What a Difference in Pressure Makes! A Framework Describing Undergraduate Students' Reasoning about Bulk Flow Down Pressure Gradients

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ABSTRACT

Pressure gradients serve as the key driving force for the bulk flow of fluids in biology (e.g., blood, air, phloem sap). However, students often struggle to understand the mechanism that causes these fluids to flow. To investigate student reasoning about bulk flow, we collected students' written responses to assessment items and interviewed students about their bulk flow ideas. From these data, we constructed a bulk flow pressure gradient reasoning framework that describes the different patterns in reasoning that students express about what causes fluids to flow and ordered those patterns into sequential levels from more informal ways of reasoning to more scientific, mechanistic ways of reasoning. We obtained validity evidence for this bulk flow pressure gradient reasoning framework by collecting and analyzing written responses from a national sample of undergraduate biology and allied health majors from 11 courses at five institutions. Instructors can use the bulk flow pressure gradient reasoning framework and assessment items to inform their instruction of this topic and formatively assess their students' progress toward more scientific, mechanistic ways of reasoning about this important physiological concept.

INTRODUCTION

Using scientific principles to reason about phenomena is central to scientific thinking (American Association for the Advancement of Science, 2011; National Research Council [NRC], 2012). Oftentimes, students focus on the surface features of a phenomenon to explain how it occurred and thus overlook the underlying principles (Chi *et al.*, 2012). In the field of physiology, Modell (2000) identified seven principles he termed "general models" that can be used to reason mechanistically about seemingly different physiological processes that are fundamentally the same. One of these general models, "mass and heat flow," can be used to describe processes as diverse as oxygen diffusing from the lungs to the blood, ions moving across cell membranes during an action potential, water uptake into plant roots, and chyme moving through the gastrointestinal tract. In each of these examples, the rate of movement of a substance is directly proportional to the magnitude of the driving force (the gradient) and inversely proportional to the magnitude of the factors that impede movement (the resistance); that is, rate of movement of a substance $\propto$ gradient/resistance (Modell, 2000; Carroll, 2001; Michael and McFarland, 2011). The mass and heat flow general model, which is a law of physics, is also conceptualized as "flow down gradients" in the physiology core concepts work (Michael and McFarland, 2011; Michael *et al.*, 2017).

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One form of mass and heat flow is "bulk flow," in which the substances moving are a mixture of molecules in a fluid rather than just one type of molecule via diffusion or osmosis. Common examples of bulk flow are blood flowing through the circulatory system, air moving through the respiratory tree, and sap flowing through the xylem and phloem of plants. In bulk flow, the gradient is a hydrostatic or atmospheric pressure gradient, defined as the difference in pressure between two places, and sources of resistance are tube diameter, tube length, and fluid viscosity (Michael *et al.*, 2017).

Applying the mass and heat flow general model to explain bulk flow phenomena is a powerful mechanistic reasoning approach for explaining a multitude of physiological processes. However, postsecondary students seldom use this general model to guide their reasoning and often struggle to apply it appropriately (Michael *et al.*, 2002). One reason students might struggle to understand bulk flow in physiology may arise from the interdisciplinary nature of fluid dynamics, which is grounded in principles of physics and is represented with multiple mathematical relationships (Wang, 2004; Michael, 2007; Breckler *et al.*, 2013). For example, bulk flow is commonly taught in biology courses using the Hagen–Poiseuille equation, often referred to as Poiseuille's law (Table 1).

Another way students struggle with applying the mass and heat flow general model is by misapplying relationships with similar variables they learned in other disciplines. For example, students may use the ideal gas law from chemistry (Table 1) to inappropriately relate the pressure and volume of liquids such as blood. Students may also use the definition of static pressure (Table 1) to incorrectly explain fluid movement along a pressure gradient (i.e., the difference in pressure between two points; Besson, 2004). In plant physiology, students may misapply the water potential equation, which governs water movement in and out of cells via water channels, to the bulk flow movement of sap (Clifford, 2002).

Students may also incorrectly relate ideas of pressure, volume, and resistance (Yip, 1998a,b; Carroll, 2001; Michael *et al.*, 2002). In a study exploring students' ideas about blood flow through the cardiovascular system, Michael *et al.* (2002) found that students thought blood flow determined a vessel's resistance (e.g., when blood flow increases, vessel resistance either increases or decreases), rather than realizing that the resistance of the vessel determines blood flow and thus blood pressure. This study also noted that students inversely related vessel pressure with blood volume, suggesting that a decrease in venous blood volume would cause an increase in venous pressure (when in fact the opposite would happen, perhaps an example of applying the ideal gas law from chemistry). Similarly, Yip (1998b) found that some students explained that blood could flow from low to high pressure in certain physiological situations. Even in a nonliving context, engineering students studying fluid mechanics struggled to relate pressure and resistance factors to explain fluid flows in pipes (Besson, 2004; Brown *et al.*, 2017; Lutz *et al.*, 2019).

Moreover, to reason about *how* fluids flow down pressure gradients, students must draw on an understanding of energy, such as Bernoulli's principle, which states that the sum of the pressure energy, potential energy, and kinetic energy of a liquid must be equal between two points, ignoring the loss of energy due to shearing friction between the flowing blood and the vessel walls (i.e., energy must be conserved for flowing fluids). As energy in fluid flow may only be addressed in advanced physiology courses (Badeer and Rietz, 1979), this leaves less advanced physiology students with uncertainty regarding the forces that govern fluid flows down pressure gradients (Besson, 2004; Vitharana, 2015).

These challenges may lead to student confusion and interfere with their ability to mechanistically reason about pressure gradients and bulk flow. For example, it may be difficult for students to accurately predict and explain perturbations to physiological systems (e.g., how changes in blood pressure can cause fainting). Faculty are often unaware of these alternative types of student reasoning. A reasoning framework is an evidence-based tool that can help faculty become aware of these alternative types of reasoning. It organizes and characterizes different ways students reason about a topic (e.g., see Scott *et al.*, 2018; Ghalichi *et al.*, 2021). By making explicit the different ways students reason, a reasoning framework can help direct changes or modifications to instruction as well as research into student reasoning (Modell *et al.*, 2015; Lira and Gardner, 2017).

