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# Using the graduated sequence instruction to combat errors in precalculus

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ARTICLE INFO	ABSTRACT
Received: 13 Feb. 2024	The position of developing countries on international mathematics assessments lends to a growing concern
Accepted: 23 Jan. 2025	among stakeholders. The factors are multifaceted. This explanatory sequential mixed research design used the graduated sequence instruction (GSI) model to help ameliorate the situation. The researchers sampled 100 students: 50 (experimental group) received GSI and the other 50 (control group) received traditional whole-class instruction. Participants were assessed before and after the treatments. Analysis of covariance was performed to determine the effects between the groups while controlling for pre-test, sex, and age. The results indicate significant differences between the groups. However, sex and age showed no significant differences. The qualitative texts confirmed conceptual errors in traditional instruction. It was recommended that the GSI model be employed in teaching and learning basic concepts in pre-calculus.
	Keywords: combat errors, experiential learning, graduated sequence instruction, pre-calculus

# INTRODUCTION

Calculus seeks to solve problems in tangent and area/volume (Berggren, 2021). Regrettably, senior high school students continue to perform abysmally (Alam, 2020; Hurdle et al., 2022; Karikari et al., 2020; National Teaching Council [NTC], 2023) due to conception, applications, and formulas (Aduko & Armah, 2022; Baidoo & Ali, 2024; Domondon & Rin, 2022; Owusu-Darko et al., 2022). West Africa Examination Council (2020) found that traditional instruction breeds rules and procedures without conceptual understanding (Ali, 2021, 2023; Owusu-Darko et al., 2023). One school (Agbofa, 2023; Bouck et al., 2019; Butakor & Dziwornu, 2018; Varaidzai & Makondo, 2020) attributed the canker to schools, and another (Abaidoo, 2018; Arhin & Hokor, 2021; Owusu-Darko, Apoenchir & Mensah, 2022) to the environment. This study believes that the graduated sequence instruction (GSI) could be the stem tide.

GSI, also known as concrete-representational-abstract, visual-representation-multi-sensory approach, peer-assisted reflection, or concreteness fading (Ali, 2024; Bustos Tiemann & Ramos Rodriguez, 2022) sequentially and gradually promotes and propels one's conceptual understanding, procedural accuracy, and multi-sensory within local context (Ali, 2023; Flores & Hinton, 2022). Moving from concrete through visual representations aids deeper understanding (Litteck et al., 2024), relates to real-world representations (e.g., graphs, tables, and diagrams) (Baidoo & Ali, 2023; Herrera et al., 2024; Hong & Lee, 2022; Parr et al., 2024), and enhances conceptual, visualization, problem-solving, and engagement skills (Lemonidis et al., 2020; Spektar, 2023).

# **Calculus as a Function**

A 'function' is an ordered pair such as that  $x \to f(a, b)$  or an input-output such that  $x \to f(x)$ . This rule assigns every element in the first set to a unique element in the second set (García-García & Dolores-Flores, 2020; Nagle et al., 2019). However, the word 'function' could ambiguously mean a social gathering, and 'input-output' could mean the value is the same (Hatisaru, 2023).

# **Calculus as a Limit**

Let f(x) be a function and L as real numbers. Then as 'x' approaches 'a', as f(x) approaches L. The notation  $\lim_{x \to a} f(x) = L$  means as x tends to 'a'  $(x \to a)$ , 'x' gets closer and closer to f(x) (Radmehr & Drake, 2020). However, students think 'never reaches' means the function cannot be evaluated (Sidelil, 2019).

# **Calculus as a Derivative**

The derivative is  $\frac{dy}{dx}$  and the integral is  $\int f(x)dx$ . Here, students cannot establish the relationship between the differential and integral (Borji et al., 2018). Consequently, they cannot conceive  $\frac{dy}{dx}$  as a 'single fraction' (Nieto et al., 2022; Radmehr & Turgu, 2024; Sie & Agyei, 2023).

