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Why do Plants Wilt? Investigating Students' Understanding of Water Balance in Plants with External Representations at the Macroscopic and Submicroscopic Levels

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ABSTRACT

In order to understand water balance in plants, students must understand the relation between external representations at the macroscopic, microscopic, and submicroscopic levels. This study investigated how Slovenian students (*N* = 79) at the primary, secondary, and undergraduate tertiary levels understand water balance in plants. The science problem consisted of a text describing the setting, visualizations of the process occurring in a wilted plant stem, and five tasks. To determine students' visual attention to the various elements of the tasks, we used eye tracking and focused on the total fixation duration in particular areas of interest. As expected, primary school students showed less knowledge and understanding of the process than the secondary school and university students did. Students with correct answers spent less time observing the biological phenomena displayed at the macroscopic and submicroscopic levels than those with incorrect answers, and more often provided responses that combined the macro-, micro-, and submicroscopic levels of thought. Learning about difficult scientific topics, such as the water balance in plants, with representations at the macroscopic and submicroscopic levels can be either helpful or confusing for learners, depending on their expertise in using multiple external representations, which is important to consider in biology and science education.

Keywords: biology, eye tracking, multiple representations, osmosis, students

INTRODUCTION

In order to understand basic biology concepts, it is important for students to develop an understanding of the transport of materials across cell membranes. Learning about the mechanisms underlying water balance in plant cells is dependent on understanding osmosis and diffusion (Malińska, Rybska, Sobieszczuk-Nowicka, & Adamiec, 2016). Diffusion is the primary method of short-distance transport in cells and cellular systems. It is defined as the random, thermal movement of molecules in which a net flow of matter moves along a concentration gradient, i.e., from an area of higher concentration toward an area of lower concentration (Sperelakis, 2012). Osmosis is used to explain water uptake by plants, turgor pressure in plants, water balance in aquatic creatures, and transport in living organisms. It is the flow of a solvent across a semipermeable membrane from a region of lower to higher solute concentration (Sperelakis, 2012).

Johnstone and Mahmoud (1980) published a very influential study about the learning difficulties encountered by Scottish secondary school students and university students in biology. Two of the most troublesome topics proved to be genetics and water transport in plants. Problems concerning water transport may result from the fact that this topic is related to the processes of diffusion and osmosis (Malińska et al., 2016). Several studies (AlHarbi, Treagust, Chandrasegaran, & Won, 2015; Malińska et al., 2016; Odom, 1995; Odom & Barrow, 1995; Odom & Kelly,

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Contribution of this paper to the literature

- Students with correct answers spent less time observing the biological phenomena displayed at the macroscopic and submicroscopic levels and they more often provided responses that combined macro-, micro-, and submicroscopic levels of thought.
- When responding to the task correctly, primary school students mostly provided answers at the macroscopic level, but when their explanations included micro- or submicroscopic levels of thought the percentage of incorrect answers increased substantially.
- The study provides evidence that students regularly use the microscopic or "cell level" of thought to explain complex biological phenomena.

2001; Sanger, Brecheisen, & Hynek, 2001; She, 2004; Tomažič & Vidic, 2012; Zuckerman, 1988) reported that students have many difficulties understanding diffusion and osmosis processes. According to these studies, difficulties with understanding diffusion and osmosis are the result of 1) confusion regarding vernacular and scientific usage of terms such as pressure, concentration, and quantity; 2) misunderstanding technical concepts such as solution, semipermeability, and molecular and net movement; and 3) insufficient abilities in terms of formal reasoning, visualization, and thinking at the molecular (or submicroscopic) level.

Panizzon (2003) wrote that one step toward a better understanding of diffusion and osmosis would be to provide students with a range of learning opportunities that would enable them to gain different experiences to explore and build their understanding of diffusion and osmosis. Tomažič and Vidic (2012) found that pre-service teachers that had actively approached the concepts of (e.g., conducted experiments on) diffusion and osmosis in upper secondary school achieved significantly higher scores on a diffusion and osmosis diagnostic test. AlHarbi et al. (2015) found that pre-service teachers' understanding of osmosis and diffusion concepts was mildly positively correlated with their understanding of particle theory. The findings suggest that greater time and attention need to be invested in teaching particle theory for students to ensure their scientific understanding of diffusion and osmosis. Sanger et al. (2001) demonstrated that students that observed computer animations depicting the molecular processes when perfume particles diffuse in air and when water osmoses through a semi-permeable membrane developed more accurate conceptions of these processes based on particulate nature and random motion of matter.