An effective way to uncover the different ways students reason about a topic is through thoughtful and timely formative assessments (Chen *et al.*, 2021). To that end, we developed open-ended, formative assessment items to elicit the kinds of reasoning that undergraduate students use to explain the rate of fluid flow through tubes due to a hydrostatic pressure gradient. Phenomena that include osmosis or oncotic pressure, such as fluid flow into and out of vessels at the capillaries (i.e., Starling forces) or into and out of phloem at the source or sink, are beyond the scope of this paper. We identified common conceptual patterns in students' reasoning about pressure gradients and bulk flow on our assessments and organized them into a bulk flow pressure gradient reasoning framework. This framework describes the different patterns in reasoning that students express about bulk flow and pressure gradients and orders those

TABLE 1. Equations related to pressure and courses students might take that typically use the equation

Name of equation	Equation	Variables	Courses using this equation
Hagen–Poiseuille equation	$Q = \frac{(\pi \Delta P r^4)}{8 \eta l}$	Q = fluid flow rate, P = pressure, r = radius of a tube, η = viscosity of the fluid, l = tube length	Physiology, fluid dynamics
Ideal gas law	$PV = nRT$	P = pressure, V = volume, n = amount of substance in moles, R = gas constant, T = temperature	Chemistry
Static pressure	$P = F/A$	P = pressure, F = force, A = area	Physics
Water potential	$\Psi_w = \Psi_s + \Psi_p$	Ψ_w = water potential, Ψ_s = solute potential, Ψ_p = pressure potential	Biology

TABLE 2. Descriptions of institutions and types of courses providing students' data[a]

Institution	Carnegie Classification	Type of course	Number of students (number of courses)		
			Pilot short answer for RQ1	Interview for RQ1	Short answer for RQ2
Associate's A	4-year, higher part-time, associate's dominant	Allied health physiology	14 (1)	3 (2)	
		Majors introductory biology	36 (2)	12 (2)	
Associate's B	2-year, higher part-time, associate's dominant	Allied health physiology			18 (1)
R1 A	Full-time, more selective, lower transfer-in	Allied health physiology	304 (1)	8 (2)	113 (1)
		Majors introductory biology	159 (2)	9 (2)	228 (1)
		Upper-division physiology		13 (3)	128 (3)
R1 B	Full-time, more selective, lower transfer-in	Allied health physiology			242 (1)
		Upper-division physiology			43 (1)
R1 C	Full-time, more selective, higher transfer-in	Majors introductory biology			158 (1)
R1 D	Full-time, more selective, lower transfer-in	Upper-division physiology			93 (2)

[a]Students providing written data for RQ2 may not have provided data at both the beginning and end of the course and may have answered both blood and phloem sap items (see Supplemental Table S1). For more information on courses for students who provided interview data, see Supplemental Figure S1.

patterns into sequential levels that instructors can use to understand how their students' ideas about bulk flow progress toward the mechanistic ideas described in Modell's general model for mass flow (i.e., using pressure gradients and Poiseuille's law). Instructors could also use this framework to inform their instructional design. Our two research questions are: RQ1, What patterns and levels of reasoning do undergraduate students use when responding to bulk flow pressure gradient assessment items?; and RQ2, Can we use the bulk flow pressure gradient reasoning framework to evaluate written assessment responses from a national sample of undergraduate biology and allied health majors?

Our research is the first to investigate biology students thinking about how pressure gradients are a main determinant of fluid flow and that the size of the gradient is dependent on the difference between those two values.

RQ1: WHAT PATTERNS AND LEVELS OF REASONING DO UNDERGRADUATE STUDENTS USE WHEN RESPONDING TO BULK FLOW PRESSURE GRADIENT ITEMS?

Methods

This study is part of a larger project we started in 2014, inspired by Modell's work on general models (2000), in which we investigated students' understanding of flow down gradients across multiple physiological contexts (e.g., plants and animals), including bulk flow, ion movement, osmosis, and diffusion. The bulk flow items we developed for this study were modeled after pressure flow illustrations found in the cardiovascular physiology chapter of many undergraduate human physiology textbooks (e.g., Figure 14.3 in Silverthorn, 2013; Figure 12.4 in Widmaeier *et al.*, 2014) and a concept check question in Silverthorn's *Human Physiology* (p 470; 2013).

To develop a framework that encompasses the full range of undergraduate students' reasoning about bulk flow pressure gradients, we needed to administer our assessment items to students at different points in their academic careers. Therefore, we administered our items to students at various time points of instruction (e.g., both pre- and postinstruction, introductory and upper-division courses) and from different populations (e.g., students at associate's-dominant and R1 institutions, biology majors and allied health majors). The ways in which students' reasoning about bulk flow pressure gradients can be affected and altered by differences in instruction, teaching strategies, and context is not within the scope of this present work. This important question will need to be addressed in the future.

We piloted one bulk flow item with 513 students from two institutions (associate's-dominant and R1 institution) from a range of academic settings (i.e., before and after college physiology course work, biology majors and allied health majors; Table 2) in the 2017–2018 academic year. The item in the pilot study consisted of a simplified diagram with a series of tubes with pressures noted at the beginning and end of the tubes. We asked students to identify which tube had the highest flow rate and to explain their reasoning.

We used the constant comparative method to develop a preliminary bulk flow pressure gradient reasoning framework. The constant comparative method is an inductive data-coding process used for categorizing and comparing qualitative data in which any newly collected data are compared with previous data (Glaser, 1965). Three researchers (J.H.D., E.E.S., J.A.C.) identified qualitatively different types of student reasoning in a subset of 200 written responses in the sample. The researchers then discussed the types of reasoning each identified until reaching consensus on a set of seven distinct reasoning patterns. The researchers then individually recategorized the same 200 written responses using the seven reasoning patterns to test their efficacy. After agreeing on the seven reasoning patterns that captured students' ideas, we grouped the different reasoning patterns into levels of a preliminary bulk flow pressure gradient reasoning framework. We used this preliminary bulk flow pressure gradient reasoning framework to code the remainder of the pilot data and did not find additional reasoning patterns.

To more deeply probe students' ideas about bulk flow pressure gradients, we collected interview data from students at the same two institutions in the 2018–2019 academic year. This was critical for developing our bulk flow pressure gradient reasoning framework, because it allowed us to differentiate between the reasoning patterns of students who picked the same pressure gradients but offered different rationales for their choices. For example, if a student explained that their choice of pressure gradient showed the "highest pressure," we were able

Blood flow item

A scientist is studying blood flow in the aorta of five different animal species of similar size and age. She found that the composition of the blood was identical in each animal as well as the diameter of their aortas, but the rate of blood flow through the aorta was different. The scientist measured the following pressures at the beginning (i.e., ascending aorta) and near the end (i.e., abdominal aorta) of the aorta.

Blood vessel	Start pressure	End pressure
Zebra	210	150
Camel	200	180
Elk	200	160
Water Buffalo	150	130
Sitka Deer	100	30

Which animal has the greatest flow rate (L/min) of blood through the aorta?

Zebra
Camel
Elk
Water Buffalo
Sitka Deer

Explain why the animal you selected has the greatest flow rate (L/min) of blood through the aorta.

Phloem sap flow item

A scientist is studying sap flow in phloem tubes in five different trees of similar size and age. She found that the composition of the sap was identical among the trees as well as the diameter of their phloem tubes, but the rate of sap flow through the phloem tubes was different. The scientist measured the following pressures at the top (i.e., in the tree crown) and near the bottom (i.e., the base of the trunk) of each phloem tube.

Phloem tube	Start pressure	End pressure
Beech	0.60	0.30
Oak	1.20	1.10
Chestnut	0.90	0.80
Maple	1.25	1.00
Hickory	0.80	0.60

Which tree has the greatest flow rate (L/hour) of sap through the phloem tube?

Beech
Oak
Chestnut
Maple
Hickory

Explain why the tree you selected has the greatest flow rate (L/hour) of sap through the phloem tube.