# **Calculus as Fundamental Theorem**

The fundamental theorem says that derivatives and antiderivatives are inverse processes. That is if f is continuous on [a, b] and F is an antiderivative of f on [a, b], then  $\int_a^b f(x) dx = F(b) - F(a)$ , and if  $\int_a^x f(t) dt$  is an antiderivative of f, then  $\frac{d}{dx} [\int_a^x f(t) dt] = f(x)$ . However, the difficulties are functional notations and lack of knowledge in 'f' and 'F' (Radmehr & Turgu, 2024).

- Therefore, this study seeks to address the following research questions:
- 1. What errors do students commit?
- 2. How does the GSI model combat the errors?

# METHODOLOGY

## **Research Design**

Domondon et al. (2022) and Spekta (2023) used quantitative-qualitative methods. Brannen (2018) used mixed-method simultaneously and sequentially. Gesser-Edelsburg et al. (2020 used a quantitative method dominated by a qualitative. This study adopts a sequential explanatory design from Creswell and Creswell (2018) to analyze both quantitative and qualitative data (Douglas et al., 2020).

# **Population and Sample**

The population was 318 students. About 100 participated in the quantitative phase through simple random sampling techniques (Nga et al., 2023). They comprised girls and boys across general science, general arts, home economics, business, and technical/vocational programs. They also included form 1, form 2, and form 3 students who hailed from rural, peri-urban, and urban, with diverse social, economic, and cultural backgrounds. These ensured the representativeness and generalizability of the findings (Nagle et al., 2019). Ten participants were used in the qualitative phase through purposive sampling techniques (Berggren, 2021).

#### **Data Collection Procedure**

The participants in the experimental group were designated SE<sub>1</sub>, SE<sub>2</sub>, SE<sub>3</sub>, ..., and SE<sub>50</sub>, and control as SC<sub>1</sub>, SC<sub>2</sub>, SC<sub>3</sub>, ..., and SC<sub>50</sub>. Even though both groups were pre-tested, only the experimental group was administered with the GSI model (Listiawati & Juniati, 2021). In the qualitative phase, the text scripts were scanned and subjected to content analysis (Schoonenboom & Johnson, 2017).

### **Sustainability and Impact**

The graduate school stipulates that a graduate research student starts the thesis after one year of coursework. So, we started the research in October 2021. By October 2023, the data collection processes had been completed. Therefore, the two-year duration was enough to create sustainability (CKT-UTAS, 2021). The researchers also maintained methodological consistency by analyzing both quantitative and qualitative data to reveal errors and guarantee research sustainability. The GSI theory is time-tested and has existed for many 25 years. The data processing, and statistical significance in the quantitative, and data categorization and excerpts in the qualitative suffice more sustainability (Torres et al., 2024).

Research impact was measured by the transformation of 55 academic literature. We had 30 on calculus, 20 on GSI, and 5 on methodology. The development of the interventions treatment activities, and social interactions foster higher-quality impact (Torres et al., 2024).

### **Data Collection Instruments**

The four sections of the instruments were demographic characteristics, causes of poor performance, the GSI model, and research sustainability. The test items were closed-ended and open-ended (Da, 2023). Items 1, 2, 3, and 16 were adapted from Sebsibe and Feza (2020), and items 6, 7, 8, 9, 10, 11, 14, 15, and 17 from West Africa Examination Council (2020). We used digital recorders, transcription software, and visual analysis software to collect qualitative data. The codes and themes were generated to observe patterns and relationships (Principe, 2022). We reduced respondent biases by avoiding sensitive or controversial questions. We reduced researcher biases with multiple coders, external reviews, triangulations, and participants' evaluations (Principe, 2022).