Teaching diffusion and osmosis should not be limited to acquiring decontextualized and unrelated facts (Odom, 1995), or to learning these concepts for their own sake (Hasni, Roy, & Dumais, 2016). To build understanding, students should be able to link new concepts with those that are already familiar to them (Marek, Cowan, & Cavallo, 1994). In the case at hand, students need to link biology processes such as water uptake by plants and turgor pressure in plants with diffusion and osmosis. Furthermore, Tomažič and Vidic (2012) made the important conclusion that observation alone at a macroscopic level of processes explained by diffusion and osmosis is not sufficient, and that a link must be made to also understand it at the submicroscopic level.

Johnstone (1991) argued that understanding of science concepts can be explained with triple levels of representations: the macroscopic, submicroscopic, and symbolic level. Taking his model into account, to understand turgor pressure in plants properly the learner should consider the macroscopic level at which biological structures are visible to the naked eye, the submicroscopic level at which the interactions between particles are shown, and the symbolic level that uses symbols, formulas, chemical equations, etc., to explain the mechanism of the phenomenon. However, Tsui and Treagust (2013) claimed that in biology four levels of representations should be considered instead of three. Due to the hierarchical organization of biological entities (e.g., cells are nested within tissues, these are nested within organs, etc.), another level of representation should be added, i.e. the microscopic level at which structures are only visible under a microscope.

According to Ainsworth (1999), translation refers to learning situations in which a student must comprehend a relation between external representations at different levels: for example, understanding turgor pressure in plants at the macroscopic level (e.g., a photo of a plant), microscopic level (e.g., a microscopic image of plant cell), and the submicroscopic level (e.g., animations of molecules and particles). Treagust and Tsui (2013) claimed that learning biology with multiple external representations enables constructing deeper understanding in terms of scientific reasoning.

Ainsworth (2008) argued that multiple representations are powerful tools, but need careful handling if learners are to use them successfully. She made the following recommendations on how to use multiple representations to support the acquisition of complex scientific knowledge. First, the minimum number of representations that are required for a learner to understand should be used. Secondly, the skills and experience with particular type of external representation (e.g., diagram, graph, equation) of the intended learners should be assessed. Thirdly, the representations should be sequenced to gradually introduce a concept, allowing learners to gain knowledge and confidence with fewer representations before introducing more. Next, it should be considered what extra support is needed for learners to overcome all the cognitive tasks related to learning with multiple representations (e.g., exercises, consistent colours, labels and symbols). And finally, it should be considered what pedagogical functions external representations have. For example, if the primary goal is to support complementary functions, then it may be sufficient that the learners understand representations individually, without understanding the relations between them. On the other hand, when the learners must understand the connections between representations, it is imperative that we find ways of signaling how to connect representations (e.g., we can use arrows).

Different methods can be used for studying students' processing of multiple representations of biological concepts. Besides examining verbal responses and achievements or using think-aloud protocols, eye tracking technology can also be used. Eye tracking makes it possible to monitor cognitive processes due to the links between eye movements and cognition (Rayner, 1998). Eye movements indicate where attention is being directed. The duration of a fixation is associated with the ongoing mental processes related to the fixated information (Henderson, 2007; Just & Carpenter, 1976). Total fixation time (i.e., cumulative duration of fixations within a region) is considered as a sign of the amount of total cognitive processing engaged with the fixated information (Just & Carpenter, 1980; Rayner, 1998).

Eye movement data can provide information about the cognitive processes of the learner (Ballard, Hayhoe, Pook, & Rao, 1997; Just & Carpenter, 1976), such as reading, language processing, scene perception, and visual search, and other information processing tasks (Rayner, 1998, 2009). Eye tracking has been used in many studies of learning and problem solving (for a review see, for example, Lai et al., 2013), and is as well a promising method for studying students' processing of various visualizations (Ferk Savec, Hrast, Devetak, & Torkar, 2016; Hinze et al., 2013; Stieff, Hegarty, & Deslongchamps, 2011). Using eye tracking, Chen et al. (2014) found that pictorial presentations appear to convey physics concepts more quickly and efficiently than do textual presentations. Hannus and Hyönä (1999) found that during learning authentic textbook materials high-ability students paid more attention to pertinent segments of an illustration than did low-ability students. In a study by Lin, Holmquist, Miyoshi, and Ashida (2017), detailed illustrations with salient features (colour and greater detail) received more of students' visual focus than simplified illustrations and seemed to better motivate students for learning, which led to the conclusion that the use of detailed illustrations may be beneficial in the early learning stage, as far as they do not introduce excessive distracting details.

Eye tracking measures were shown to differentiate between novices and experts (Tai, Loehr, & Brigham, 2006), high- and low-ability students (Hannus & Hyönä, 1999), and successful and unsuccessful problem solvers (Hegarty, Meier, & Monk, 1995). In a meta-analysis on expertise differences in the comprehension of visualizations, Gegenfurtner, Lehtinen, and Säljö (2011) concluded that experts have shorter fixation durations, more fixations on task-relevant areas and fewer fixations on task-redundant areas than novices.