FIGURE 1. Bulk flow pressure gradient assessment items.

to probe to see whether "highest pressure" meant "highest starting pressure," "highest average pressure," or "least pressure change." We could also ask students why they did not choose the other pressure gradients. The interviews also allowed us to validate that the preliminary patterns we found from the piloted bulk flow item recurred in additional populations of students.

We interviewed 34 biology majors and 11 allied health majors recruited during different points in their academic careers (Table 2 and Supplemental Figure S1). Students were recruited via emails from their instructors asking for volunteers. Given the large enrollment at the R1 institution, we limited interviews to the first five volunteers per course. At the associate's-dominant institution, we interviewed all volunteers. For some courses, there were fewer than five volunteers. Students were interviewed at one or two time points for a total of 70 interviews (Supplemental Figure S1). Twenty-five of the 45 students were interviewed twice. Students who were interviewed once fell into in one of three categories: students interviewed after their 400-level physiology class (these students were seniors who preferred to be interviewed just once), students interviewed before Introductory Biology I (a course that did not include physiology), and a few students who chose not to schedule a second interview.

For the interviews, we developed two assessment items that were based on the structure of our pilot item but were situated in either an animal (i.e., blood flow through the aorta) or plant (i.e., sap flow through phloem) physiological context (Figure 1). When selecting organisms for each item, we chose organisms of a similar size and taxa (e.g., zebra and elk but not a mouse or rabbit). Students were asked to reason about only one of the items in each interview. Items were randomly assigned to students, stratified by course. If students were interviewed twice, they received one item during the first interview and the other item on the subsequent interview. To elicit more student ideas, we followed up the question with prompts related to students' answers. For each student who explained or implied fluids flowed from high to low pressure, we also followed up by asking why they thought fluids flowed from high to low pressure. Additionally, we asked students' to describe their ideas about why fluids flow down gradients. This provided us with greater insight into the mechanisms students considered when thinking about bulk flow and how their thinking influenced their responses to our items. These bulk flow items were part of a larger interview protocol that asked students multiple plant and animal physiology questions. Interviews were 45–60 minutes long. Students received a $25 gift card in exchange for their time for each interview.

We used the preliminary bulk flow pressure gradient reasoning framework derived from the pilot written data as a foundation for identifying reasoning patterns and levels in the interview responses to the bulk flow items. Based on our analysis of the student interviews, we revised the bulk flow pressure gradient reasoning framework and created a coding rubric to code all interviews by pattern and levels of reasoning.

We tested and calibrated the coding rubric with four researchers (J.H.D., E.E.S., J.A.C., M.P.W.) who each scored eight interview transcripts. After this calibration phase, the four researchers coded the rest of the interviews in pairs. When there were

TABLE 3. Three-level bulk flow pressure gradient reasoning framework describing common conceptual patterns in students' reasoning about bulk flow of fluid through a tube in an organism[a]

Level	Description
Level 3	"Flow down gradients": The magnitude of the pressure difference is proportional to the rate of fluid flow (i.e., Poiseuille's law).
Level 2	"Emerging mechanistic reasoning": A variety of emerging mechanistic ideas about pressure and flow.
	Sublevel 2.1. "Pressure causes":
	Pressures at a single location along the tube, not the pressure gradient, determine fluid flow.
	2.1A. High pressure values cause a large force "pushing" on the fluid.
	2.1B. Low pressure values at the end of a tube push back less, causing a low resistance to flow.
	Sublevel 2.2. "Pressures indicate":
	The magnitude of pressures are only a result, not the cause, of fluid flow.
	2.2A. A small difference between pressure values at the start and end of a tube indicates that flow is maintained,
	2.2B. Pressure magnitude indicates the volume of blood that is flowing or has flowed (e.g., high pressures indicates high volumes are flowing, low pressures indicate a high volume of fluid has flowed out of tube).
	2.2C. A small difference between pressure values at the start and end of a tube indicates that the tube has a low resistance, thus higher flow.
Level 1	"Nonmechanistic ideas": Ideas about characteristics and behaviors of organisms

[a]Though this nomenclature may indicate one sublevel is above the other, in fact we do not ordinate sublevels, as we feel one way of demonstrating emerging mechanistic reasoning is not necessarily "better" than another.

disagreements in coding, the researchers discussed the differences until consensus on a particular code was reached. Consequently, the interrater reliability for interview coding was 100%.

Results

We developed a three-level reasoning framework that describes the different ways students reason about bulk flow pressure gradients (Table 3). Each level incorporates increasingly more mechanistic ideas that are consistent with Modell's general model for mass flow in physiology. Specifically, at the lowest level, we identified one pattern of reasoning. At this level, students either used pressure as a measure that indicates how organisms are functioning or had only limited ideas about what the pressure values represented. At the middle level, we identified five patterns of reasoning in which students used a mix of correct and incorrect ideas about how pressure was a driving force that caused fluid flow. At the highest level, we identified one pattern of reasoning. At this level, students consistently reasoned that pressure gradients caused fluids to flow.

In the following sections, we present in greater detail the kinds of ideas students used at each level of the bulk flow pressure gradient reasoning framework. We also present excerpts from our interviews with students enrolled in introductory- to advanced-level biology courses at an associate's-dominant college and an R1 university as exemplars of the different reasoning patterns we found. These excerpts provide rich insight into students' thought processes. We use bolding to emphasize ideas critical to, or a hallmark of, a reasoning pattern. Though the scenarios used in the interview question were of blood flowing through blood vessels and sap flowing through phloem in plants, we did not see any indication that the context of the question influenced how students answered. We will further explore the influence of the context of scenario as well as the influence of varying the starting and ending pressures in a future research publication.

Level 1: Pressure as a Measure of Organism Function. At the lowest level of the framework, student explanations contained physiology ideas about pressure that were unrelated to pressure gradients and were nonmechanistic in nature. For example, S44 interpreted the difference between the two pressure numbers across different tree species (Figure 1) as indicating the time it takes fluid to travel.

> **S44:** The difference between the start and the end [pressure] is smaller so it's faster for the thing [i.e., sap] going through from the top to the bottom.

> **Interviewer:** If we're looking at the oak again and if this end pressure was also 1.20, so it was the same as the start pressure, would that be even faster or what would that mean? If, instead of this end pressure being 1.10 it was the exact same as the start pressure?

> **S44:** It's super-fast … It just goes straight "boom" … I think it's going to be really super-fast with the oak tree.

S44's interpretation of the pressure values as representing fluid travel times led them to mistakenly view pressures with the least difference as signifying the fastest flow rate of tree sap, which is contrary to an understanding based on how pressure gradients work.

Level 1 explanations also referenced characteristics and behaviors of organisms that were presented in the assessment items rather than referencing principles that govern fluid movement. For example, when asked which of five different animals had the greatest flow rate (liters/minute) of blood (liquid) through their aorta (Figure 1), S38 responded, "So I'm not sure what the normal pressures are for animals … I know that humans have, you know, a regular pressure would be 100 over 70." This student's focus on what a "normal pressure" would be for an animal suggested S38 was accessing knowledge about how organisms function to address the task rather than noting changes in pressure that impact fluid movement.

