articles, and that they need to only fill out information for the first article that each one reads. 20 minutes.

Split the class into groups of four to five students per group and have students share within their groups the information they summarized from the articles. Groups should discuss which water contaminants seem to be the most serious health concerns and which seem to be the most commonly found in water supplies. Are there any contaminants that may possibly be in the water supplies of their community? Students should fill in the information summarized by two other students within their groups on pages two and three, and answer the questions at the bottom of page three. 20 minutes.

Day 2: Water quality

Prior to class, set up a chart on the board to fill in as students share information from the article excerpts. Include the following categories for each article: (1) contaminant; (2) biological or chemical; (3) source; (4) mode of movement (surface runoff, groundwater, or both); and (5) health risks. In a different location on the board, write down the Clean Water Act of 1972 and the Environmental Protection Agency (EPA). Have students share their information from the articles with the class. As each student shares his/her article, fill in the information on the board. Place a check next to a contaminant that has already been listed each additional time it is mentioned. Prompt students for a summary of the Clean Water Act of 1972 and the role the EPA plays with respect to the Clean Water Act. 35 minutes.

Next, have the class vote on the three biological and the three chemical contaminants that they believe to be the most prevalent water contaminants across the
country based upon the frequency of mention in the articles, and the seriousness of health risks. Each student gets three votes for each type of contaminant, biological and chemical. Add up the total votes for each contaminant to determine which three biological contaminants and which three chemical contaminants the class voted as the most serious threats to water supplies. 15 minutes.

Record the contaminants voted on by the class to review the next day.

Day 3. Water quality

Rewrite the contaminants that the class prioritized as the most serious on the board. Discuss the each of the contaminants, their sources, modes of water contamination, and health risks. Summarize the Clean Water Act of 1972 and discuss the role that the EPA plays in monitoring water quality. 20 minutes.

Have students record the information off of the board on page four of their handouts. Make sure that they are aware that this is the information they will be responsible for during student assessment. 20 minutes.

Finally, have students revisit the survey questions on page one of the handout and ask if their opinions on water quality have changed since reviewing articles about water contamination. Do they have any concerns about the water they use in their homes or in the community? 10 minutes.

Day 4: Lab: What is a ppm?

List of materials for lab activity (per group):

2 – 500mL beakers

1 – 100 mL graduated cylinder
28% acetic acid (or substitute vinegar)
methylene blue (or substitute blue food coloring)
water

Pass out to each student a copy of Lab: What Is A ppm? Split students into groups (sizes of groups depend on materials available). Have the students read the overview and the problem. Then take the methylene blue and the acetic acid concentration around the class to let students see and smell the chemicals. Make sure students waft fumes instead of sniffing the container directly. Next, have students write their hypotheses on the handout. They may then follow the procedures and record their results in the results section. After clean-up, students may answer the questions. Assign the lab report for homework. 50 minutes.

Day 5: Biological contaminants and water quality analysis

Collect lab reports from What Is A ppm? 5 minutes.

Background Information: LaMotte’s Green Water Monitoring Kit is an inexpensive water quality testing kit, complete with instructions. The teacher should read through the instructions prior to organizing a field trip or lab activity, and select which methods he/she will use for collection of water samples and selection of which tests will be performed. You may wish to have students read through the instructions prior to this activity since the instruction booklet gives a very good summary of what the tests are for and what the most common sources of contamination are. Cases of bottled water are excellent for field trips. They provide water for students, and the empty bottles may be
used for collection of water samples at sites. This is an inexpensive way to obtain containers and keep students focused on “water”.

Pass out the handout titled *Biological Contaminants*. Students should read through and either highlight or underline the major biological contaminants of water. They need to also highlight or underline the contaminants and their concentrations that are used for identifying contaminated water. 15 minutes.

Next, pass out the handout titled *Lab: Water Quality Analysis*. Explain to students that they will be collecting water samples from various locations and conducting water quality tests in the classroom. Read through the handout with the students and emphasize the importance of proper collection so that the samples themselves do not get contaminated from the collection process, and so that oxygen is not dissolved into the water. The teacher may wish to do collection as a field trip, or have students bring in samples from home. 15 minutes.

Finally, split students into groups and pass out the instruction booklets from the LaMotte Green Water Monitoring Kits. Students need to write down the procedures for collection and testing for each test you decide to run. For the lab handout provided, the following tests are conducted: pH, phosphates, nitrates and coliforms. 20 minutes.

*Day 6: Lab: water quality analysis*

Have students split into their groups and conduct the tests on their water samples using the LaMotte Green Water Monitoring Kits. They need to record their results on their handouts. Write a chart on the board with the sample numbers and different tests so as groups complete their tests, they may fill in the chart on the board so other groups may
write down the results. Results of the coliform tests will not be ready until the next day, so do not start this test on a Friday. 50 minutes.

Day 7: Lab report: water quality analysis

Complete filling in the results on the chart on the board as students analyze their coliform tests. 10 minutes.

After students complete the coliform tests, summarize the results from the chart on the board. Identify positive tests for the tests the class performed and ask them if there is any pattern to the locations of where the positive test samples were taken. 10 minutes.

Have students begin writing their lab reports on the water quality analysis. They should complete the lab report for homework. 30 minutes.

Day 8: Review: water quality

Collect lab reports on Water Quality Analysis. 5 minutes.

Pass out the handout titled Review: Water Quality. Allow students to use their notes to fill in the review. 45 minutes.

Day 9: Test: water quality

Pass out the handout titled Test: Water Quality for student evaluation. 50 minutes.

Section 4: Water In The Future

Student Objectives. The student will be able to:

1. Research trends in population growth and water use, and predict future water uses and availability.
2. Describe a technology or invention that may be used in the future to meet the needs of a limited water supply.

3. Prepare and present a presentation to class about future water needs and availability that includes map(s), charts and/or graphs, and future technology.

Arizona State Science Standards Addressed: Grade 7, S1 (all concepts), S2 (all concepts), S3 (all concepts), and S4C3(PO2-6).

Day 1: Water in the future

Pass out the handout titled Water In The Future. Read through the directions with the class, explaining the responsibilities of the different team members and the objectives of team presentation (see handout). Organize students into groups of four. Each group needs to have students assigned the following specialist positions: population analyst, hydrologist, cartographer and engineer. 15 minutes.

Allow teams the rest of the period to research information on projected world population growth, and current water use and water availability of the continents. 35 minutes.

Day 2: Water in the future

Materials: poster board, markers, and copies of map of continents.

Allow teams the class period to prepare materials and information for their class presentations. 50 minutes.

Day 3: Water in the future

Teams present to the class on Water In The Future. Use the Water Presentation Score Sheet to evaluate each team’s presentation. 50 minutes.
Day 4:  Wrap up of water unit

Pass out the handout titled Water Use Survey and have students complete the survey. Collect the surveys. 15 minutes.

After collecting surveys, review the unit by asking students for comments about the unit, what they believed to be important information that they learned, etc. 15 minutes.

Pass out the handout titled Writing Prompt and have students write a short story based on the prompt. 20 minutes.
References


Arizona Department of Water Resources: http://www.azwater.gov

Arizona Geographic Alliance: http://aliance.la.asu.edu

Centers of Disease Control and Prevention: http://www.cdc.gov

Garry Lab, Tulane University: http://www.virology.org


The Manduca Project: Retrieved from (http://insected.arizona.edu/Manduca).


The Virtual Virus Experience: Retrieved from (http://www.thinkquest.org).


APPENDIX A

Student Handouts

for

Effects of Antennal Removal in *Manduca sexta*
Basic Anatomy of the \textit{Manduca} Pupa and Adult

Activity 1

The two pictures below are of a pupa and an adult \textit{M. sexta} moth. On each photo, label the following parts: eyes, legs, antennae, proboscis, wings, head, thorax and abdomen.

\textit{Manduca sexta} pupa

\textit{Manduca sexta} adult
Activity 2

Look carefully at the two photos. List characteristics that are similar between the pupa and the adult in the left column of the chart below. List characteristics that are different between the pupa and the adult in the right column.

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Activity 3

Share your observations with the class. Add any observations from other students that you think are important to your chart.
What Do The Antennae Do?

**Directions**

Read the short explanation about the *Manduca* antennae below, and then answer the questions that follow. Some questions you will need to answer by reading articles on the internet or finding the information on *The Manduca Project* CD or visit the website (http://insected.arizona.edu/Manduca/). Other questions will be answered by experimental investigation.

When the adult moths come out of the pupal stages, eclosion, they have just a few days left to live. They must find the right species of plant (members of the family Solanaceae) to lay their eggs on, so that after hatching, the developing larvae will have enough food. The adults use their antennae to sense volatile chemicals (olfaction) that plants emit into the air. This is the main sense that the moths use to find the correct plants, and to find a mate. Just as you can distinguish between the odor of bacon and the odor of popcorn, moths can detect many different odorants with their antennae. Just how sensitive are their antennae?