Based on the above mentioned studies it is reasonable to expect that eye movement data can also provide important information about students' understanding of diffusion and osmosis and can be helpful in investigating processing of multiple representations of these concepts in students with different expertise. We found two studies that addressed this issue using eye tracking technology. Cook, Carter, and Wiebe (2008) examined how high school students' prior knowledge of diffusion and osmosis influenced the way they observed and interpreted a static visual representation of cellular transport processes. They found that students with high prior knowledge oriented their visual attention to conceptually relevant features, whereas students with low prior knowledge focused more on surface features of the graphics. Cook, Wiebe, and Carter (2008) presented students a graphic containing three macroscopic representations of the diffusion process and three corresponding submicroscopic (molecular) representations. They found that high prior knowledge students transitioned more frequently between the submicroscopic representations, whereas low prior knowledge students transitioned more frequently between the macroscopic ones.

Aims and Research Questions

The aim of our study was to extend the work done by Cook, Wiebe, and Carter (2008) on the differences in the learners' distribution of visual attention when interpreting multiple representations. We wanted to study more closely how students at various educational levels (primary, secondary, and undergraduate tertiary) understand water balance in plants and the process of osmosis. In particularly, we explored which thought levels (i.e., the macroscopic, microscopic, submicroscopic, and symbolic levels; Johnstone, 1991; Tsui & Treagust, 2013) students use in their explanations of turgor pressure in plants, whether they are capable of comprehending the relation between external representations at different levels, and how they transition between different levels of representations and shape their responses when asked to explain what exactly is going on during the biological process that causes plant wilting. A dynamic animation was used to present the submicroscopic level of the process instead of static images. To gain a better insight into students' cognition while solving a task on osmosis, eye-tracking measures (fixation times) were combined with behavioural measures (response time, accuracy, and content).

With education, students' understanding of biological concepts develops from naïve to expert. In our study, the following research questions were defined:

1. How are the differences between the groups of students at different levels of education in their knowledge and understanding of the water balance in plants reflected in the way they are solving an authentic biological problem? Which thought levels do students at different levels of education use to explain water balance in plants? Do they link different thought levels and how?
2. What are the differences between students that solved the tasks about water balance in plants correctly and those that did not in the time they spent observing biological phenomenon displayed at the macroscopic and submicroscopic levels?

METHODS

Participants

Slovenian primary school students (n = 30), secondary school students (n = 29), and pre-service teachers (n = 20) participated in the study. Primary school students (age 12 or 13) were attending the seventh grade of public primary school in Ljubljana. Secondary school students (age 15 to 17) were attending the first year of secondary school in Ljubljana. Pre-service teachers (age 22 to 25) were in their fourth year at the University of Ljubljana's Faculty of Education, working on a degree in two science subject areas (double-subject teacher of biology and chemistry) leading first to the bachelor's degree (a 4-year program) and compulsory continuation at the master's level (a 1-year program).

The Science Problem

The selected science problem was presented on a computer screen in a form of a text describing the setting, visualizations of the process occurring in a wilted plant stem, and five tasks on two PowerPoint slides. Both slides contained the same photo of a wilted plant stem of grape hyacinth (*Muscari botryoides*). On both PowerPoint slides there were also three dynamic animations specially made for the purpose of this study. Two out of three animations incorrectly displayed turgor pressure in a plant cell. Animations included a submicroscopic level with particles representing the process of osmosis. The upper part of the display contained an introductory text, explaining what is presented in the photo and represented in the animations. Separate from the introductory text, the tasks were presented (**Figure 1**). On Slide 1, the task was to describe why the plant had wilted (Task 1). On Slide 2, four tasks were presented. Participants needed to list the compounds that were represented with circles (Task 2) and ellipses (Task 3) in the animations. Then they had to choose the animation correctly representing the process of osmosis (Task 4) and provide the reasons for their choice (Task 5). Animation 2 showed the process of osmosis correctly, whereas Animations 1 and 3 were incorrect representations of this process.