Students' Ideas about Why Fluids Move along Pressure Gradients at Level 1. When asked why fluids move along pressure gradients, explanations at level 1 continued to frame the question around organismal functioning (i.e., meeting demands of daily living that the animal may encounter). For example:

> **S38:** I mean I know **in the fight or flight response it [blood pressure] will go up**. It's kind of like they stay at certain rates for the body to get what they need at the right times. I guess in an animal it would be at a certain rate so they could get nutrients to run or you know, things like that, so a sloth would probably be pretty slow.

Instead of reasoning with fundamental principles of fluid movement, S38 drew on ideas about what blood pressure at "certain rates" enables animals to do, such as having a "fight or flight response," getting "what they need at the right times," and having "nutrients to run." Consequently, S38's continued framing of the tasks as being about how or why organisms function may have prevented broader reasoning about why fluids move along pressure gradients.

Level 2: Emerging Principle-Based Reasoning. Students' explanations at the second level of the framework demonstrated emerging mechanistic ideas relating pressure and flow. Many of these ideas were linked to scientific relationships that included pressure but were misapplied to the given tasks. Explanations at this level also drew incompletely, or inaccurately, on scientific ideas to explain why fluids move down gradients. We organized students' explanations at this level into two sublevels, sublevels 2.1 and 2.2. Explanations in sublevel 2.1 reasoned that differences in the magnitudes of pressure at a single location along the tube, not differences in pressure gradient, caused differences in flow rate. Explanations in sublevel 2.2 reasoned that differences in the magnitudes of pressure are a result, not the cause, of fluid flow. Though this nomenclature may indicate one sublevel is above the other, we do not ordinate sublevels, as we currently have no evidence to demonstrate that one type of emerging mechanistic reasoning is "better" than another.

Sublevel 2.1 Reasoning Pattern 2.1A: Higher Pressures Cause Higher Bulk Flow Rates. One set of student explanations reasoned that tubes with the highest pressure values would cause greater bulk flow rates, because these tubes had the most "force pushing" on the fluid. For example, S8 suggested the tree with the highest start pressure, the maple tree, would also have the highest sap flow rate: "Because if it has a higher pressure and they all have the same diameter of the [tubes], then it's probably moving more at one time than the trees with the lower pressure." We found that students explained which system had the "highest pressure" in different ways; some explanations used the magnitude of the start pressure as the most important value, such as S7 who said: "Well, my instinct is just to say the highest number. So, the zebra at the beginning." S7 reasoned that: "If you turn a hose on really high, you're going to get more water out of it than if it's just lower." Other explanations calculated the average of the starting and ending pressures and selected the option with the highest value or viewed the two numbers as a ratio and selected the greatest ratio as correct. Some students chose the tube with the highest starting and ending pressure, because a high pressure along the tube meant that the pushing force was maintained along the entire tube. For example, S37 reasoned:

> The camel has the **largest end pressure** which means that, I guess, for whatever reason, it's … the **blood is continuing to push equally hard** when it reaches the end of the animal as … or very close to when it reaches the end of the animal as when it left.

The idea that stronger forces will cause fluids to flow at higher rates is consistent with how physics defines static pressure as equal to the amount of force applied to a particular surface area (P = force/area). Indeed, S19 explained why a high force causes a greater flow rate by reasoning: "It's force over area and then force is due to acceleration and mass … So, this is why I'm assuming that a greater pressure will mean a greater heart rate." Using this kind of reasoning may prime students to focus on one pressure value, either measured (i.e., the largest start pressure) or derived (i.e., the highest average pressure), as being most important for determining the driving force behind bulk flow rather than the difference in pressure between the beginning and end of the tube. Thinking about pressure as the force applied to a certain area is productive, in that it helps students conceptualize pressure as a force. However, this definition of pressure alone is unreliable as a reasoning strategy to address fluid flows in tubes; a tube with high pressures at both ends of the tube (e.g., Zebra in Figure 1) will have a lower rate of fluid flow compared with a different tube that has a low beginning pressure but significantly lower ending pressure; that is, a greater pressure gradient (e.g., Sitka deer in Figure 1).

Sublevel 2.1 Reasoning Pattern 2.1B: Higher Pressures at the End of the Tube Cause Resistance To Bulk Flow. Another set of student explanations suggested lower pressures at the end of the tube caused higher rates of fluid flows because that lower pressure would provide less resistance or less force to be overcome for fluids to flow. For example, S23 selected the beech tree as having the greatest flow of sap, not because it had the greatest pressure gradient but rather because it had the lowest end pressure (0.30). They used their knowledge of the cardiovascular system to reason:

> The **pressure in the extremities will determine the amount of flow**, so resistance can determine blood flow to a certain part … so the beech tree, the end pressure is 0.3 lower than the start pressure. And so therefore … **it's not going to have to overcome as much pressure** when the plant glucose is moving down the phloem as opposed to the oak and chestnut, which is only 0.1 difference which is—that's higher.

In these explanations, a large pressure gradient meant less pressure at the end of the tube for the fluid flow to overcome. Thus, pressure at the end of the tube was viewed as an inhibiting force, rather than the difference in pressure being a driving force for fluid movement.

Sublevel 2.2 Reasoning Pattern 2.2A: Maintenance of Pressure Indicates Higher Bulk Flow Rates. Another set of student explanations explained that the greatest flow rate occurred when the pressure on the fluid was "consistent" or "maintained" between the two points, no matter whether the pressures were high or low at each end. Students reasoned that this consistent pressure indicated (higher) flow rates were maintained. For example, when S11 was asked why they thought the oak tree (with the smallest pressure gradient) would have the greatest flow rate of sap, they responded: "Well, it's just the most constant throughout. So, that's what I would think. Like it, it [pressure] doesn't really change." When asked what the flow rate would be in an instance where the start and end pressure were the same, S11 replied:

> **S11:** Okay, now I would say that that has the most [flow].
>
> **Interviewer**: Why would you say that?
>
> **S11:** Because it doesn't change at all. It's the same throughout.
>
> **Interviewer**: Right. And how does it not changing indicate that it's the most flow?
>
> **S11:** I don't, it's just constant. Like the **constant amount of pressure**. So it's the **same amount being pushed** is what my thinking is. And there's going to be a lot more at the end if it's constant compared to a lot more at the beginning and a little bit at the end.

Students interpreted the similar pressure values as indicating little change to the system, and therefore little change to fluid flow, because the system was "able to maintain the pressure the whole entire time," according to S28.

Sublevel 2.2 Reasoning Pattern 2.2B: Pressures Indicate the Volume of Blood That Has Flowed. Student explanations using this pattern reasoned that flow rate is a measure of the volume of fluid moving through a tube (accurate) and different volumes of fluid cause different pressures (accurate); therefore, pressure can be used to infer flow rate (in inaccurate ways). Students used this reasoning in several ways.

In one way, students explained that organisms with high-pressure values from high blood volumes have the greatest flow rates. S30 explained this by saying:

> Pressure in the beginning of the aorta probably means that you have **some volume of blood being pushed into that area**. If you have a **larger volume, you could have a larger pressure** … I'm going to go with the zebra just because it has the highest starting pressure.