**Questions**

1. What are the main plant species *M. sexta* larvae feed on?

2. How do male and female moths find each other? Are there any differences in the odors that they are attracted to?

3. In comparison to our nose, how sensitive are the moth’s antennae?

4. Does removal of portions of the antennae prior to eclosion have an effect on olfaction, and if so how much effect?
5. What do the following words mean?
   a. olfaction:
   b. eclosion
   c. pheromones

6. In the space below, design an experiment that would test the moth’s olfaction abilities.
Effects of Partial Deantennation on Olfaction in *Manduca sexta*

Overview

You and your partner(s) will work together to investigate the ability of *M. sexta* to detect odors. You will be addressing two questions in one experiment: (1) how odor-sensitive are the antennae of female *M. sexta* adults; and (2) what effect, if any, does removal of approximately half of each antenna have on the ability to detect odors? By the end of this investigation, you should have gained enough knowledge and experience to accomplish the student objectives.

Student Objectives. The student will be able to:

1. Identify the following anatomical structures of *M. sexta* pupae: eyes, legs, antennae, proboscis, wings, head, thorax and abdomen.
2. Determine sex of pupae.
3. Surgically remove parts of antennae in pupae.
4. Calculate and mix different concentrations of plant leaf extract in parts per million (ppm).
5. Collect and analyze behavioral data on adult *M. sexta*.
6. Evaluate experimental methods and make changes to improve experimental design.
7. Design an experiment that further tests the functions of antennae in *M. sexta*.

Experimental Design

Each team will have a control group of *M. sexta* adults that have their complete antennae, and a variable group that have had approximately half of each antenna removed. Each student in each team will have an unaltered control animal and a test animal which he/she has removed the antennae on. Removal of antennae needs to be done in the late pupal stages instead of on adults because the adults are too easily damaged. The pupae are anesthetized by placing in ice for 20 minutes to reduce movement, injury and stress. Each team will have a rearing (moth) box to place their pupae in and to conduct their experiments in. Two to three days after eclosion, the adults will be offered varying concentrations (1 part per million (ppm), 10 ppm, 1000 ppm and 100,000 ppm) of tomato leaf extract to test for any differences in sensitivity. Teams will record the movements of adults to different locations within the box. Class data will be compiled for analysis by the teams.
2

Activity 1

In your team, discuss the two questions posed in the overview. Write your team’s hypotheses, both the null and the alternative, for each question.

1. How odor-sensitive (in parts per million) are the antennae of female *M. sexta* adults?
   
   \( H_A: \)

   \( H_0: \)

2. What effect, if any, does removal of approximately half of each antenna have on the ability to detect odors?
   
   \( H_A: \)

   \( H_0: \)

Activity 2

Read through the handout titled Deantennation Procedures and discuss within your team the sequence your team will use to perform the surgeries. Each team member must do his/her own deantennation. Each member must be familiar with the procedures and the entire team prepared and ready to complete all of the surgeries within a class period.
Tomato Leaf Extract Procedures

Materials Needed

Tomato plants
Mortar and pestle
Graduated cylinder
4 beakers or cups with covers (plastic wrap)
Refrigerator

Overview

You are going to make four different concentrations of tomato leaf extract by diluting pure tomato leaf juice in water. Many substances are measured in units called parts per million (ppm) because they are found in such small concentrations in nature. A part per million in solution of water can be measured out as one gram in one million milliliters of water 1g/10^6 mL, or 1 mL/ 10^6 mL. There are many organisms that can detect chemicals or are harmed by chemicals that occur in concentrations of less than 10 ppm. You are going to make the following concentrations of tomato leaf extract: 100,000 ppm, 1000 ppm, 10 ppm and 1 ppm.

Procedures

1. Crush tomato leaves with the mortar and pestle until there is a little over 5 mL of green juice in the bottom of the mortar.

2. Measure out 5 mL of tomato leaf juice and pour into a beaker or cup. Add 50 mL water and mix to make a 1 part to 10 parts solution. *This is not a 10% solution. Why?

   How many parts per million is this solution?

   Label the concentration in this beaker with masking tape and pen marker.

3. Measure out 1 mL of the solution above and pour it into a second beaker. Label this beaker 1000 ppm tomato leaf extract. How many milliliters of water do you need to add to make this a 1000 ppm solution?
4. Using the same dilution techniques as in the previous steps, take 1 mL of the 1000 ppm solution and prepare a 10 ppm solution in another beaker. Make sure this beaker is labeled correctly. How many mL of water do you need to add to 1 mL of the 1000 ppm solution to make a 10 ppm solution?

5. How many mL water do you need to add to 1 mL of the 10 ppm solution to make a 1 ppm solution? Prepare this solution in another beaker and label it. Cover all four of your beakers and place them in a refrigerator until the adult moths emerge and are ready for testing.
Artificial Tomato Plant Procedures

Overview

When the adult moths emerge from their pupae cases (eclosion), they are not very active. Their wings need to unfold and dry. It takes two to three days before the adults begin to fly around in search of a host plant. You will need to have a structure that simulates plants in your box to test for sensitivity to the different concentrations of tomato leaf extract. Follow the procedures below to construct an artificial tomato plant, but do not put the leaves on the stem until the moths are flying and your team is ready to conduct the tests.

Materials

- Filter paper (or stiff, porous fiber art paper)
- Clay
- Floral wire and floral tape (or suitable substitutes)
- Two 1/8 inch wood dowels 12–18 inches long (must fit upright in box) or suitable alternatives.
- Artificial Tomato Leaf template.

Procedures

1. Cut the wood dowel so it will fit upright in the moth box and wrap the dowel with floral tape. Masking tape will work if floral tape not available.
2. Make a cone shaped base out of clay that will hold the wood dowel upright at a 90° angle and poke the dowel into the clay base. The base needs to large enough that the dowel will not easily fall over.
3. Cut out the tomato leaf design that accompanies this handout (Artificial Tomato Leaf) and use it to trace leaf borders on filter paper or any other thick, porous fiber paper. The paper needs to be absorbent and somewhat stiff even when soaked. Trace and cut out four paper leaves.
4. Cut four pieces of floral wire (or other thin wire) about eight inches long. Place a piece of wire along the center petiole of each paper leaf and tape the wire to the leaf. Leave extra wire towards the base to tape to the wood dowel (stem).
5. Use pencil to label each leaf with the solution concentration that it will be soaked in on the side with the wire and tape (underside of leaf).
6. The day after the adults emerge from their pupae, place the tips of each leaf in its corresponding tomato leaf extract to begin soaking.
7. When the adult moths begin to fly around (2-3 days after eclosion), begin the experiment. See the procedures for testing the leaf extracts in the handout titled *Testing Moth Antennae*. 
Artificial Tomato Leaf

Wire and tape
Testing Moth Antennae

Overview

When the adult moths begin to fly (2-3 days after eclosion), your team will need to test the extracts to determine which concentrations the moths can detect. You will need to carefully observe and record the behaviors of both the control animals and the deantennated animals. Your team members must first determine how to tell the difference between the control and test animals. Then, you must decide the methods you’ll use to test the four different concentrations of tomato leaf extract. How will you record results that can be measured in some manner and compared to other results? Will all of the teams in your class use the same methods, or each team try a different method, to see if the results change or not with the methods used?

Activity 1

Review the hypotheses that your team wrote on the handout titled Effects of Partial Deantennation on Olfaction in Manduca sexta. Discuss with your team the methods that your team will use to test your hypotheses. On a separate piece of paper, write down the procedures your team will use.

Activity 2

Share your methods with the other teams, and discuss the weaknesses and the strengths of the different methods. Decide whether all of the teams are going to use the same methods (adapted using the strong points from the procedures from all of the teams), or whether the teams will use different methods.

1. What are some good reasons for all of the teams using the same methods?
2. What are some good reasons for all of the teams using different methods?
3. On a separate piece of paper, write down the final draft of the procedures your team will use. Include any charts or organizers that you will use to record your data. Also include the methods by which you intend to compare your data. You must compare the control animals to the test animals, and you must either compile or compare data collected by the different teams.
Effects of Partial Deantennation Results

Activity 1

Discuss your results with your team members. Are there any significant patterns? If so, what are they?

Activity 2

Discuss your team’s results with the other teams. Are there any similarities or differences in the results obtained? Are there any significant patterns? Is there any conclusive evidence or any tendencies that support any of the hypotheses?

Activity 3

Answer the following questions on a separate piece of paper.

1. What did you learn from this experiment?

2. If you were going to continue this investigation, what changes would you make in your experimental design, methods, questions asked, hypotheses, etc.?

Activity 4

Answer one of the following questions on a separate piece of paper. Include the question(s) proposed, the null and alternative hypotheses, the materials needed to conduct the experiment, and the methods or procedures to implement and evaluate the results of the experiment.

1. Design an experiment that would test the ability of the human tongue to detect different concentrations of chemicals.

2. Design an experiment that would further test the sense of olfaction in antennae of Manduca sexta.
APPENDIX B

Student Handouts

for

Transmissible Diseases PBL
Dear Student:

Congratulations! You have just been drawn in our grand opening promotional to receive one free pet from IMACON.COM PET STORE. Because we are limited in certain animals, please write down the names of three animals you would like to own as pets in order of priority.

1.

2.

3.

If your first priority were available, write a brief paragraph explaining why you would like to own one of these animals for a pet.

Press the tips of your fingers and thumbs from both hands onto the blank space below. Look for any red paprika (tick and flea powder) smudged onto the paper. If you have paprika on your fingers, you just caught a disease. I lied (this is IMACON), paprika doesn’t represent tick and flea powder – it represents a harmful pathogen.
Part III

1. Was your pet already infected with this pathogen (paprika) when you received it from IMACON.COM., or did you or your animal get it from another animal? If you got it from a different animal, which animal do you think it was?