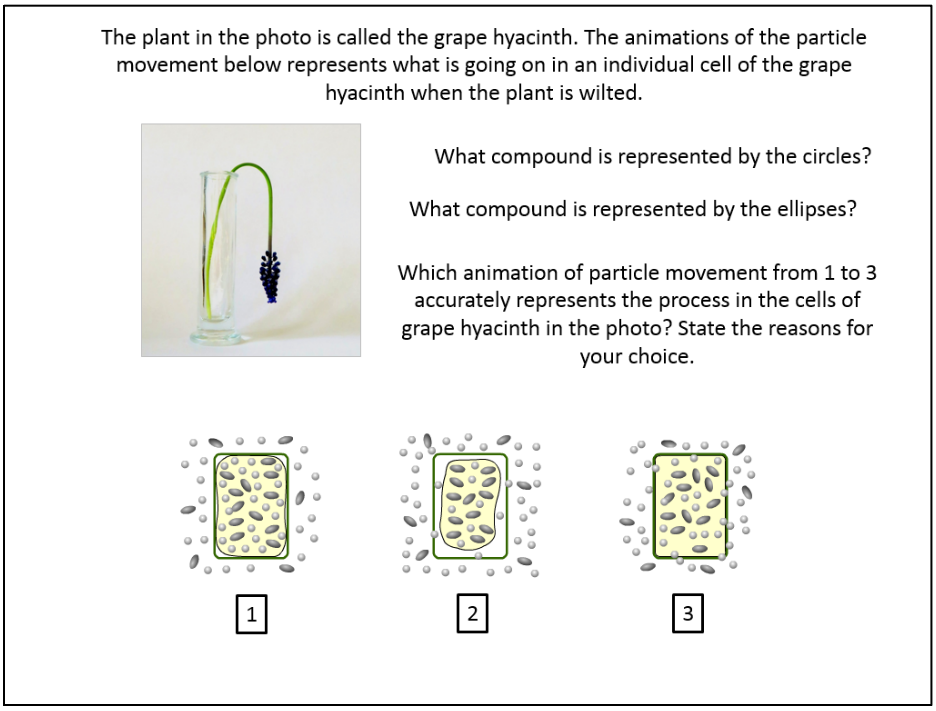

Figure 1. Screenshot of Slide 2. Slide 1 looked the same, except that the tasks right of the photo (Tasks 2–5) were replaced with the following text (Task 1): "Explain why the plant wilted."

Eye-Movement Measures

To determine students' visual attention towards different elements of slides while solving the tasks, we focused on the total amount of time (total fixation duration; in some studies, also referred to as dwell time) spent in particular areas of interest (AOI). For this purpose, the tasks displayed on the computer screen were divided into several AOIs with regard to the placement of the parts investigated. Fixations refer to maintaining the visual gaze on a certain location, and fast eye movements from one location to another are called saccades (Susac, Bubic, Kaponja, Planinic, & Palmovic, 2014). The identification of saccades or fixations is based on the motion of gaze during each sample collected. When both the velocity and acceleration thresholds (in our case: 30 degrees per second and 8,000 degrees per second squared) are exceeded, a saccade begins; otherwise, the sample is labelled as a fixation.

Data Collection

The selected science problem is one of 11 science problems that were tested in the project Explaining Effective and Efficient Problem-Solving of the Triplet Relationship in Science Concepts Representations. This was a broader project on students' understanding of authentic problems in the area of chemistry, physics, and biology. Each problem was context based and required students to link different levels of representations in order to understand and explain the science concept under consideration. The participants had no time limit, and it took them approximately 30 minutes to solve 11 science problems. Prior to the testing, the participants were informed about the purpose of the study, the method used, and their role in it. They sat in front of a screen in a faculty laboratory and had to place their heads in a special head-supporting stand to ensure stability and gather the most optimal recordings. The distance between the screen and the eyes was 60 cm. After the initial calibration and validation (through a nine-point algorithm), participants solved the science problems out loud and the experimenter wrote their answers down. The science problems were presented in the same order for all participants. We followed their eye-movement measures with eye tracker EyeLink 1000 (35 mm lens, horizontal orientation) and used the associated software (Experiment Builder to prepare the experiment and a connection with EyeLink; Data Viewer for obtaining the data and basic analysis) for the recordings and data analyses. Data on corneal reflection and pupil responses were collected from the right eye (monocular data collection) at 500 Hz.

Data Analysis

We decided to focus only on the problem of osmosis in the present paper. Data analysis was performed using R (R Core Team, 2016). Statistical hypotheses were tested at 5% alpha error rate if not mentioned differently. Testing of the hypotheses on the difference between groups was non-directional.

First, we analyzed the accuracy of students' responses to different tasks. For each task, frequency analysis was performed to describe the percentage of students in different groups providing a correct answer. Fisher's exact test was used to test the difference in accuracy among the three groups of students, using Šidák's correction for multiple (i.e., five) tests, which resulted in a .0102 alpha-error rate for an individual test. Cramér's V coefficient was calculated as a measure of effect size. *Post hoc* pairwise Fisher's exact tests with correction for multiple comparisons were calculated with the R 'rcompanion' package (Mangiafico, 2017).

Next, we examined how much time students fixated on particular AOIs within each slide, i.e., on the photo, each of the three animations presented, and other parts of the slide (instructions, questions, animation numbers, or elsewhere). Because the frequency distributions of total fixation duration across individuals showed a large asymmetry or kurtosis, robust measures were used to describe it: median (*Mdn*) was calculated as a measure of central tendency, and median absolute deviation around the median (*MAD*) was used as a measure of dispersion. The Wilcoxon rank sum test was used for comparing eye-tracking variables in different groups of students, using Šidák's correction for multiple (i.e., five) tests, which resulted in a .0102 alpha-error rate for an individual test. A coefficient r was calculated as a measure of the effect size (a Z value resulting from the Wilcoxon rank sum test was divided by the square root of total sample size).