This type of reasoning may be based on students' understanding that increasing the volume of fluid in a compartment will cause an increase in pressure in that compartment. Therefore, these explanations suggest that a high flow rate will cause a high volume of fluid in that space, which in turn causes the high pressure. Given the high pressure, there must have been a high flow rate into that area. Student explanations in this group focus on pressure as a measure of the amount of fluid moving rather than a driving force for fluid movement. It is correct that higher fluid volumes exert more pressure on the walls of a tube; however, there will be only limited fluid flow if the pressure gradient between the beginning and end of the tube is small.

In another way, student explanations described lower pressures at the end of the tube, or large pressure differences, as indicating that a greater volume of blood had *left* the tube. This was exemplified when S29 said:

> If the starting and ending pressures were pretty similar, that would indicate more of a constant flow of blood and maybe **not as much volume of blood flow through**. If there was **a greater pressure difference, maybe there was a lot of blood that traveled which is why the [end] pressure is so much different than the starting pressure**.

These explanations interpreted the low pressures as indicating a loss of fluid volume due to the fact that the fluid had already flowed out of the area in question. Consequently, the lower volume of remaining fluid created less pressure on the tube. Similar to the first way of inaccurately connecting pressure, volume, and flow, student explanations using the second way of reasoning described pressure as being directly related to volume with a loss of volume causing the lower pressure.

Another set of student explanations discussed pressure and volume as being inversely related. To justify this reasoning, some explanations cited the ideal gas law ($PV = nRT$), likely because it was a well-known relationship in which "pressure" was a key variable. For example, S35 said: "More pressure is happening when just … less volume. So the volume and the pressure. The **formula between both the volume and the pressure,** PV[=]nRT, if you know it from chemistry." Although using the indirect relationship between pressure and volume from the ideal gas law frequently led students to select the largest pressure gradient as having the greatest fluid flow, their rationales were not based on pressure gradients as driving forces.

Sublevel 2.2 Reasoning Pattern 2.2C: Small Pressure Differences Indicate Low Resistance, which Indicates High Bulk Flow. This set of student explanations inaccurately linked two accurate understandings. The first accurate understanding is that decreased resistance along a path will lead to a smaller pressure drop along that path. The second accurate understanding is that decreased resistance along a path will cause increased flow along that path. By connecting these two understandings, students reasoned that a smaller pressure drop along a path indicates a decreased resistance, which causes a greater flow. For example one student explained, "I wasn't completely sure but I chose the camel because there is only a small decrease in [pressure] from the ascending aorta to the abdominal aorta so the resistance in the aorta and maybe the rest of the arteries and arterioles are low, which would increase flow by the equation." However, as the question stated that each tube had the same diameter, length, and blood viscosity (i.e., the same resistance), this sequence of reasoning was inaccurate. While these student explanations correctly noted that resistance moderates fluid flow, they did not attend to the pressure gradient as the driving force for fluid flow.

Students' Ideas about Why Fluids Move along Pressure Gradients. Level 2 explanations generally noted that driving forces caused materials to flow, which represented a shift to a more mechanistic understanding of fluid movement rather than the purpose-driven explanations we found at level 1. However, some explanations revealed that students were struggling to conceptualize these driving forces as energy gradients. Instead, explanations often referenced molecular mechanisms in line with diffusion to explain bulk fluid flows along pressure gradients. For example, explanations contained ideas like materials going to equilibrium (e.g., "If you are going along the concentration gradient, where you're just trying to equilibrate on both sides, you don't want to lose any energy so you have to follow the concentration gradient," S29) or that molecules in liquids move to places that were less "crowded" (i.e., "If you have a bunch of molecules in a really tight space, but they have the chance to escape, then they're going to want to disperse evenly comparative to their environments," S39).

When explanations did describe energy as playing a role in bulk fluid flows, the ideas were imprecise or vague. For example, some explanations described the challenges associated with moving against gradients (e.g., "You can't push against a gradient," S11) or simply mentioned that energy was involved, such as S16, who said: "High to low pressure because just the **thermodynamics** of it … If there's a high number here and a low number here, then that's **the path of least resistance** for molecules to move." S37 had a relatively sophisticated understanding about the role of energy in pressure gradients, saying:

> Because the universe is always trying to decrease potential energy. So, basically, anything at any time is going to go… somewhere that decreases potential energy. So if you have a cliff, and there's a liquid on it, anything that encourages the water to jump off that cliff is going to be totally fine with water.

However, they had previously explained that organisms where high pressure was maintained (and therefore the gradient was small) would exhibit the greatest flow. When confronted with their previous explanation in the face of their energy explanation, they struggled to reconcile the two competing ideas:

> **Interviewer:** If we go back to the camel that was the 200 to 200, it's maintaining its, I think you said, potential energy across that distance?

> **S37:** Oh, hm. So I don't think of it maintaining the potential—well, oh, actually, yes, I do think it would … so if the camel was standing on all fours, I think the blood that was going down to its feet would have less potential energy. But since the pressure is still up, it sounds like the potential energy is being maintained despite that. Yeah.

S37 in many ways epitomizes what is characteristic about students who provided responses at level 2; they used scientific ideas that relate to gradients but were uncertain how to apply those ideas to the bulk flow assessment items.

Level 3: Principle-Based Reasoning with Pressure Gradients as a Driving Force. At the highest level of the framework, student explanations consistently identified organisms with the largest pressure difference as experiencing the greatest fluid flow. Moreover, the explanations used pressure gradients as driving forces that mediated fluid movement. The following exchange with S4 demonstrates this kind of reasoning, going so far as to explicitly cite the general relationship for bulk flow as part of their reasoning:

> **S4:** The deer… I see that, even though the start and end pressures are relatively lower compared with the other animals, there's a greater difference. And so I'm just looking at the differences between start and end pressure. And you would look to the zebra, and that has a difference of 60, but you also see that the Sitka deer has a difference of 70, which to me indicates a higher flow rate.

> **Interviewer:** And why does that indicate a higher flow rate?

> **S4:** Hm, that's a good question. Again, I will think back to my flux model … So we're assuming that, in all of these animals, you have the same amount of resistance, so that shouldn't have an impact. So what you're looking at then are your driving forces, which would come from this start and end pressure. And so if you have a greater difference, then you have a greater numerator, which makes your flux larger.

S4 acknowledged that the start and end pressures of the Sitka deer are "relatively lower compared to other animals," indicating they noticed the different magnitudes of pressures across the animals, but S4 focused on the pressure differences as the most important consideration when making a selection. In the latter part of the response, S4 confirms their selection by drawing on the bulk flow relationship (bulk flow ∝ gradient/resistance), recognizing that when resistance is held constant, the driving forces—as indicated by the pressure gradient—must be driving fluid movement. By using the bulk flow relationship as a reasoning tool, S4 identified the most salient features of the system that would lead to a scientifically correct understanding of the task. Similarly, S31 explicitly used Poiseuille's law to examine their initial ideas about flow rates:

> **Interviewer:** Would any of them [the animals] have a higher flow rate, given that they have the same size aorta, same composition of blood, but these different pressures is the only difference?