2. What does the term “pathogen” refer to? List as many pathogens as you can think of.

3. Have you recently heard in the news of any diseases that are spread by animals, or do you know of any from past occurrences? List the diseases and the animals that transmit the disease.

Part IV

Summarize what your group thinks the disease may be and what animal or animals transmit it.
Part V

Read the articles Beyond Cute: Exotic Pets Come Bearing Exotic Germs by Denise Grady and Lawrence K. Altman; and Cute but Wild: The Perilous Lure of Exotic Pets by Mark Derr.

Answer the following questions and be ready to discuss these articles in our next class.

1. What pathogen causes SARS and what animals do scientists believe to be the hosts?

2. What pathogen causes monkeypox and what animals are the hosts?

3. How did monkeypox get to the United States?

4. Why do you think people want exotic pets as animals? Does your answer in Part I fit any of the theories mentioned, or do you agree with any of the theories?
5. If you were on a committee making policy for regulating the pet trade, what recommendations would you make? Keep in mind economics, public safety, consumer demand, and feasibility.
Introduction to Trainees

You are a member of an investigative team with the Center for Disease Control (CDC). Your job is to help other team members obtain information about outbreaks of disease in different parts of the world, and to make recommendations to control the spread of the diseases. An epidemic has broken out in San Francisco. Time is of the essence! You and your team must work quickly and efficiently to:

- Discover the source of the pathogen
- Determine how it is being spread
- Limit the spread of the disease
- Educate the public as to how to prevent future occurrences

It is your team’s responsibility to carefully analyze the information available to insure and accurate diagnosis. The disease agent could be anything from the common flu virus up to and including the fast-spreading SARS virus. You will be given a list of diseases to research and a description of the patients’ symptoms. Members of your team will specialize in various aspects of disease transmission. A group commander will oversee what you are doing and make sure you are taking your job seriously. Sloppy work can lead to further disease spread and can even endanger your team.

Team Composition

Commander
Disease Specialist
Insect Specialist
Animal Specialist
Food- and Water-borne Disease Specialist
Mysterious Outbreak Sickens Chinatown Congregation

San Francisco – Several members of a Chinatown church, including elderly members and very young children, have been hospitalized in the past two weeks with a mysterious illness. Authorities from the state Health Department report that five members of the Hill Street Chinese Baptist Church in Chinatown have been hospitalized with symptoms of high fevers and extreme weakness. Two elderly women are in comas.

Health Department agent Rudy Gonzalez reported Thursday that all victims of the mysterious illness had attended a three-day retreat in Napa County approximately one week before falling ill. Victims include a four month-old infant and three other children, several adult relatives, and the two elderly women. A member of one of the affected families has recently returned from visiting Hong Kong, stirring concerns that a disease has been introduced from that part of the world.

Authorities are trying to determine the identity of the illness and to prevent its possible spread to the remainder of the community. Any community members who have been ill with high fevers, weakness, chills, or headaches are urged to contact the Health Department at 424-SICK.
Commander: Day One

Your team will be assigned to you.

Assemble the team in a location where you can discuss your mission. Discuss the newspaper report on the case and ask each agent to write down his/her initial impressions about the case. In order to prevent premature judgments, ask team members to refrain from discussing their ideas until the information-gathering phase is ended.

Assign roles to the other members of the team in whatever way you feel is best and distribute assignments and research packets. Each agent will be responsible for gathering the information he/she feels is relevant from the most reliable sources possible. Your job is to insure that each agent understands his/her role and that information gathered is as up-to-date and accurate as possible. You will be the team member who presents the group’s consensus at the daily press conferences. You will also be responsible for the safety of the team when they visit the site of the outbreak.

The information phase of the investigation will be conducted at CDC headquarters in Atlanta. Subsequently, the team will travel to the site of the outbreak to conduct tests and to interview patients.

Ultimately, your team must identify the cause of the outbreak using information gathered by your agents and medical procedures and tests, explain the pattern of spread of the disease, develop strategies to prevent further spread, and make recommendations to public health officials that will prevent further outbreaks.
Commander/Safety Information

1. Review the four levels of hazards when dealing with contagious diseases and summarize the procedures that protect personnel from disease.

2. For each level of hazard, give an example of an infectious agent that is handled with that level of precaution.

3. For each level of hazard, summarize the equipment required to protect your team from harm.

References for Information Sources:
Disease Specialist

Your background in public health and clinical medicine makes you an essential member of this team. Because you have been working in an unrelated field for several years, you must refresh your memory about the symptoms, similarities, and differences between the following diseases:

- Cholera
- West Nile virus encephalitis
- SARS
- Influenza
- Monkeypox

Use the following as a guide for information you need to obtain on each disease:

1. Common Name of Disease:
2. Causative Agent:
3. Disease Symptoms:
4. Treatments for Disease Victims:
5. Is the Disease Lethal? If so What Percent?
6. Is the disease transmissible between humans? If yes, how?
7. Is the disease transmissible by insects or other animals? If yes, what kind?
8. Is the disease spread by water or foods?

References for Information Sources:
Insect Specialist

Your background in entomology and disease transmission make you essential to this team. You will use your background to help your team narrow down the likely causes of the outbreak. However, you have been working in another area recently, so you will have to refresh your memory about insects that carry diseases.

1. Find information on at least five insects that can spread infectious diseases.
2. Determine if any of the insects you are investigating are likely to be found in the San Francisco Bay Area, where the outbreak occurred.

Use the following as a guide for information you need to obtain on each insect:

1. Common Name of Insect:
2. Scientific Name of Insect:
3. What type of habitat or environment is it found in?
4. Is the insect commonly found in northern California?
5. What diseases does it spread?
6. What are the symptoms of the diseases?
7. What is the incubation time for the disease?
8. Effective disease prevention procedures:

References for Information Sources:
Animal Disease Specialist

Your background in zoology and disease transmission make you essential to this team. You will use your background to help your team narrow down the likely causes of the outbreak. However, you have been working in another area recently, so you will have to refresh your memory about animals that can spread diseases to humans.

1. Find information on at least five animals that can spread infectious diseases.
2. Determine if any animals you are investigating are likely to be found in the San Francisco Bay Area, where the outbreak occurred.

Use the following as a guide for information you need to obtain on each insect:

1. Common Name of Animal:
2. Scientific Name of Animal:
3. What type of habitat or environment is it found in?
4. Is the animal commonly found in northern California?
5. What diseases does it spread:
6. What are the symptoms of the diseases?
7. What is the incubation time for the disease?
8. Effective disease prevention procedures:

References for Information Sources:
Food-/Water-borne Disease Specialist

Your background in microbiology and disease transmission make you essential to this team. You will use your background to help your team narrow down the likely causes of the outbreak. However, you have been working in another area recently, so you will have to refresh your memory about microorganisms in food or water that can spread diseases to humans.

1. Investigate at least five food- or water-borne diseases.
2. Determine whether any of the diseases you investigate are likely to be found in Northern California.

Use the following as a guide for information you need to obtain on each insect:

1. Common Name of Disease:
2. Scientific Name of Disease:
3. What microorganism cause the disease?
4. What kinds of food or water does the microorganism inhabit?
5. What are the symptoms of the diseases?
6. What are common treatments for victims?
7. What is the incubation time for the disease?
8. Effective disease prevention procedures:

References for Information Sources:
Commander: Day Two

Assemble your team and review the findings of the specialists. What diseases can you eliminate from consideration, based just on the short news article and the research findings? You and the team should discuss the evidence and come to a consensus about the diseases that must be considered as possible causes of this outbreak.

You and your team will travel tonight to San Francisco to interview victims and to conduct laboratory testing.

Before you depart, you and your team must come up with a safety plan that will ensure that you neither expose yourselves to the disease, nor spread it from the victims to the general public. Work with your team to put together a list of the safety equipment you will need to take with you and any other safety precautions the team will make.

You and your team must also take the equipment needed to test for the infectious agents. Work with the different specialists to compile a list of the testing equipment you will take with you and to make a plan for processing samples in central lab facilities if necessary.

Once you and your team have formulated the safety and testing plans and have assembled the safety and testing equipment, you will travel to San Francisco and begin interviewing victims of the outbreak and conducting tests.
Patient Interview: The Cho Family

Mrs. Cho is a thirty-four year-old woman with two children, ages six and four. She and her husband own a printing business and she works there half-time, in the mornings, when her children are in school.

Mrs. Cho reports feeling achy and feverish about, one week after returning from a church retreat in Napa Valley. She briefly had a rash on her abdomen, but all the symptoms were gone within five days. She has had no lasting effects from her infection.

Mrs. Cho’s youngest son, Alex, also became ill around the same time as his mother did. His symptoms were more serious: high fever, headache, disorientation, and muscular weakness. He was taken to the hospital and treated for an unspecified viral illness. With constant care and IV fluids, he improved and he returned home after four days in the hospital. His muscular weakness has improved but has not completely disappeared.

Mr. Cho and the couple’s older son were not affected by this illness.

None of the family were able to recall any encounters with animals or unusual insects during the several weeks before they became ill. Both boys, as well as their mother, reported being bitten by mosquitoes while at the Napa Valley campground where the church held the retreat. The tent was pitched near the river and they felt they were bitten both inside the tent and outside during meetings. Mrs. Cho, but not her husband, had tasted wine at one local winery while at the camp.