## **Potential Scalability and Adaptability**

Scalability is the ability of an instrument to effortlessly transition back and forth from micro to macro (Flyvbjerg, 2020; Milat et al., 2020). The researchers adopted the Vaughan-Lee et al. (2018) framework involving success, adaptability, sustainability, and high effectiveness. We expanded the scope and content of the study (Bulthuis et al., 2022; Vaughan-Lee et al., 2018). The GSI model

## Table 1. Key errors

Error		GSI function
	Dropping the constant	
	Dropping negative signs	_
Carelessness	Writing questions wrongly	Corrective feedback, collaboration, and re-enforcement
	Sloppy handwriting	
	Not stating units	_
Due ee duwel	Incorrect computation	
Procedural	Overgeneralization writing $\infty^0 = 1$ and $\frac{\infty}{\infty} = 1$	<ul> <li>Corrective feedback and practice</li> </ul>
	Taking infinity as numbers	
	Writing $\frac{0}{0} = 0$ and $\frac{\infty}{0} = \infty$	
Conceptual	Confusing functional values of limits	<ul> <li>Modelling, formulation, and practice</li> </ul>
conceptual	Misunderstanding limits as unreachable, and infinite	- modeling, formulation, and practice
	Confusing notation $f^{-\prime}(x)$ as $f^{\prime}(x)$	
	Linguistic issues	_

was made adaptable to different contexts, content, and target groups (Milat et al., 2020). We also collected data for two years to sustain horizontal (quantitative) and vertical (qualitative texts) studies. The scalability was mostly observed in the piloting, text scripts, and GSI intervention model (Palmie et al., 2023).

### **Data Analysis Procedures**

The quantitative data was analyzed t-test and analysis of covariance (Hurdle et al., 2022). The qualitative phase was analyzed by codes and plausible themes. Excerpts were scanned and pasted for comparative analysis.

#### **Reliability and Validity**

We checked the reliability coefficient with the Kuder-Richardson formula 20 (Baidoo & Ali, 2023). We also satisfied content, construct, and criterion-related validities, and internal and external threats to validity (Moloto & Machaba, 2021; Sie & Agyei, 2023). The researchers randomly assigned participants to both experimental and control groups to ensure comparability and eliminate biases and triangulated them with the traditional and GSI models (Ali, 2024).

## **Ethical Considerations**

Approval was obtained from the Ethical Committee of the University of Education, Winneba. The following guidelines were adopted by CKT-UTAS (2021):

- 1. The purpose of the study and the participants' rights
- 2. Participation was strictly voluntary by signing the consent form.
- 3. Participants were fully informed of their right to withdraw from the study at any time.
- 4. Participants were fully informed that they would take assessments.
- 5. The schools were written to for permission.
- 6. The privacy and confidentiality of participants were assured

### **Challenges During Implementation**

The major hiccups in the implementation emanated in the GSI model during the training of moderators, matching, and triangulation.

# **RESULTS AND DISCUSSION**

In the first part, we present the quantitative results. In the second section, we present the text analysis.

# **Research Question One: Students' Errors**

In **Table 1**, the students encountered real-life settings, modelling, problem-solving, communication, and collaborative skills. The GSI model was responsive, conducive, and viable.

#### **Research Question Two: Minimizing Errors**

This research question examined the cause-effect relationships with a hypothesis:

H<sub>0</sub>: There is no significant difference before the GSI model.

The analysis satisfied the following assumptions:

- 1. Linearity of model
- 2. Linearity between covariates and outcomes
- 3. No interaction between factors and covariates

## Table 2. Descriptive statistics (N = 50)

Group	Pre-	test	Post-test (u	nadjusted)	Post-test (adjusted)		
Group	М	SE	М	SE	М	SE	
Experimental	51.64	0.87	80.10	1.27	79.91	0.83	
Control	51.16	0.86	58.90	1.03	59.09	0.83	

#### Table 3. Independent samples t-test for post-test

Levene's test for equality of var	evene's test for equality of variances			46	Sig. (2-tailed)	Mean difference	CE difforence	95% CI of th	e fifference
	F	Sig.	ι	u	Sig. (2-tailed)	Mean unierence	SE unierence	Lower	Upper
Equal variances assumed	1.345	0.249	12.993	98.000	0.000	21,20000	1 (2170	17.96194	24.43806
Equal variances are not assumed			12.995	94.088	0.000	21.20000	1.63170	17.96026	24.43974

#### Table 4. Analysis of covariance for covariates

Source	Type III sum of squares	df	Mean square	F	Significance	Partial eta squared
Pre-test	2,560.086	1	2,560.086	75.261	0.000	0.442
Sex	207.169	1	207.169	6.090	0.015	0.060
Age	25.143	1	25.143	0.739	0.392	0.008
Group	10,812.443	1	10,812.443	317.864	0.000	0.770
Error	3231.509	95	34.016			