> **S31:** **No, they wouldn't**. Because the flow rate, the **flow rate would be Q**, I don't remember the exact formula. But I know it's, well, it's **change in pressure divided by … 8 pi R to the fourth**. And if you're saying the radius isn't changing, because they all have the similar diameter … Then, hmm. **Flow rate would be proportional to the change in pressure. So if you have a larger change in pressure, you would expect to have a larger flow, right?**

By using Poiseuille's law to mechanistically reason about blood flow, S31 realized their initial answer of no differences in blood flow among the different animals was incorrect and that, in fact, the animal with the largest pressure difference would have the most flow.

Students Ideas about Why Fluids Move along Pressure Gradients. When students were asked why fluids move down pressure gradients, energy-related explanations were more likely to be associated with level 3 explanations of bulk flow rather than level 2 explanations of bulk flow (i.e., 26% of level 3 interview explanations mentioned energy, whereas only 10% of level 2 interview explanations mentioned energy; the other 74% of explanations were similar to those of level 2, using "equilibrium" and "crowdedness" ideas). However, the energy ideas used were similarly vague or imprecise regardless of the associated bulk flow explanation. Several students mentioned "entropy" as important but were unclear exactly how that played a role in fluid movement. Other students suggested systems moved to lower energy states, like S10 who said: "Things tend towards equilibrium because it's a lower energy level." S2 showed one of the more nuanced understandings of the way energy is involved in bulk flow, saying:

> **S2:** Because you have a greater force at the high-pressure end. As you go down to a lower pressure, it can't go back up. Otherwise … **you would need energy to go from low to high pressure.**
>
> **Interviewer**: Why don't you need energy to go from high to low pressure?
>
> **S2**: Because there's a concentrate—or, **there's a pressure gradient existing. So that's basically your energy**.

S2 recognized that pressure gradients are also energy gradients, which was uncommon in the students we questioned. Students' uncertainty with the link between energy and pressure gradients was not prohibitive to their ability to reason productively about bulk flow in a physiology context. However, there may be other contexts where students' confusion about the link between energy and pressure gradients might be prohibitive.

Table 4 presents the frequency of levels observed during our interviews. This information is provided to show the range and frequency in our sample; it is not meant to indicate the prevalence of ideas that might occur in a classroom or show how students' ideas change over a term, as we only interviewed a small number of students from each course (Supplemental Figure S1).

RQ2: CAN WE USE THE BULK FLOW PRESSURE GRADIENT REASONING FRAMEWORK TO EVALUATE WRITTEN ASSESSMENT RESPONSES FROM A NATIONAL SAMPLE OF UNDERGRADUATE BIOLOGY AND ALLIED HEALTH MAJORS?

Methods

To further validate the bulk flow pressure gradient reasoning framework, we recruited students from 11 courses at five institutions across the United States taking a course that included instruction on bulk flow. These students took our assessment items as low-stakes, formative assessments. This allowed us to obtain responses to our assessments from an additional, larger group of students. We did this in order to investigate whether the framework captured the diversity of student reasoning in this larger group of responses and collect validity evidence based on response processes (American Educational Research Association *et al.*, 2014). It also provided us with a snapshot of reasoning levels pre- and postinstruction in different student groups. Four of the five institutions were public R1s, very high, research-active, "more selective" institutions (Carnegie Classification of Institutions of Higher Education, n.d.; Table 2).

TABLE 4. The number of student interview responses at each level of the bulk flow pressure gradient reasoning framework at the start and end of the term (numbers in parentheses show how total level 2 responses are distributed across different patterns)

	Start of term	End of term
Level 1: Nonmechanistic	5	—
Level 2: Emerging mechanistic	16	10
Sublevel 2.1: Pressure causes		
2.1A: High pressure pushes	(6)	(4)
2.1B: Low pressure is less resistance	(2)	(1)
Sublevel 2.2: Pressures indicate		
2.2A: Pressure maintained	(4)	(4)
2.2B: Pressure indicates volume	(2)	(1)
2.2C: Pressure indicates resistance	(2)	—
Level 3: Flow down gradients	16	23

In each course, students were given an online assessment of six short-answer physiology items. One of the six items was a bulk flow pressure gradient item similar to our interview items (Figure 1) but with values for three rather than five tubes in a plant or animal scenario (Supplemental Figure S2). The instructors selected the most appropriate scenario(s) to give to their students. The other five items were part of a different study.

We collected students' written responses at the beginning and/or end of a term for three student groups: students in introductory physiology courses for allied health majors, students in introductory biology courses for majors, and students in upper-division physiology courses (Table 2). We collected 1050 responses from 935 students in 11 courses at the beginning of the term and 882 responses from 752 students in 11 courses at the end of the term (see Supplemental Table S1 for sample sizes by item, time point, and course). While physiological topics dealing with the concept of bulk flow were presented in each of these classes, the data collected are meant to serve as validity evidence to evaluate whether or not the bulk flow pressure gradient reasoning framework can capture the breadth of reasoning used in a diverse sample and are not intended to assess specific instructional practices.

We used the coding rubric created for analyzing the interview data for RQ1 to identify the patterns and levels of reasoning in students' short-answer responses. We calibrated coding on students' responses by having two researchers (J.A.C. and a research assistant) use the coding rubric to code 114 responses into one of the seven patterns. If two independent coders coded the students' responses to the same reasoning pattern, this was considered a match. Interrater reliability for this calibration phase was greater than 90% agreement. After this calibration phase, one researcher (research assistant) coded the rest of the responses, with a second researcher (J.A.C.) coding 10% of those data. Final agreement was greater than 90%.

Results

We collected data from 11 courses across five institutions to confirm that our reasoning framework could be used to categorize

TABLE 5. Example student responses from each pattern and level from the national validation sample for RQ2

Level and pattern	Blood flow item: zebra, 106–102; camel, 93–91; elk, 83–75	Phloem sap flow item: American beech, 0.60–0.35; white oak, 1.20–1.00; American chestnut, 0.90–0.80
1: Nonmechanistic	Elk: "I compared it to what I know about humans. People with a high blood pressure usually have a heart that beats faster. The heart, although it beats faster, pumps less blood which is probably why it beats faster, to compensate for the difference."	American beech: "It has the greatest number of vessels."
2.1A: High pressure pushes	Zebra: "The zebra has the greatest flow rate because there is more pressure which pushes blood through the aorta faster."	White oak: "There is highest pressure both at the beginning and the end so the sap in the phloem will be pushed more to move faster."
2.1B: Low pressure is less resistance	Elk: "It has the greatest flow rate since it has the least pressure in the aorta so it has the least resistance to the blood flow."	American beech. "Lowest pressures mean the least resistance. Therefore, the sap would be flowing the fastest because the size of the 'tubes' are generally the same size in all of the trees."
2.2A: Pressure maintained	Camel: "The camel was able to maintain almost the same blood pressure meaning that the blood pressure remained high."	American chestnut: "I think the American chestnut has the greatest flow because it has the smallest difference in start and end pressure."
2.2B: Pressure indicates volume	Zebra: "Since the pressure is the greatest, I assume that it means that there is more blood in the area, meaning that it has the greatest flow rate."	American beech: "Because it had the greatest loss in pressure over the same amount of time as the other trees, meaning that more sap flowed out flowed through the vessel over the given time, and thus faster than the others."
2.2C: Pressure indicates resistance	Camel: "The pressure from the beginning of the vessel to the end of the vessel decreased the least, meaning resistance is the least in this animal, which means that decreased resistance will increase flux."	American chestnut: "The rate of flow would be greatest at the tree with the least resistance. Since the starting and ending pressure in the American chestnut is similar, the resistance must have been low."
3: Flow down gradients	Elk: "Elk has the greatest flow rate due to having the largest difference in start pressure and end pressure."	American beech: "There is a greater pressure gradient in the American beech. So the sap will flow down its pressure gradient faster."