The couple’s children attend the Happy Heart preschool and kindergarten with the children of several other families in the congregation, several of whom had reported being ill during the same period as the Cho family.
Patient Interview: Mr. Chen

Mr. Chen is a sixty-seven year old man who has been hospitalized in a coma for four days. He lived alone but near his son, and was a retired teacher.

His son says he reported feeling achy and "flu-ish" for three days prior to his hospitalization. He was admitted to the hospital after becoming weak, disoriented, and dehydrated. He was rehydrated in the hospital but developed paralysis of his left arm and slurring of his speech and then lost consciousness. His coma is beginning to lighten and his doctors suspect he will soon regain consciousness.

Mr. Chen attended the church retreat to Napa Valley but returned early on the second day, dissatisfied with the amenities at the campground, saying that is was too hot and buggy and there were dead animals in the campground. His symptoms began around ten days after his return home. His son had no idea whether he had contact with any live animals while there or whether he encountered any insects. As far as he knew, none of Mr. Chen’s close acquaintances had become ill.
Patient Interview: The Tsing Family

Xiaobo and Adam Tsing live in Chinatown with their three children: Lin, four years; Mika, two years; and Elen, four months (who is breastfeeding exclusively). Lin and Elen have been ill, and Mr. Tsing reports having felt tired and run-down, but otherwise normal. Mrs. Tsing had no symptoms of an infection.

The family lives in Daly City but still attends church in San Francisco at the Chinese Baptist Church. Xiaobo and Adam attended the first two days of the church retreat, and Adam’s mother and aunt stayed with the children. Mr. and Mrs. Tsing both report having been bitten by mosquitoes at the retreat.

Lin and Mika attend day care at Child Success Preschool in Daly City; Mrs. Tsing’s mother stays with Elen so Mrs. Tsing can work at a department store. Mr. Tsing works at a water treatment plant as a treatment engineer.

Lin’s illness consisted of nausea and slight fever; she became ill seven days after her parents returned from the church retreat. She recovered with no treatment in just a day and is feeling essentially normal.

Elen, the four-month old, developed a high fever five days after her parents returned and was cranky and out of sorts for two days before Mrs. Tsing took her to the pediatrician. The pediatrician sent them directly to the hospital and Elen was there for five days before returning home. At the hospital, Elen received IV fluids and was briefly on oxygen. Aside from weight loss, she seems to have suffered no lasting effects.
Margaret Otis is a fifty-seven year old woman who works at the River Shadows campground and resort, where the Chinese Baptist Church held their annual retreat. She cooks the meals at the camp and also supervises the operation of the store and raft rental concession. She is divorced, with no children, and usually of good health.

Mrs. Otis developed a high fever and a stiff neck approximately ten days before the Chinese Baptist Church retreat. When she also developed muscle tremors in her legs, she saw a doctor. She spent the night in the hospital for observation, but returned home when her fever decreased. Her headache persisted for two more days and the muscle tremors have decreased in severity and frequency.

Mrs. Otis reports that she has observed several dead birds on her perimeter walks around the campground and surroundings in the past month, five or six dead jays and two dead crows. She has not touched the birds directly but has removed the carcasses immediately when she finds them. She has not been in contact with any other animals except for the horses kept at a neighboring stable for trail rides. She was concerned about the eight new prairie dogs she had purchased from a pet dealer in Texas. She wants to start a prairie dog town at the campsite for visitors to enjoy. Some of the prairie dogs didn’t seem very healthy to her for a few days after they arrived, but seem to be doing better now. She guesses that the stress of being transported by truck from Texas to California was what made them sick.

Mrs. Otis states that a month of unseasonable rains, followed by hot weather, have left the campground with a big mosquito problem. Several of the campers have left early due to the large number of mosquitoes, which are not normally numerous in this relatively dry area. She says that some of the horses at the neighboring stables have been sick and their owner speculates that the mosquitoes may have something to do with their illness. Another neighbor has taken her horses to pasture in a higher, dryer place away from the river to keep the mosquitoes from bothering them.
Patient Interview: Rose Ng

Rose Ng is a twenty-eight year old sanitation worker and the mother of one six-year old son, David. Her husband is deceased (not recently). Her mother May, 70, lives with Rose and her son. All three attended the church retreat. While there, David suffered from an upset stomach, but recovered in less than a day.

Rose and David developed chills, fevers, and Rose had a headache and an extremely stiff, sore neck about ten days after the church retreat. After two days of illness, both started to improve and both are now without symptoms.

May became ill a few days before Rose and David developed the symptoms and was admitted to the hospital with respiratory distress. She was given antibiotics for her respiratory infection but slipped into a coma and is currently on a ventilator. Her doctors suspect that she had a viral infection and that the bacterial respiratory infection was secondary. However, because of the severity of the infection, her prognosis is not good.

David attends the Happy Heart preschool with the children of Mr. And Mrs. Cho. Rose and David rode horses while at the church retreat and suffered from a few mosquito bites. They had petted a couple of the young prairie dogs that were in the campground store. Neither has been in contact with any other animals, to the best of their knowledge.
Viral Diseases and Viruses

Focus

Students conduct antigen-antibody testing of blood samples (simulated blood) from infected patients to identify the disease they have been tracking down as members of a CDC Investigative Team. This activity carries over from a unit on transmissible diseases and leads into a lesson on viruses. Upon learning the method in which viruses are replicated (DNA and RNA), this unit will tie into the study of cells through a genetic approach.

Audience and Time

These activities are designed to introduce middle school students to structure and replication of viruses. It is designed for students that have no prior knowledge in virology. The unit will require two to three 55 minute class periods, or two block periods.

Major Concepts

RNA and DNA are considered life’s molecules. Viruses lie somewhere between the living and the non-living, relying on living cells to replicate their genetic material and propagate more viruses. The interaction between viruses and host cells exemplifies the very basics of genetic replication and the versatility of RNA and DNA.

Objectives

The student will be able to:

1. Conduct a simulated blood antigen test and correctly identify samples containing certain antibodies.
2. Identify the main components of a virus and explain their functions.
3. Demonstrate how viruses are produced (replicated).
4. Discuss why viral infections cause certain symptoms (fever, headache, drowsiness, etc.) in multicellular organisms.

Arizona State Standards Addressed:

Standard 1 Science as Inquiry

1SC-E1. Identify a question, formulate a hypothesis, control and manipulate variables, devise experiments, predict outcomes, compare and analyze results, and defend conclusions.

PO 3 Analyze results of an experiment
PO 4 Defend conclusions drawn from the analysis

1SC-E4. Identify and refine questions from previous investigations.

PO 2 Refine a hypothesis from a previous investigation

**Standard 3  Personal and Social Perspectives in Science and Technology**

3SC-E2. Develop and use a systematic approach to analyze the risks associated with natural and biological hazards.

PO 1 Analyze the risk factors associated with natural and biological hazards

**Standard 4  Life Science**

4SC-E5. Describe changes or constancy in groups of organisms over geologic time.

PO 2 Identify environmental factors that may determine adaptations or constancy of an organism over geologic time

4SC-E6. Describe the role of genes in heredity.

PO 1 Explain the basic principles of heredity and genetics

PO 3 Describe information carried in a gene

4SC-E7. Explain and model the interaction and interdependence of living and non-living components within ecosystems, including the adaptations of plants and animals to their environment.

PO 1 Explain the role of living/non-living components in an ecosystem

**Prerequisite Knowledge**

Students should have completed investigations in a Transmissible Diseases unit and have proposed their hypotheses as to which pathogens are responsible for a mock epidemic they have investigated.

**Introduction/Teacher Background Information**

West Nile Encephalitis Virus, SARS, and Monkeypox are just a few diseases that have been reported in various news media. These three diseases are caused by viral agents, but each has its own unique host reservoir and mode of transmission to humans. Students should have already researched these and similar diseases. For more information about these diseases, visit the Center for Disease Control (CDC) website at: http://www.cdc.gov/

To test for and to identify infection of specific viruses, researchers often test blood samples for antibodies that are produced by the blood to destroy viruses. These antibodies are specific to the virus, as they are made by specialized white blood cells. To test for and to identify infection of specific viruses, researchers often test blood samples for antibodies that are produced by the blood to destroy viruses. These antibodies are specific to the virus, as they are made by specialized white blood cells that identify the specific antigens (or viruses) that are foreign to the body. So, a known antigen can be added to a blood sample to see if the antibody is present to bind up with...
the antigen. If so, then we say that the blood sample tested positive for the antibody, meaning that the person has previously been infected with the antigen (virus).

Blood type kits test for the antibodies common in human blood. On the surface of the red blood cells there may be one or more proteins, called antigens. These antigens are called A and B. Antibodies are produced in the blood plasma against these A and B antigens, and continue to be produced throughout a person’s life. A person normally produces antibodies against the antigens that are not present on his or her red blood cells. For example, a person with antigen A on his red blood cells will produce anti-B antibodies; a person with antigen B will produce anti-A antibodies; a person with neither A or B antigens will produce both anti-A and anti-B antibodies; and, a person with both antigens A and B will not produce these antibodies. If blood cells are mixed with antibodies the cells will clump together. This is called agglutination.