We also analyzed the frequency distribution of different levels of thought reflected in students' responses to Tasks 1 and 5.

RESULTS AND DISCUSSION

Table 1 shows the percentage of students that provided correct answers to each of the five tasks. Even though there was an overall increase of percent correct with educational level as expected, the result of Fisher's exact test did not reach statistical significance for Task 1, asking about the causes of the plant wilting, nor for Task 4, which required students to choose the correct visualization of the process of osmosis. The percent correct differed across groups statistically significantly on Tasks 2 and 3, which asked about the chemical compounds represented in the visualizations by the circles and ellipses. The primary school students solved these tasks less accurately than the secondary school and university students. In Task 5, which required the students to give the reasons for choosing a specific animated visualization of the process of osmosis, the accuracy of the three groups was statistically significantly different as well, with primary school students less accurate than secondary school and university students (even though approximately one-third of university students also failed to provide correct arguments for their choice of animation). The significant differences in accuracy between the seventh-grade primary school students group and the other two groups of students is most probably a result of limited experiences of the first group with higher levels of explanation (i.e., the submicroscopic level) and the fact that the Slovenian primary school science curriculum introduces animations of particles in the eighth-grade chemistry course (Bačnik et al., 2011). Longer education provided older students with more knowledge about various science concepts, as expected. In addition, at the end of primary school, a transition between the concrete operational level and formal operational stage occurs and students develop the ability to think about abstract concepts and become capable of deductive reasoning (Inhelder & Piaget, 1958). Datta and Dutta Roy (2015) suggested that abstract reasoning is also related to spatial visualization ability. In our study, better-developed deductive reasoning and spatial visualization ability might have enabled older students to relate more effectively the photo showing the result of the wilting process at the macroscopic level and the animations of this process at the submicroscopic level, and provide a larger percentage of correct responses.

The association between the age group and the accuracy of responses was moderate (see the column Cramér's V in **Table 1**) but, overall, the older the students were, the higher was the percentage of those that solved the tasks correctly. The research question of how accuracy of response affects the time spent observing the phenomena displayed at the macroscopic and submicroscopic levels is thus inherently related to the question of how these times differ between age groups, even though the two questions do not overlap completely (Cramér's V was quite low for some of the tasks). In subsequent analyses, we decided to focus more on the accuracy of response and its relation to eye-tracking measures.

Table 1. Comparison of percent of correct answers for different tasks in different groups of students

	Total sample (*N* = 79)		**Group 1: Primary school students (*n* = 30)**		**Group 2: Secondary school students (*n* = 29)**		**Group 3: University students (*n* = 20)**		**Cramér's V**	**Fisher's exact test *p***	**Results of *post hoc* tests**
Task	*f*	%	*f*	%	*f*	%	*f*	%			
Task 1	55	70	19	63	18	62	18	90	0.26	.064	
Task 2	51	65	10	33	24	83	17	85	0.51	< .001	1 < 2,3
Task 3	39	49	8	27	18	62	13	65	0.36	.007	1 < 2,3
Task 4	56	71	20	67	18	62	18	90	0.25	.084	
Task 5	31	39	3	10	15	52	13	65	0.48	< .001	1 < 2,3

Table 2. Descriptive statistics for the time different groups of students spent on Slide 1 areas of interest

	Total sample (*N* = 79)		**Task 1 solved incorrectly (*n* = 24)**		**Task 1 solved correctly (*n* = 55)**		**Wilcoxon rank sum test**		**Effect size *r***
Area of interest	***Mdn***	***MAD***	***Mdn***	***MAD***	***Mdn***	***MAD***	***W***	***p***	
Total duration of fixations (in sec)									
Photo	5.5	3.9	7.1	5.8	4.4	2.9	796	.148	–.16
Animation 1	9.3	7.1	11.7	10.9	8.8	5.9	800	.138	–.17
Animation 2	20.2	13.2	22.9	11.2	18.2	13.1	712	.585	–.06
Animation 3	4.5	3.3	6.0	4.1	3.7	2.3	857	.036	–.24
Other parts	19.8	7.4	22.3	7.2	18.5	7.0	831	.069	–.21
Percent fixation duration									
Photo	.079	.057	.084	.048	.076	.061	711	.593	–.06
Animation 1	.158	.085	.186	.092	.151	.077	717	.550	–.07
Animation 2	.302	.161	.275	.139	.325	.163	555	.267	.13
Animation 3	.069	.044	.090	.076	.066	.035	712	.585	–.06
Other parts	.309	.113	.289	.088	.319	.110	583	.417	.09