reasoning offered by a larger and more varied group of students. We found examples of all the reasoning patterns described in our bulk flow reasoning framework in all three student groups at both the beginning and the end of term (Table 5 and Figure 2). Additionally, we were able to use the bulk flow pressure gradient reasoning framework to code all student responses collected. We noticed that fewer than 10% of all students reasoned at level 1 at the beginning or the end of the term, while more than 50% of students enrolled in majors introductory biology or upper-division physiology courses began the term using level 3 reasoning. We also found that, regardless of when the items were given (i.e., the beginning or end of a term), a greater proportion of students in allied health physiology courses reasoned at level 2 compared with students enrolled in majors introductory biology or upper-division biology courses. Within these level 2 responses, we found most students used reasoning pattern 2.1A (higher magnitude pressures cause higher bulk flow rates) with the second largest proportion of responses coded as 2.2A (maintenance of pressure indicates higher bulk flow rates). By the end of the term, students in allied health physiology courses reasoned at roughly equal proportions for levels 2 (44%) and 3 (49%), whereas students enrolled in majors introductory biology or upper-division biology courses reasoned predominantly at level 3 (60% and 70%, respectively).

DISCUSSION

We developed the first reasoning framework that describes how undergraduate students reason about the role of pressure gradients in determining the rate of fluid flow through tubes. The bulk flow pressure gradient reasoning framework has three levels and is based on 70 interviews and 2445 responses to short-answer assessment items from biology and allied health majors in introductory to upper-division courses. Our framework focuses on a simple bulk flow system and targets students' understanding of how the magnitude of the pressure gradient determines the flow of fluids through tubular structures of similar length and diameter. Despite this constrained focus, we found that student explanations displayed a diversity of ideas concerning the concept of pressure and how pressure gradients influence fluid movement.

Students providing bulk flow explanations at level 1 in the framework used nonmechanistic ideas about pressure, often relying on ideas about characteristics and behaviors of organisms. As with many science concepts (e.g., energy, evolution), pressure is both a scientific term and a term used commonly in everyday language (Pramling, 2009; Jin and Anderson, 2012; Slominski *et al.*, 2020). Pressure is used colloquially (e.g., people are under pressure to meet a deadline), in medical situations (e.g., systolic and diastolic blood pressure), and in relation to water flow in homes (e.g., lack of water pressure causing low shower output). Students providing explanations at level 1 draw on these surface understandings. Therefore, instructors can be aware that students may be interpreting their words through a different lens than intended. Providing assignments to compare and contrast the colloquial and scientific use of the word "pressure," in addition to teaching Poiseuille's law may be helpful.

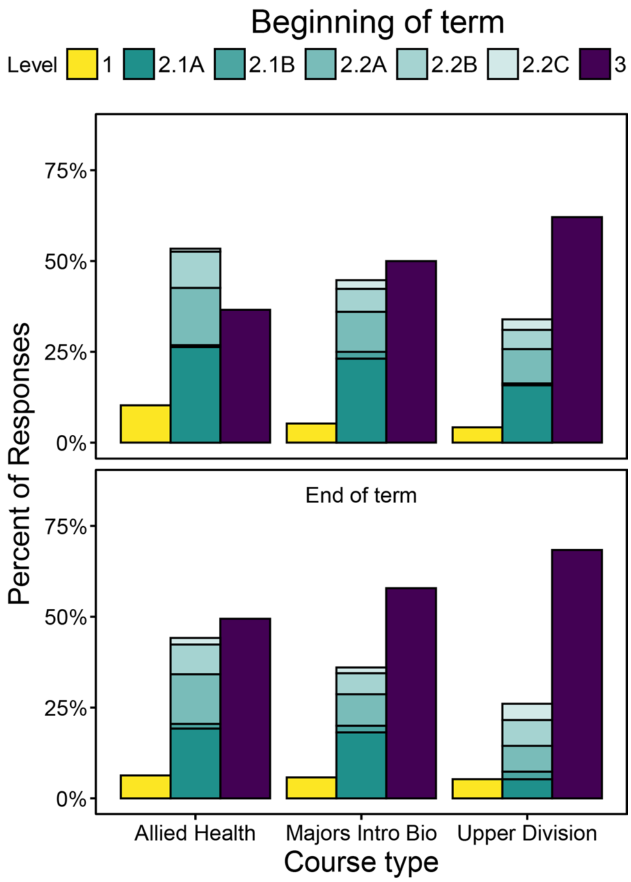

FIGURE 2. Pattern and level of reasoning in written responses from the national validation sample described in Table 2. Responses are grouped by time point (beginning and end of the term) and course type (introductory physiology for allied health majors, introductory biology for majors, and upper-division physiology for majors). Data from blood and phloem sap items were combined.

We found that, when explaining blood flow through vessels or sap flow through phloem, students using level 1 reasoning often relied on teleological reasoning. In these cases, students reasoned the blood had to flow because the animal needed to deliver the blood with all its nutrients and oxygen to the tissues to keep the animal alive. Students used similar logic for the delivery of sap with its water and nutrients to the various parts of a plant. We were not surprised by this teleological thinking, as we see it in the students in our classes and it has been well documented in a robust body of literature from the fields of biology and physiology education research (Richardson, 1990; Tamir and Zohar, 1991; Michael, 1998; Mohan *et al.*, 2009; Slominski *et al.*, 2020).

Students using any of the five patterns of reasoning seen in level 2 demonstrate emerging ideas about how pressure relates to flow. Many of these ideas were linked to scientific relationships that included pressure but were misapplied to the given tasks.

Students using reasoning pattern 2.1A explained that tubes with the highest pressure values, be that the highest starting pressure, highest average pressure, or highest ratio of pressures, would cause greater bulk flow rates, because these tubes had the most force "pushing" on the fluid. This pattern is similar to one that Brown and colleagues (2017) found in some engineering university students; even after completing a course on fluid mechanics, students can reason that a high pressure at one point is a pushing force causing fluid flow. Students using reasoning pattern 2.1B explained that lower pressure at the end of the tube causes higher rates of fluid flows, because there is less resistance to overcome. These students see the ending pressure as a source of resistance to flow, yet pressure and resistance are independent variables in the bulk flow equation. This connection between a change in pressure causing a change in resistance was also found by Michael *et al.* (2002). Students using reasoning patterns 2.1A and 2.1B might understand the pressure vocabulary used in class (e.g., high pressure, low pressure, mm Hg) and interpret pressure as a force that impacts fluid flow, but may not be cueing into the instructors' emphasis on pressure gradients.