The lab activity uses a simulated blood typing kit to identify antibodies in blood samples. Instead of using the kit to test for blood types, the antigens and antibodies are relabeled to represent the viruses and their respective antibodies of the diseases studied by the students.

The students will be able to test their hypotheses of which disease(s) is responsible for a mock epidemic.

Viruses are unique structures in that they contain the genetic material DNA or RNA. However, they themselves are incapable of reproduction, movement, respiration and other processes associated with life. They require host cells to replicate their genetic code and make more of them, resulting in the destruction of the cell. The second activity in this unit helps students gain an understanding of what viruses are and how they replicate. The information for this activity was adapted from The Virtual Virus Experience online at http://www.thinkquest.org/

**Materials and Preparation**

You will need to prepare the following materials before conducting these activities:

**Activity 1: Antibody Testing for Viral Infection**

- Teacher’s directions for altering simulated blood typing activity (in Procedure)
- Simulated blood typing kit (order from any major science supply catalogue – about $35 for two classes of 35-40 students)

**Activity 1: Viruses: Structure and Function**

- Student copies of the handout Viruses
✓ Computer and multimedia projector with screen or whiteboard; the Viruses presentation (either Powerpoint or Keynote) or outline from the Viruses presentation.
✓ Student copies of the handout Assignment: What Are Viruses?

Procedure

1. **Day 1.** Students should have completed research on various infectious diseases (as members of CDC investigative team) which should include SARS, West Nile Virus Encephalitis (WNV), Western Equine Encephalitis (WEE), and Monkeypox. Begin this unit with Activity 1: Antigen Testing for Viral Infection.

Re-label (replace blood type with patient’s name, and antibody with viral antibody) the simulated blood and the antigen antibodies in the kit with the following labels:

<table>
<thead>
<tr>
<th>Blood Sample From:</th>
<th>Use Blood Type:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alex Cho</td>
<td>A</td>
</tr>
<tr>
<td>Mr. Chen</td>
<td>A</td>
</tr>
<tr>
<td>Elen Tsing</td>
<td>O</td>
</tr>
<tr>
<td>May</td>
<td>A</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antibody:</th>
<th>Viral antibody:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-B</td>
<td>West Nile Virus</td>
</tr>
<tr>
<td>Anti-A</td>
<td>Monkeypox</td>
</tr>
<tr>
<td>Anti-A &amp; Anti-B</td>
<td>SARS</td>
</tr>
<tr>
<td>Anti-B &amp; Rh⁺</td>
<td>Western Equine Encephalitis</td>
</tr>
</tbody>
</table>

2. Write the charts above on the board. Explain to students that when testing blood samples for specific viral infections, that when the antibodies to those viruses are added to the blood, they cause the blood cells to stick together (agglutinate). If the blood agglutinates, the test is positive for that virus. If not, the test is negative for that virus. Explain to students that you only have samples for the people listed in the chart. Then have students get into their teams (CDC investigative teams) and have them design their tests. As each team agrees on their tests, have the commander come up and collect the blood samples and antibodies needed for the team’s tests. Teams may then conduct their tests.

3. For homework, each student should write his/her own report on the team’s findings and suggestions for actions that should be taken to prevent the
spread of the disease. Reports should include hypotheses, testing methods, results, conclusions and discussions.

4. **Day 2.** Pass out the handout *Viruses*. Have students fill in the handout as you go through Powerpoint or Keynote presentation. Pick students to read different slides, and help with pronunciation when necessary. Discuss the information with the class before proceeding to the next slide.

5. After completing the presentation, discuss any questions students may have with the class. Then pass out the handout *Assignment: What Are Viruses?* Discuss with the class what is expected on the assignment, then let them start on the assignment. Posters, concept maps or models and the handout should be due the following class period.

**Questions for Students**

1. Why do we get fevers, chills, headaches, nausea, etc. when we have an infection?
2. How do cells, or multicellular organisms protect themselves against viruses? If they didn’t have protective measures, what would happen?
3. How do cells replicate or reproduce themselves?
4. What is the difference between DNA and RNA?
5. Is genetic material ever swapped between viruses and cells?

**Potential Extensions**

- Do a unit on blood typing.
- Have students research antibodies and antibody resistance.

**Helpful Websites**

- Centers of Disease Control and Prevention: [http://www.cdc.gov/](http://www.cdc.gov/)
- The Garry Lab, Tulane University: [http://www.virology.org](http://www.virology.org)
- The Virtual Virus Experience: [http://www.thinkquest.org/](http://www.thinkquest.org/)

**Student Masters**

- *Viruses*
- *Assignment: What Are Viruses?*
VIRUSES

Student Objectives. The student will be able to:

1. Describe or produce a model of the structure of a virus.
2. Diagram and/or explain the viral cycle, and viral replication inside a cell.
3. Define the following terms: bacteriophage, capsid, replication, nucleic acid, DNA, RNA, amino acid, protein, and nucleotides.

Structure of a Virus (T-Even Bacteriophage)

Adapted from: http://www.virology.org
nucleic acid.

_________________________ is a rod like structure that consists of a retractable sheath surrounding a central hallow core. It is attached to the capsid.

_________________________ is at the very end of the core. It is a spiked plate carrying 6 slender tail fibers which help anchor the virus to its host.

Nucleic Acids

_________________________ are considered life’s master molecules because they code for all of the molecules that make up and operate the cell, or in this case, the virus. There are two nucleic acids.

1. _________________________ (deoxyribonucleic acid)
2. _________________________ (ribonucleic acid)
3. The nucleic acid of a virus - the viral chromosome - may be either DNA or RNA, single stranded or double stranded, circular or linear. Viral chromosome vary greatly in size, from some 5,400 nucleotides (4 bases A,T,G,C) to 180,000 for the T-Even Bacteriophage.
4. _________________________ are molecules which contain a sugar (ribose or deoxyribose), a phosphate group, and a base (A,T, G, C, U). They hook (bond) together to make up the RNA or DNA molecules.

Adapted from: http://www.virology.org
Function of RNA and DNA

The RNA or DNA supplies the codes for building the protein coat (capsid) and for producing certain enzymes needed to replicate more viruses. The codes also provide enzymes that allow the newly built viruses to do something called lyse (or break through the cell). Which in turn totally ruptures the cells outer membrane, thus totally destroying it.

The Viral Cycle

[Diagram showing the viral cycle with labels: Host Cell, Cell DNA, Viral DNA, New Viruses being created.]

Adapted from: www.thinkquest.org

Phase 1

Phase 2
Phase 3

Inserting the Virus DNA into the Cells DNA

After the viral DNA enters the cell, it will find its way to the cell’s DNA and literally insert its DNA into the chain of the cell’s DNA. This alters the cell’s "instruction manual," telling it to create more viruses rather than working for the cell!

Creating the Proteins for the new Viruses

1. _________________________ strand is transcribed from a DNA template.

2. _________________________ molecule with its amino acid plugs into a slot (known as an anticodon) momentarily with the codon on the mRNA.

Adapted from: www.thinkquest.org
3. _________________________ moves along the mRNA strand so that another tRNA can come into the next slot with an anticodon that matches the codon on the mRNA.

4. _________________________ next to each other form a polypeptide bond, creating a protein chain. This protein will then be used to create the capsid and other parts of the next generation of viruses.
Assignment: What Are Viruses?

1. Draw and label a diagram, or produce a model of the structure of a virus.
2. Diagram and/or explain the viral cycle, and viral replication inside a cell.
3. In your own words, define the following terms:

Bacteriophage:

Capsid:

Replication:

Nucleic acids:

DNA and RNA:

Amino acids:
Proteins:

________________________________________________________________________

Nucleotides:

________________________________________________________________________

Questions For Discussion

4. How do cells, or multicellular organisms protect themselves against viruses? If they didn’t have protective measures, what would happen?

5. Why do we have the symptoms of fever, headache, nausea, etc. when we have a viral infection?

6. How do cells replicate or reproduce themselves?
APPENDIX C

Student Handouts for

Water in Arizona PBL
Suggested Websites and Resources for Water Use Unit


http://geochange.er.usgs.gov/sw/impacts/hydrology/water_use/


Water As A Transporter

**Student Objectives.** The student will be able to:

1. Describe three historical events that relied on water as a means of transporting people or goods.
2. Identify three different geographic locations and describe how and when water is used as a means of transportation with respect to the limitations of water resources within each region.
3. Describe two ways in which water is used to transport materials mechanically.
4. Describe one way in which water is used to transport materials chemically.

**Arizona State Science Standards Addressed:** Grade 7, S2C1(PO1-PO4), S2C1(PO1-PO3).

**Methods:** Class discussion: student generated responses listed, then prioritized by students.

**Historical Use Of Water As A Transporter**

**Question:** What major events do you know of in mankind’s history when the use of water to transport people or goods was a major factor in shaping the future of civilization?