Table 3. Descriptive statistics for the time different groups of students spent on Slide 2 areas of interest

	Total sample (*N* = 79)		**Tasks 4 and 5 solved incorrectly (*n* = 49)**		**Tasks 4 and 5 solved correctly (*n* = 30)**		**Wilcoxon rank sum test**		**Effect size *r***
Area of interest	***Mdn***	***MAD***	***Mdn***	***MAD***	***Mdn***	***MAD***	***W***	***p***	
Total duration of fixations (in sec)									
Photo	1.9	1.8	2.2	2.4	1.4	1.4	958	.025	–.25
Animation 1	9.8	9.6	16.3	13.0	5.3	3.6	1177	< .001	–.50
Animation 2	29.7	19.3	27.5	20.1	30.3	15.6	624	.597	.11
Animation 3	5.0	5.0	8.2	7.8	2.2	1.5	1195	< .001	–.52
Other parts	27.5	10.3	29.3	9.3	24.9	12.1	865	.192	–.15
Percent fixation duration									
Photo	.020	.020	.022	.027	.016	.019	875	.168	–.16
Animation 1	.131	.108	.177	.131	.075	.060	1136	< .001	–.46
Animation 2	.374	.193	.289	.184	.459	.124	363	< .001	.42
Animation 3	.060	.055	.097	.066	.032	.018	1188	< .001	–.51
Other parts	.331	.094	.316	.086	.363	.138	534	.042	.23

We compared the eye-tracking measures in students that solved tasks correctly and in those that were not able to solve the tasks correctly. The differences in eye-tracking measures could indicate that these two groups processed data at a different thought level. The time an individual spends on a certain AOI may indicate both the speed of processing as well as how much attention he or she needs to devote to the AOI to solve the task. For example, two students may both spend 20% of their time on a certain AOI, yet one may process the information more slowly and will therefore need to spend more absolute time on the AOI. It is thus important to compare both the absolute and relative amount of time spent on a certain AOI between groups because the relative amount may be more indicative of which AOI is more important for solving the task. Tables 2 and 3 contain both types of data.

For Slide 1, we split the entire sample of students into two groups: the group of students that solved Task 1 correctly (i.e., they accurately explained why the plant wilted) and the group of those that did not provide a correct answer. Overall, students that provided a correct response needed less time to derive their answer (*Mdn* = 61.3 s, *MAD* = 30.3 s) than students that failed to answer correctly (*Mdn* = 80.8 s, *MAD* = 24.9 s), Wilcoxon rank sum test $W = 901$, $p = .010$, $r = -.29$. Even though in **Table 2** a tendency can be observed that students that solved Task 1 correctly spent less time on Slide 1 AOIs than students that did not solve this task correctly, the differences did not reach statistical significance ($p > .0102$) for any of the AOI examined, neither when total duration of fixation was compared between the two groups nor when the percentage of time fixating on a certain AOI was compared between the groups.

For Slide 2, we split the students into two groups: ones that solved both Tasks 4 and 5 correctly (i.e., they chose the animation representing the osmosis process correctly and accurately explained what was going on during this process) and those that did not solve Tasks 4 and 5 correctly. In primary school group, three students (10%) solved both tasks correctly. Fourteen (48%) secondary school students and 13 (65%) university students solved both tasks correctly.

Overall, students that solved Tasks 4 and 5 correctly needed less time to derive their answer (*Mdn* = 64.2 s, *MAD* = 23.0 s) than students that failed to provide correct answers (*Mdn* = 90.1 s, *MAD* = 34.4 s), Wilcoxon rank sum test $W = 1034$, $p = .002$, $r = -.34$. **Table 3** shows that, in comparison to the group of students that did not solve Tasks 4 and 5 correctly, the group of students that solved both tasks correctly spent relatively less time on incorrect animations (Animations 1 and 3) and relatively more time on the correct animation (Animation 2). Eye movements during solving tasks on Slide 2 therefore differentiated between successful and unsuccessful problem solvers, similar as in the study by Hegarty et al. (1995).

Out of 30 students that responded correctly to Tasks 4 and 5, 15 students (50%) provided responses that combined the macro-, micro-, and submicroscopic level of thought, such as "The amount of water outside the cell decreased. That's why the water left the vacuole. The vacuole contracted because of the osmotic pressure." Eight students (27%) provided responses at the macro- and microscopic level of thought; for example, "The plant wilted because inside of the cell the concentration was higher than outside and the water went out for the concentration to be equalized." Three students (10%) used a combination of micro- and submicroscopic level, such as "The water goes to the environment because the molecules of water go out of the plant's cells. The cell membrane disassembles from the cell wall." Two students responded at the macroscopic level ("Because the water went out.") and two students responded at a combined macro- and submicroscopic level ("The particles that are soluble in water can cross the membrane, whereas others cannot"). No student used the symbolic level of explanation.