Students using reasoning pattern 2.2A explained that little to no pressure difference between the start and the end of the tube indicated that the flow was maintained (i.e., at a high level). These students are using pressure in the tube as a measure of flow rather than as a cause for it. This use of pressure as indicators and not causes of flow was similar to what we observed in students using reasoning pattern 2.2B. Some students explained that a high flow rate will cause a high volume of fluid to accumulate in a space, which in turn causes the high pressure, so given the high pressure at the end of the tube, there must have been a high flow rate into that area. Other students explained that lower pressures at the end of the tube, or large pressure differences, indicated that a greater volume of blood had *left* the tube. A third group of students reasoned that pressure and volume are inversely related, as in the ideal gas law. This incorrect use of an inverse relationship between pressure and volume for liquids was also found by Michael *et al.* (2002). All three groups of students using 2.2B reasoned that flow rate is a measure of the volume of fluid moving through a tube per unit time, and because different volumes of fluid cause different pressures, pressure can therefore be used to infer flow rate. These students are using pressure as an indicator of volume and fluid flow rather than focusing on the pressure gradient as a driving force for fluid flow.

Students using reasoning pattern 2.2C reasoned that a smaller pressure drop along the tube indicates a decreased resistance and decreased resistance will cause a greater flow. However, in this case, the students failed to note that the question specifically stated that the tubes were identical (i.e., have the same resistance) and that any difference in flow would be due to the difference in the stated pressure gradients. Like students who used reasoning pattern 2.1B, these students focused on their accurate understanding of the inverse relationship between resistance and flow rate, but failed to incorporate the direct relationship between pressure gradients and flow rate.

Students providing level 2 explanations are calling on multiple resources about pressure, volume, and resistance that they have accumulated through their academic or everyday life. As many students in biology and physiology classes have taken physics or chemistry either in high school or college, they may have encountered many of the principles associated with the concept of pressure (e.g., Poiseuille's law, ideal gas law, static pressure; Table 1). Instructors should therefore be very clear on what principles are appropriate for hydrostatic fluid flow in organisms and provide students an opportunity to practice applying these principles in situations with pressure gradients of different magnitudes to confirm that the students are using the proper principle. It may also be beneficial for biology and physiology instructors to build collaborations with their colleagues who are teaching introductory physics courses to coordinate how principles such as Poiseuille's law are taught. Such interdisciplinary collaborations have been shown to be quite beneficial to both the instructors and the students who take these courses (Redish and Cooke, 2013).

The results from our national validation sample indicate that only 10% of our sample (predominantly from R1 institutions) reasoned at level 1 at the start of term. This suggests that, by the time students reach the undergraduate level at R1 institutions, most have an emergent mechanistic understanding of pressure rather than an indicator of an organism's functioning. We also found that, as students experience more biology and physiology courses, many gain an understanding that a pressure gradient rather than just pressure is the driving force for fluid flow. These are the 70% of upper-division students who provided level 3 reasoning when explaining fluid flow. However, this development is not inevitable, as indicated by the 30% of upper-division students who continued to demonstrate uncertainty about how pressure gradients work (i.e., reasoned using levels 1 or 2) even at the end of the term.

LIMITATIONS

We acknowledge that our national sample for validation is composed almost exclusively of public R1 institutions. To get a more comprehensive data set, we need to expand our sample to include more associate's-dominant institutions as well as regional public institutions and private schools.

Although we did not observe a difference in the pattern or type of student reasoning between the blood flow and phloem sap flow items, we realize that context may influence the resources students call on to answer a question (Nehm and Ha, 2011; Slominski *et al.*, 2020). That is, students may draw from different patterns or levels of reasoning as they reason through problems in different contexts (Lira and Gardner, 2020). To that end, we are currently analyzing a new set of data from items that not only vary the context of the organism and the magnitude of the pressure gradient but also investigate changing or keeping constant starting pressures. Additionally, the ways in which students' reasoning about bulk flow pressure gradients can be affected and altered by differences in instruction and teaching strategies is not within the scope of this present work. This important question will need to be addressed in the future.

Conclusions and Implications for Teaching and Research

Pedagogical content knowledge (PCK) is "information about typical difficulties students encounter as they attempt to learn about a set of topics; typical paths students must traverse in order to achieve understanding; and sets of potential strategies for helping students overcome the difficulties that they encounter" (NRC, 2000). There are several different types of knowledge that make up PCK: knowledge of students, assessment knowledge, content knowledge, curricular knowledge, and pedagogical knowledge (Carlson *et al.*, 2019). Of particular relevance for this study, "knowledge of students" includes instructors' ability to anticipate how students are likely to reason and what students will find confusing or challenging about a topic (Ball *et al.*, 2008). Assessment knowledge includes knowing how to design formative assessments and make changes to instruction based on responses to these assessments (Chan and Hume, 2019). We propose that instructors can use our bulk flow pressure gradient assessment items to make student reasoning visible and provide instructors with greater knowledge of their students' current understanding of the topic. Our bulk flow pressure gradient framework can guide the instructors' interpretation of the reasoning students offer when solving bulk flow problems, which in turn can inform instructors' design of future formative assessments and course activity (i.e., assessment knowledge; Auerbach *et al.*, 2018; Chan and Yung, 2018).

We realize it is often challenging to untangle students' explanations of physiological phenomena. However, by taking the time to dissect their reasoning, instructors can gain valuable insight into student thinking about the relation of variables to one another, as well as students' misinterpretations of what is a cause and what is an effect of a change in a physiological variable. Our bulk flow pressure gradient reasoning framework provides some guidance as to the multitude of ways students use pressure, volume, flow, and pressure gradients. We suggest instructors can use our formative assessments and reasoning framework to enhance their teaching of bulk flow down pressure gradients in three ways: 1) Use our bulk flow pressure gradient reasoning framework to anticipate the kinds of ideas students will bring to the classroom or laboratory and plan instruction accordingly. 2) Have students take a bulk flow pressure gradient item as a low-stakes formative assessment near the beginning of the unit to reveal students' incoming ideas about bulk flow along pressure gradients. Instructors can then modify planned instruction to meet the learning needs of their students. 3) Have students take a bulk flow pressure gradient item again at the end of the unit or term and use the responses to reflect on the impact of the teaching methods used. By uncovering and summarizing patterns in students' explanations, our bulk flow pressure gradient reasoning framework provides faculty with the opportunity to more effectively design their course topics about pressure and pressure gradients and thus enhance their PCK and thus their students' mechanistic reasoning on this challenging topic (Ergönenç *et al.*, 2014).

In the beginning of the *Discussion* and in the paragraphs above, we have endeavored to provide suggestions for how faculty might respond if they uncover their students using the different types of reasoning in our bulk flow pressure gradient reasoning framework. At this point, these instructional strategies are mostly our own personal suggestions based on our years of teaching. We suggest that biology education researchers could use our bulk flow pressure gradient reasoning framework as a tool to assess the distribution of patterns and levels of reasoning in different populations of students, including investigating the effectiveness of new teaching strategies focused on bulk flow pressure gradient reasoning.

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