**Historical Event 1:** _________________________________________________

Description of Event:

__________________________________________________________________
__________________________________________________________________
__________________________________________________________________

Date of Event: __________________

Geographic Location(s):

__________________________________________________________________

Impact on Future Civilization:

__________________________________________________________________
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<td>Geographic Location(s):</td>
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Use Of Water Geographically

**Question**: How is water used differently as a transporter in different regions of the world?

**Geographic Location 1**: 

Limitations on Water Use:

Technologies or Adaptations Used:

**Geographic Location 2**: 

Limitations on Water Use:

Technologies or Adaptations Used:
Geographic Location 3:

Limitations on Water Use:

__________________________________________________________________
__________________________________________________________________
__________________________________________________________________

Technologies or Adaptations Used:

__________________________________________________________________

Water As A Mechanical Transporter

**Question**: How is water used to mechanically move things?

**Mechanical Use 1**: _______________________________________________

Explanation:
__________________________________________________________________
__________________________________________________________________
__________________________________________________________________

**Mechanical Use 2**: _______________________________________________

Explanation:
__________________________________________________________________
__________________________________________________________________
__________________________________________________________________

Water As A Chemical Transporter

**Question**: How is water used to move chemicals?

**Chemical Use**: _________________________________________________

Explanation:
__________________________________________________________________
__________________________________________________________________
__________________________________________________________________
__________________________________________________________________
__________________________________________________________________
Water Use

Student Objectives. The student will be able to:

1. Identify and quantify his/her own personal use of water.
2. Estimate quantity of home water use.
3. Convert gallons to acre-feet and km³
4. Identify and estimate water use in a community.

Arizona State Science Standards Addressed: Grade 7, S1C1(PO1-PO3), S1C2(PO1-PO5), and S1C1(PO1-PO7).

Personal Water Use Log

Prediction: How much water do you think you use each day?

Directions: Use the Individual Water Use Chart to record the type of use and the estimated quantity used each time you use water in one day (~24 hours). Estimate in gallons how much water is used each time you use it.

Results: How did the actual amount of water you used in one day compare to your prediction?

___________________________
___________________________
___________________________

Domestic Water Use

Prediction: How much water do you think your family uses in a year?

Directions: Discuss the following questions with your family to come up with an accurate count of activities that require water.

1. How many loads of dishes are washed by hand in the kitchen sink each week?
2. How many loads of dishes are washed in a dishwasher each week?
3. How many loads of laundry are washed in a washing machine each week?
4. How many hours is a garden hose run each week for watering plants, lawns, washing cars, or any other uses of the hose?
5. How much water is used to fill pools, fountains, fish tanks, animal water tanks, etc., and how often are they filled?

Estimate family (domestic) use of water for a year by doing the following calculations.

1. Multiply number of family members (including yourself) by the number of gallons of water you used as an individual in one day. Then multiply that product by 365 days to estimate personal use in your family for a year.
2. Multiply the number of loads of dishes washed in the sink each week by 20 gallons (national average). Then multiply that product by 52 to estimate yearly use for dishes.
3. Multiply the number of loads of dishes washed in a dishwasher each week by 15 gallons (national average). Then multiply that product by 52 to estimate yearly use for dishwasher.
4. Multiply the number of loads of laundry washed each week by 40 gallons (national average). Then multiply that product by 52 to estimate yearly use for washing machine.
5. Multiply by 600 gallons the number of hours the garden hose is used each week for watering lawns, gardens, or washing cars, etc. Multiply this product by 52 to estimate yearly use.
6. Add the totals for each category of use above to estimate total domestic use by your family per year. You may need to add additional categories for swimming pools, spas, farm animals, etc.

Results: How does the amount of water your family uses in a year compare to your prediction?

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

Research: Find your state and national averages for the amount of water used each year per person. Find the population of your community. Estimate the total water use in your community per year for domestic use. How much is used by businesses, industry, and agriculture each year in your community? What is the total water use in your community each year? How many gallons are in one acre-foot? How many gallons are in one cubic kilometer?
### Individual Use of Water

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Water Use</th>
<th>Amount (gallons)</th>
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<td>Total</td>
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</table>
Average Daily Water Consumption

The figures below are the average daily amounts of consumption in the United States for common uses. Data are from the Environmental Protection Agency.

**Average Household**

- Bath = **36 gallons**  
- Brushing Teeth with Tap Running = **2 gallons**  
- Dishwasher = **15 gallons**  
- Hand Washing with Tap Running = **2 gallons**  
- Shaving with Tap Running = **20 gallons**  
- Shower (5 min.) = **25-35 gallons**  
- Toilet Flush = **5-7 gallons**  
- Washing Car with Tap Running = **180 gallons**; Stopping Tap = **40-50 gallons**  
- Washing Dishes by Hand with Tap Running = **20-30 gallons**; Wash, Rinse in sink = **5 gallons**  
- Washing Machine (Top Loading) = **40 gallons**; (Front Loading) = **25 gallons**  
- Watering Lawn with Tap Running = **10 gal/min**

The average household of four people in this country uses about **243 gallons** of water per day.  
The average water use per person (including non-home water use) is over **100 gallons** per day.
Water Availability

Student Objectives. The student will be able to:

1. Draw and label a diagram showing a cross-section of a watershed that includes surface water, groundwater, water table, groundwater recharge and groundwater removal.
2. Describe what an aquifer is.
3. List the number of gallons in one acre-foot of water.
4. List the number of MAF (millions of acre feet) in a cubic kilometer (Km³).
5. List the estimated amount of water used (MAF) in Arizona each year and the estimated amount of water available in Arizona.
6. List the estimated amount of water used (Km³) in the United States each year and the estimated amount of water available in the United States.
7. List the estimated amount of water used (Km³) worldwide each year and the estimated amount of water available worldwide.
8. Identify the three continents or landmasses with the most available fresh water, and the three continents that use the most water.
9. List the estimated amount of water used in the San Pedro River Basin each year and the amount of water available.
10. Identify the major categories of water use locally, statewide, nationally and worldwide.

Directions. Research current water resources data on the internet. Visit the USGS and the Arizona Department of Water Resources sites on the internet. Share the information in class and agree on rounded figures for the class to use before filling in the information below.

1. ____________________ is the average amount of domestic water use per person in Arizona.
2. ____________________ is the total amount of estimated water use in the San Pedro River Basin.
3. ____________________ is the total amount of water estimated to be in the San Pedro Basin aquifer.
4. ____________________ is the percent of water used for in the San Pedro Basin for agriculture.
5. ____________________ is the percent of water used for industry and business in the San Pedro Basin.

6. ____________________ is the percent of water used for mining in the San Pedro Basin.

7. ____________________ is the percent of water used for domestic purposes in the San Pedro Basin.

8. ____________________ is about 326,000 gallons.

9. ____________________ is the total estimated amount of water used each year in Arizona in millions of acre feet (MAF).

10. ____________________ is the total estimated amount of water used in the United States in MAF for the year 2000.

11. ____________________ is the total estimated amount of water used worldwide in Km$^3$.

12. ____________________ is the number of acre-feet in a Km$^3$.

13. The two top categories of water use in Arizona are:
    A. ____________________ B. ____________________

14. The two top categories of water use in the United States are:
    A. ____________________ B. ____________________

15. The two top categories of water use globally are:
    A. ____________________ B. ____________________

16. The three continents with the greatest amount of available fresh water are:
    A. ____________________ B. ____________________
    C. ____________________
17. The three continents that use the most water are:

A. ____________________  B. ____________________  C. ____________________

18. Define or describe each of the following terms.

A. Aquifer:

B. Groundwater:

C. Aquifer Recharge:

D. Aquifer Withdrawal:

E. Water Table:

F. Surface Water:

G. Domestic Use:

H. Watershed:

19. Draw and label a diagram showing a cross-section of a small watershed. Show (a) how water is deposited into the watershed, (b) how it flows above ground, (c) how it flows below ground, and (d) how it is withdrawn for use.
Water Availability Key

1. **18,000 gal/yr** is the average amount of domestic water use per person in Arizona.

2. **78,000 acre-feet** is the total amount of estimated water use in the San Pedro River Basin.

3. **59 MAF** is the total amount of water estimated to be in the San Pedro Basin aquifer.

4. **67%** is the percent of water used for agriculture in the San Pedro Basin.

5. **17%** is the percent of water used for industry and business in the San Pedro Basin.

6. **2%** is the percent of water used for mining in the San Pedro Basin.

7. **14%** is the percent of water used for domestic purposes in the San Pedro Basin.

8. **One acre-foot** is about 326,000 gallons.

9. **7 MAF** is the total estimated amount of water used each year in Arizona in millions of acre feet (MAF).

10. **460 MAF** is the total estimated amount of water used in the United States in MAF for the year 2000.

11. **4000 Km³** is the total estimated amount of water used worldwide in Km³.

12. **810,000** is about the number of acre-feet in a Km³.

13. The two top categories of water use in Arizona are:

    A. Agriculture  B. Industry

14. The two top categories of water use in the United States are:

    A. Agriculture  B. Thermoelectric

15. The two top categories of water use globally are:
16. The three continents or landmasses with the greatest amount of available fresh water are:
   A. Antarctica  
   B. Greenland  
   C. North America

17. The three continents that use the most water are:
   A. Asia  
   B. North America  
   C. Europe

18. Define or describe each of the following terms.
   A. Aquifer:
   B. Groundwater:
   C. Aquifer Recharge:
   D. Aquifer Withdrawal:
   E. Water Table:
   F. Surface Water:
   G. Domestic Use:
   H. Watershed:
19. Draw and label a diagram showing a cross-section of a small watershed. Show (a) how water is deposited into the watershed, (b) how it flows above ground, (c) how it flows below ground, and (d) how it is withdrawn for use.
Review: Water Use and Availability

1. ____________________ is the average amount of domestic water use per person in Arizona.