These findings support the statement made by Tomažič and Vidic (2012) that observation alone at a macroscopic level of the processes is not sufficient, and that a link must be made with the submicroscopic level. The results also show that students regularly use the microscopic level or a combination of the microlevel with the macroscopic and submicroscopic levels to explain the biological phenomena studied. What is unique for biology learning is complex, hierarchical organization of life and a nested knowledge domain, which, according to Treagust and Tsui (2013), provides a rationale for using four levels of external representation (macro-, micro-, submicro-, and symbolic levels) instead of three levels (macro-, submicro-, and symbolic levels) as proposed by Johnstone (1991) in chemistry education.

We also compared the percentage of time spent on the photo and on the animations (all animations combined) in the group of students that provided a correct response at the macroscopic level and the group of students that provided a correct response including other levels of thought. The percentage of time spent on the photo when solving Task 1 was larger in students that provided the response at the macroscopic level ($n = 29$, *Mdn* = .124, *MAD* = .101) than in students that provided the response at some higher level ($n = 26$, *Mdn* = .052, *MAD* = .026), Wilcoxon rank sum test $W = 583$, $p < .001$, $r = .47$. In addition, the percentage of time spent on the animations when solving Task 1 was smaller in students that provided the response at the macroscopic level ($n = 29$, *Mdn* = .513, *MAD* = .186) than in students that provided the response at some higher level ($n = 26$, *Mdn* = .643, *MAD* = .086), Wilcoxon rank sum test $W = 185$, $p < .001$, $r = -.44$. Therefore, students that responded to the question "Why did the plant wilt?" with a correct answer at the macroscopic level paid relatively more attention to the photo and relatively less attention to the animations than those whose response contained higher levels of thought.

In addition, the total score for responding correctly to all four tasks on Slide 2 was calculated (min = 0, max = 4 points). The correlation between the total score and percentage of time spent on different AOIs on Slide 2 is shown in **Table 4**. As can be seen, the Slide 2 total score was positively related to the percentage of time spent on the correct animation and negatively related to the percentage of time spent on incorrect animations of the osmosis process. Participants that understood the process of osmosis better observed the correct animation relatively more and spent less time on the incorrect animations. Most likely, when describing the processes represented in Animation 2 (Task 5), these students were also observing this animation for a longer period.

Table 4. Correlations between total score on Slide 2 tasks and the percentage of time spent on different areas of interest on Slide 2

	1	2	3	4	5
1. Score on Slide 2					
2. Percent fixation on the photo	−.18				
3. Percent fixation on Animation 1 (incorrect)	*−.31***	.19			
4. Percent fixation on Animation 2 (correct)	.45***	−.33**	−.62***		
5. Percent fixation on Animation 3 (incorrect)	−.44***	.11	*.32***	−.40***	
6. Percent fixation on other parts of Slide 2	.01	−.03	*−.32***	*−.26**	*−.26**

Note: Spearman correlation coefficients are shown. Coefficients in italics would not be considered statistically significant after using the Šidák correction for multiple tests, resulting in a .0034 single-test alpha error rate.
*p < .05. **p < .01. ***p < .001.

Table 5. Levels of correct and incorrect responses to Tasks 1 and 5

	Incorrect response						Correct response					
	Primary school students		Secondary school students		University students		Primary school students		Secondary school students		University students	
Level of thought	*f*	%	*f*	%	*f*	%	*f*	%	*f*	%	*f*	%
Task 1												
No response	1	3	1	4	0	0	–	–	–	–	–	–
Macroscopic	2	7	2	7	1	5	15	52	8	29	6	30
Macro- & Submicroscopic	3	10	1	4	0	0	1	3	0	0	0	0
Macro- & Microscopic	5	17	4	14	1	5	2	7	6	21	4	20
Macro- & Micro- & Submicroscopic	0	0	1	4	0	0	1	3	1	4	1	5
Microscopic	0	0	1	4	0	0	0	0	3	11	7	35
Micro- & Submicroscopic	0	0	1	4	0	0	0	0	0	0	0	0
Task 1: Total	11	37	11	38	2	10	19	63	18	62	18	90
Task 5												
No response	3	10	2	7	0	0	–	–	–	–	–	–
Macroscopic	3	10	1	3	2	10	2	7	0	0	0	0
Macro- & Submicroscopic	2	7	1	3	3	15	0	0	0	0	0	0
Macro- & Microscopic	7	23	1	3	1	5	0	0	6	21	2	10
Macro- & Micro- & Submicroscopic	1	3	0	0	0	0	0	0	0	0	3	15
Microscopic	6	20	8	28	0	0	0	0	9	31	7	35
Submicroscopic	2	7	1	3	0	0	0	0	0	0	0	0
Micro- & Submicroscopic	3	10	0	0	1	5	1	3	0	0	1	5
Task 5: Total	27	90	14	48	7	35	3	10	15	52	13	65