2. ____________________ is the total amount of estimated water use in the San Pedro River Basin.

3. ____________________ is the total amount of water estimated to be in the San Pedro Basin aquifer.

4. ____________________ is the percent of water used for in the San Pedro Basin for agriculture.

5. ____________________ is the percent of water used for industry and business in the San Pedro Basin.

6. ____________________ is the percent of water used for mining in the San Pedro Basin.

7. ____________________ is the percent of water used for domestic purposes in the San Pedro Basin.

8. ____________________ is equal to 325,851 gallons.

9. ____________________ is the total estimated amount of water used each year in Arizona in millions of acre feet (MAF).

10. ____________________ is the total estimated amount of water used in the United States in MAF for the year 2000.

11. ____________________ is the total estimated amount of water used worldwide in Km$^3$.

12. ____________________ is the number of acre-feet in a Km$^3$.

13. The two top categories of water use in Arizona are:
    A. ____________________ B. ____________________
14. The two top categories of water use in the United States are:
   A. ____________________  B. ____________________

15. The two top categories of water use globally are:
   A. ____________________  B. ____________________

16. The three continents with the greatest amount of available fresh water are:
   A. ____________________  B. ____________________  C. ____________________

17. The three continents that use the most water are:
   A. ____________________  B. ____________________  C. ____________________

18. Define or describe each of the following terms.
   A. Aquifer:

   B. Groundwater:

   C. Aquifer Recharge:

   D. Aquifer Withdrawal:

   E. Water Table:

   F. Surface Water:

   G. Domestic Use:
H. Watershed:

19. Draw and label a diagram showing a cross-section of a small watershed. Show (a) how water is deposited into the watershed, (b) how it flows above ground, (c) how it flows below ground, and (d) how it is withdrawn for use.

20. Describe what the term “sustainable” means with respect to water usage.

21. Explain how water budgets may be used for local, state or national planning.
Test: Water Use and Availability

1. ____________________ is equal to 325,851 gallons.

2. ____________________ is the total amount of estimated water use in the San Pedro River Basin.

3. ____________________ is the total amount of water estimated to be in the San Pedro Basin aquifer.

4. ____________________ is the percent of water used for domestic purposes in the San Pedro Basin.

5. ____________________ is the percent of water used for agriculture in the San Pedro Basin.

6. ____________________ is the percent of water used for industry and business in the San Pedro Basin.

7. ____________________ is the percent of water used for mining in the San Pedro Basin.

8. ____________________ is the average amount of domestic water use per person in Arizona.

9. ____________________ is the number of acre-feet in a Km$^3$.

10. ____________________ is the total estimated amount of water used each year in Arizona in millions of acre feet (MAF).

11. ____________________ is the total estimated amount of water used in the United States in MAF for the year 2000.

12. ____________________ is the total estimated amount of water used worldwide in Km$^3$.

13. The two top categories of water use in the United States are:
    A. ____________________  B. ____________________
14. The two top categories of water use globally are:
   A. ____________________  B. ____________________

15. The two top categories of water use in Arizona are:
   A. ____________________  B. ____________________

16. The three continents that use the most water are:
   A. ____________________  B. ____________________  C. ____________________

17. The three continents with the greatest amount of available fresh water are:
   A. ____________________  B. ____________________  C. ____________________

18. Define or describe each of the following terms.
   A. Aquifer:

   B. Groundwater:

   C. Aquifer Recharge:

   D. Aquifer Withdrawal:

   E. Water Table:

   F. Surface Water:

   G. Domestic Use:
H. Watershed:

19. Draw and label a diagram showing a cross-section of a small watershed. Show (a) how water is deposited into the watershed, (b) how it flows above ground, (c) how it flows below ground, and (d) how it is withdrawn for use.

20. Describe what the term “sustainable” means with respect to water usage.

21. Explain how water budgets may be used for local, state or national planning.
Water Quality

Student Objectives. The student will be able to:

1. List three chemical pollutants of water, identify their sources, explain their mode of movement into water supplies, and list the health problems associated with each.
2. List three biological contaminants of water, identify their sources, explain their mode of movement into water supplies, and list the health problems associated with each.
4. Explain the role of the Environmental Protection Agency with respect to water quality.

Arizona State Science Standards Addressed: Grade 7, S1C1(PO1-PO3), S1C2(PO1-PO5), and S1C1(PO1-PO7).

Directions. For the questions below, circle the answers that best match your opinion on water.

1. How important is it for you to have clean water?
   Not that important  Somewhat important  Very important

2. How serious of an issue do you think water pollution is in our country?
   Not that serious  Somewhat serious  Very serious

3. How concerned are you about the quality of the water you drink?
   Not too concerned  Somewhat concerned  Very concerned

4. Can you tell the quality of water by its taste?
   Yes  No
Directions. Read the brief excerpts of the articles handed to you and fill in the answers to the following questions for each article.

Title of Article 1:
________________________________________________________________________
________________________________________________________________________

Newspaper Title:
________________________________________________________________________

Location of Problem (City and State or Country):
________________________________________________________________________

Brief summary of article:
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

List of pollutants in water:
________________________________________________________________________
________________________________________________________________________

Source of pollutants:
________________________________________________________________________
________________________________________________________________________

How pollutants spread:
________________________________________________________________________
________________________________________________________________________

Where pollutants found:
________________________________________________________________________
________________________________________________________________________

Health problems associated with pollutants:
________________________________________________________________________
________________________________________________________________________

Questions you have about article:
________________________________________________________________________
________________________________________________________________________

Title of Article 2:
________________________________________________________________________
________________________________________________________________________

Newspaper Title:
________________________________________________________________________
Location of Problem (City and State or Country):
________________________________________________________________________

Brief summary of article:
________________________________________________________________________
________________________________________________________________________
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List of pollutants in water:
________________________________________________________________________
________________________________________________________________________

Source of pollutants:
________________________________________________________________________
________________________________________________________________________

How pollutants spread:
________________________________________________________________________
________________________________________________________________________

Where pollutants found:
________________________________________________________________________
________________________________________________________________________

Health problems associated with pollutants:
________________________________________________________________________
________________________________________________________________________

Questions you have about article:
________________________________________________________________________
________________________________________________________________________

Title of Article 3:
________________________________________________________________________
________________________________________________________________________

Newspaper Title:
________________________________________________________________________

Location of Problem (City and State or Country):
________________________________________________________________________

Brief summary of article:
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
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________________________________________________________________________
List of pollutants in water:

Source of pollutants:

How pollutants spread:

Where pollutants found:

Health problems associated with pollutants:

Questions you have about article:

Directions. Share the information you found with other students within your group. After all members of your group have shared their information, answer the following questions as a group.

1. List any commonly found pollutants:

2. List the most common sources of pollution:

3. List the most serious health hazards associated with pollutants:

4. What role does the Environmental Protection Agency (EPA) play in water quality?

**Directions.** Share your group’s summary with the other groups in class. As a class, prioritize the water pollutants by health risk and frequency of occurrence. Discuss the importance of the Clean Water Act and the EPA to public health. What historical trends, if any, have occurred with respect to water quality? Do you have any questions or concerns about the quality of your water? Revisit the questions asking your opinions on different water quality issues. Have your opinions changed?
Lab: What Is A ppm?

Overview

Many of the articles we read about toxic chemicals mention concentration levels at which the toxins become serious health risks. The Environmental Protection Agency (EPA) measures toxins in the air and in the water in units called parts per million (ppm) and parts per billion (ppb). There are some chemicals that have very serious effects on biological systems (cells) in very small amounts. You may see maximum safe levels for some of these chemicals in only 10-20 ppm. So what is a ppm?

Problem

Can you detect a dye (methylene blue) or aromatic compound (acetic acid) at a concentration of 1 ppm with your own senses of eyesight and smell?

Hypothesis

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

Procedures

1. Measure 100 mL of water in a graduated cylinder and pour it into a 500 mL beaker.
2. Measure 1 mL of methylene blue and pour it into the 100 mL of water and stir. This gives you a concentration of one part per hundred. Can you still see the blue coloring of the water?
3. Rinse the graduated cylinder with water and measure another 100 mL of water and pour into a clean 500 mL beaker. From the first beaker measure 1 mL of diluted methylene blue (one part per hundred) and pour it into the 100 mL of clean water. Stir. This gives you a concentration of one part per 10,000. Can you still see a blue color to the water? Rinse the first beaker (one part per hundred methylene blue) with water.
4. Repeat procedure three, diluting the one part per 10,000 mixture of methylene blue into 100 mL of clean water to obtain a one part per million (ppm) concentration. Can you still see the blue color in the water?
5. Rinse all beakers and graduated cylinder with water and repeat procedures one through four using acetic acid. At which concentration can you not smell the acetic acid any more?

Results

<table>
<thead>
<tr>
<th>Methylene Blue Concentration</th>
<th>Can see blue coloring?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/100</td>
<td>Yes</td>
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<tr>
<td>1/10,000</td>
<td>Yes</td>
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<tr>
<td>1/1,000,000</td>
<td>Yes</td>
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<tr>
<th>Acetic Acid Concentration</th>
<th>Can smell acetic acid?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/100</td>
<td>Yes</td>
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<tr>
<td>1/10,000</td>
<td>Yes</td>
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<tr>
<td>1/1,000,000</td>
<td>Yes</td>
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</table>

Conclusion

Can you detect the methylene blue at a concentration of 1 ppm? If not, at which concentration could you still detect it?

Can you detect the acetic acid at a concentration of 1 ppm? If not, at which concentration could you still detect it?

Would you be able to detect a hazardous chemical in your water at a concentration of 1 ppm?

Lab Report

Write a lab report which includes your problem, hypothesis, procedures, results and conclusion.
Water Quality Analysis

Directions for Collection of Water Samples:

Collect water samples from various locations around your community. Try to get samples from wells, streams, and ponds as well as from city water supply. If there are major water sources (lakes or rivers), you may wish to take samples from various locations along the water source. Unless conducting water tests on site on a scheduled field trip, students should collect water samples in clean plastic containers and fill to top so that no air (oxygen) dissolves into the water.

<table>
<thead>
<tr>
<th>Sample #</th>
<th>GPS coordinates Or location</th>
<th>Elevation (ft)</th>
<th>Description of Site</th>
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Directions for Water Quality Analyses:

Each group will do the following water quality tests using Green Water Monitoring kits from LaMotte: pH, nitrates, phosphates and coliform bacteria. Follow the kit instructions for each test. Samples will be divided among the different groups to decrease time required. Fill in the data sheet on the back of this handout as you obtain results (get data from other groups after completion of analyses).
### Summary of Data:

Write a lab report which includes the following: (1) an introduction explaining the tests performed and what a positive test indicates; (2) general location of sampling sites; (3) collection procedures; (4) testing procedures; (5) results with chart; and (6) a conclusion which identifies any sites that show signs of contamination, paying special attention to any patterns to those locations.

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>Phosphates</th>
<th>Nitrates</th>
<th>Coliforms</th>
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</table>
Review: Water Quality

1. List three chemical pollutants of water. For each pollutant, identify its source, explain its mode of movement into water supplies, and list the health problems associated with it.

   A. Pollutant: _________________________
      Source(s): ______________________________________________________
                       __________________________________________________________________
      Mode of movement into water supply: ________________________________________________
                       __________________________________________________________________
      Health risks: ________________________________________________________________
                       __________________________________________________________________

   B. Pollutant: _________________________
      Source(s): ______________________________________________________
                       __________________________________________________________________
      Mode of movement into water supply: ________________________________________________
                       __________________________________________________________________
      Health risks: ________________________________________________________________
                       __________________________________________________________________

   C. Pollutant: _________________________
      Source(s): ______________________________________________________
                       __________________________________________________________________
      Mode of movement into water supply: ________________________________________________
                       __________________________________________________________________
      Health risks: ________________________________________________________________
                       __________________________________________________________________

2. List three biological pollutants of water. For each pollutant, identify its source, explain its mode of movement into water supplies, and list the health problems associated with it.

   Pollutant 1: _________________________
      Source(s): ______________________________________________________
                       __________________________________________________________________
      Mode of movement into water supply: ________________________________________________
                       __________________________________________________________________
      Health risks: ________________________________________________________________
                       __________________________________________________________________
Pollutant 2: _________________________
Source(s): ______________________________________________________
__________________________________________________________________
Mode of movement into water supply: ______________________________
__________________________________________________________________
Health risks: ______________________________________________________
__________________________________________________________________

Pollutant 3: _________________________
Source(s): ______________________________________________________
__________________________________________________________________
Mode of movement into water supply: ______________________________
__________________________________________________________________
Health risks: ______________________________________________________
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4. Explain the role of the Environmental Protection Agency with respect to water quality.
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________________________________________________________________________
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5. Describe what pH is an indicator of in water quality testing.
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6. Describe what nitrate is an indicator of in water quality testing.

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7. Describe what phosphate is an indicator of in water quality testing.

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8. Describe what coliform bacteria are indicators of in water quality testing.

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
Test: Water Quality

1. List three chemical pollutants of water. For each pollutant, identify its source, explain its mode of movement into water supplies, and list the health problems associated with it.

   A. Pollutant: _________________________
      Source(s): ______________________________________________________
      Mode of movement into water supply: ______________________________
      Health risks: ______________________________________________________

   B. Pollutant: _________________________
      Source(s): ______________________________________________________
      Mode of movement into water supply: ______________________________
      Health risks: ______________________________________________________

   C. Pollutant: _________________________
      Source(s): ______________________________________________________
      Mode of movement into water supply: ______________________________
      Health risks: ______________________________________________________

2. List three biological pollutants of water. For each pollutant, identify its source, explain its mode of movement into water supplies, and list the health problems associated with it.

   A. Pollutant: _________________________
      Source(s): ______________________________________________________
      Mode of movement into water supply: ______________________________
      Health risks: ______________________________________________________
B. Pollutant: _________________________
Source(s): ______________________________________________________
__________________________________________________________________
Mode of movement into water supply: ______________________________
__________________________________________________________________
Health risks: ______________________________________________________
__________________________________________________________________

C. Pollutant: _________________________
Source(s): ______________________________________________________
__________________________________________________________________
Mode of movement into water supply: ______________________________
__________________________________________________________________
Health risks: ______________________________________________________
__________________________________________________________________

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4. Explain the role of the Environmental Protection Agency with respect to water quality.
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________________________________________________________________________
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7. Describe what phosphate is an indicator of in water quality testing.

________________________________________________________________________
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________________________________________________________________________
________________________________________________________________________

8. Describe what coliform bacteria are indicators of in water quality testing.

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
Name:
Science
Date:

Water In The Future

Student Objectives. The student will be able to:

1. Research trends in population growth and water use, and predict future water uses and availability.
2. Describe a technology or invention that may be used in the future to meet the needs of a limited water supply.
3. Prepare and present a presentation to class about future water needs and availability that includes map(s), charts and/or graphs, and future technology.

Directions:

You are a member of a team that includes the following specialists: (a) population analyst (responsible for researching and predicting population trends), (b) hydrologist (responsible for researching and predicting water use and water quality trends), (c) cartographer (responsible for preparing maps, graphs and charts from data), and (d) engineer (responsible for designing new technologies). Your team needs to work together to present a prediction of what human civilization will be like in the year 2105 AD with respect to water resources, and provide some solutions to possible problems that may need to be addressed.

Your presentation should include the following:

1. A graph showing world water use and predicted trends, with notations on predicted water quality. 20 pts.
2. A graph showing world population growth and predicted trends. 20 pts.
3. A global map showing predicted populations for each continent for the year 2105 AD, with predicted water use and availability overlaid on each continent. 20 pts.
4. A picture or diagram of a new technology or invention that will address water use needs in the year 2105 AD. 20 pts.
5. Your group presentation to class will be worth 20 pts. (10 pts. for organization of information presented and 10 pts. for participation of all members of team).
Water In The Future Presentation

__________ Graph showing world water use and predicted trends (15 pts), with notations on predicted water quality (5 pts). 20 pts possible.

__________ Graph showing world population growth and predicted trends. 20 pts possible.

__________ Global map showing predicted populations for each continent for the year 2105 AD (10 pts), with predicted water use and availability overlaid on each continent (10 pts). 20 pts possible.

__________ Picture or diagram of a new technology or invention that will address water use needs in the year 2105 AD (10 pts), and explanation (10 pts). 20 pts possible.

__________ Group presentation to class will be worth 20 pts. (10 pts. for organization of information presented and 10 pts. for participation of all members of team). 20 pts possible.

__________ Total Points for Presentation
Directions. For the questions below, circle the answers that best match your opinion on water.

1. How important is it for you to have clean water?
   Not that important  Somewhat important  Very important

2. How serious of an issue do you think water pollution is in our country?
   Not that serious  Somewhat serious  Very serious

3. How concerned are you about the quality of the water you drink?
   Not too concerned  Somewhat concerned  Very concerned

4. Can you tell the quality of water by its taste?
   Yes  No

5. Can your senses detect a chemical in water at a concentration of 10 ppm?
   Yes  No

6. Do you think water will always be available in quantities to meet all of society’s needs?
   Yes  No

7. How concerned are you about the availability of water in your future?
   Not too concerned  Somewhat concerned  Very concerned

8. Are aquatic ecosystems (rivers, lakes, streams, etc.) good indicators of water availability and/or water quality?
   Yes  No

9. In Arizona, is groundwater being recharged as rapidly as it is being withdrawn?
   Yes  No
2

10. Worldwide, what do humans use the most water for?

<table>
<thead>
<tr>
<th>Drinking</th>
<th>Bathing</th>
<th>Agriculture</th>
<th>Industry</th>
<th>Other</th>
</tr>
</thead>
</table>

Writing Prompt: Camping Trip

Directions:

Read the situational prompt below and write a short story (fiction) based on the situation. Make it as real-life as possible using what you have learned about water quality, but add drama. Use all of the elements of effective writing. Your story will be graded using the six traits writing rubric.

Prompt:

You and your family went up to the mountains for a camping trip over the weekend. You set up camp along the side of a little creek that flows out of canyon. Around your camp are large ponderosa pine trees, and blue spruce trees. You look up the creek and see where it leads into a golden aspen thicket as the canyon narrows towards the top of the mountain. You decide to hike up the creek to the aspen thicket and see what is in the canyon. As you begin to enter the aspen thicket, you notice an old 50 gallon drum near the creek bed. It is rusted through, and not much left to it. You begin to notice that there is a yellowish coloring to the soil below the drum and …