A closer look at the responses of different age groups of students to Task 1 (see **Table 5**) revealed that, when responding to the task correctly, the majority of primary school students provided answers at the macroscopic level, such as "The water went out," whereas among the secondary school and university students a large percentage of higher-level responses or combinations of different levels of responses could be observed. For example, some of these students provided responses at the microscopic level, such as "The amount of water outside the cell decreased. That's why the water left the vacuole. The vacuole contracted because of the osmotic pressure," and some provided a response that was a combination of the macro- and microscopic level of thought, such as "The plant wilted because it did not have enough water and thus the vacuole contracted." Individual students across all age groups provided a combination of the macro-, micro-, and submicroscopic levels of thought (e. g., "The plant wilted because water particles and solute particles exited the cells due to the turgor pressure").

In Task 5, similar patterns in the distribution of correct responses across different age groups could be observed as in Task 1. Whereas primary school students' answers to Task 5 mostly contained inaccuracies, the correct responses in secondary school and university students all included the microscopic level of thought. One of the responses that included this level only was: "The water goes out of the cell. The vacuole shrinks. The cell membrane disassembles from the cell wall." An example of the combination of the microscopic level with the macroscopic one was: "The water exits the cell and the plant wilts." Individual students also used a combination of micro- and submicroscopic levels, such as "The particles that are soluble in water can pass through the membrane, whereas others cannot." A higher percentage of older students combining the macroscopic level with some other level is consistent with Larkin, McDermott, Simon, and Simon (1980), who report that experts think about and can respond to questions at many levels.

Different types of incorrect responses to Tasks 1 and 5 were observed across all age groups. Whereas in Task 1 incorrect responses of primary school students mostly included the macroscopic level (either alone or combined with a higher level of thought), in Task 5 a larger amount of incorrect answers at the micro- and submicroscopic levels could be observed (**Table 5**). This can be a result of the task explicitly orienting primary school students' attention towards the animations and the movement of particles, but because these students have limited experience with higher levels of explanations their higher-level responses were mostly wrong. These results confirm Ainsworth's (2008) findings that complicated scientific concepts, represented with multiple forms of external representations, can offer unique benefits; however, there is considerable evidence to show that learners often fail to exploit these advantages, and in the worse cases this can completely inhibit learning. Therefore, she recommended that these powerful tools need careful handling and often considerable experience before learners can use them successfully, which is probably why primary school students had the most difficulties.

CONCLUSIONS

This article provides evidence to suggest that the learning of difficult scientific topics, such as water balance in plants, with multiple representations at the macroscopic and submicroscopic levels can be either helpful or confusing for a learner, depending on the individual's level of development of scientific reasoning. As expected, primary school students showed less knowledge and understanding of water balance in plants than secondary school and university students. Students that solved the questions about water balance in plants correctly spent less time observing biological phenomena displayed at the macroscopic and submicroscopic levels than those that were unable to answer correctly. Our focus was on thought levels students used to explain water balance in plants. Students with correct answers more often provided responses that combined the macro-, micro-, and submicroscopic levels of thought. A closer look at the responses of different groups of students revealed that, when responding to the task correctly, the majority of primary school students provided answers at the macroscopic level, but when their explanations included higher levels or combinations of different levels of thought the percentage of incorrect answers increased substantially.

These findings suggest that beginners (i.e., primary school students) using multiple representations at the macroscopic and submicroscopic levels do not achieve the same level of knowledge and understanding as more experienced secondary school and university students. Therefore, as suggested by Ainsworth (2008), one should consider how these visualizations can be designed to allow beginners to develop their expertise in using multiple external representations, which are a prerequisite for in-depth learning about complex scientific topics. Furthermore, a teacher should support students in interpreting multiple external representations so that they will be able to use them while learning about complex biological phenomena.

The study provides evidence that students regularly use the microscopic (cellular or subcellular) level of thought to explain biological phenomena. This indicates that we should use four levels of external representation (the macro-, micro-, submicro-, and symbolic levels) in designing biology textbooks, online resources and in biology lessons. Our suggestion for implementation of multiple external representations would be to use arrows (as suggested by Ainsworth, 2008) and zooming-in, which would explicitly show gradual transitions from a macroscopic to a submicroscopic level of representation (or even further to a symbolic one). New digital technologies are very handy for applying zooming-in in online (electronic) educational materials, allowing students to independently progress through different levels of external representations in their own pace. This should help students establish links between different external representations and develop comprehensive understanding of biological concepts.

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