Note. <sup>a</sup>R squared = .818 (adjusted R squared = .810)

### Table 5. Texts on limits

14			Α		В		С		D	Non-res	pondents
Items		N	P (%)	Ν	P (%)						
	1.1	2	4	16	32	4	8	28*	56	0	0
-	1.2	0	0	1	2	2	4	47*	94	0	0
Experimental	1.3	0	0	46*	92	0	0	4	8	0	0
	1.4	4	8	1	2	41*	82	3	6	0	0
-	1.5	2	4	1	2	3	6	43*	86	1	2
	1.1	3	6	38	76	4	8	2*	4	3	6
-	1.2	3	6	2	4	1	2	43*	86	1	2
Control	1.3	15	30	14*	28	3	6	18	36	0	0
-	1.4	11	22	6	12	24*	48	2	4	7	14
-	1.5	7	14	1	2	27	54	15*	30	0	0

Note. P: Percentage

#### 4. Equal variances

5. Normality of residuals

In **Table 2**, the experimental (mean [M] = 51.64, standard error [SE] = 0.87) and control (M = 51.16, SE = 0.86) groups are nearly the same. However, the unadjusted experimental group (M = 80.10, SE = 1.27) was greater than the control group (M = 59.90, SE = 1.03).

# **Tests of Hypotheses**

 $H_{01}$ : There is no statistical significance between the group performance.

This t-test compared the performance before the effect of the pre-test, age, and sex were statistically removed.

In **Table 3**, the t-test (t [98] = 12.993, p = 0.000) indicates that there are group mean differences. The null hypothesis was therefore rejected since the experimental group outperformed the control group.

Ho2: There is no statistical significance between the group performance.

The ANCOVA was used to remove the statistical effects of the pre-test, age, and sex.

In **Table 4**, the results (F [1, 95] = 317.864, p = 0.000) indicate there is a significant difference. We therefore reject the null hypothesis as the students in the GSI model outperformed their peers. However, the effect of sex (F [1, 98] = 6.090, p = 0.015) and pre-test (F [1, 98] = 75.261, p = 0.000) were significant.

# **Qualitative Text Analyses**

In **Table 5**, item 1.1 scored 28 (56%) for the experimental group in limit value and the function whereas only 2 (4%) for the control group. Item 1.2, 46 (94%) of the experimental group and 43 (86%) in the control group solved the at x = 3. Item 1.3 was well understood by the experimental group 46 (92%) as compared to only (8%) in the control group in the  $\frac{0}{0}$  is undefined.

**Figure 1** indicates that items 1.4 and 1.5 record 41 (82%) and 43 (86%) correct responses for the experimental group and 14 (28%) and 15 (30%) in the control group. Two major difficulties were taking infinity as a number and generalizing  $\frac{\infty}{\infty} = 1$  or  $\frac{\infty}{\infty} = \infty$ .

In Figure 2, the errors are both procedural and conceptual difficulties.

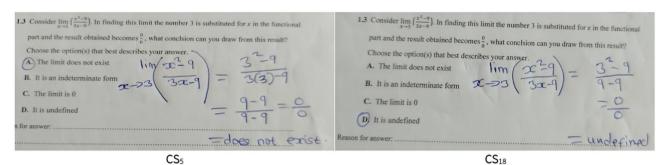


Figure 1. Two excerpts in the control group-1 (Source: Field study, Control Participants 5 & 18)



Figure 2. Three excerpts in the control group (Source: Field study, Control Participants 23, 27 & 48)

Itomo			Α		В		с		D	Non-res	pondents
Items		Ν	P (%)	Ν	P (%)						
	2.1	1	2	48*	96	0	0	1	2	0	0
-	2.2	42*	84	0	0	7	14	1	2	0	0
Experimental	2.3	1	2	3	6	46*	92	0	0	0	0
-	2.4	5	10	39*	78	2	4	2	4	2	4
-	2.5	4	8	2	4	3	6	37*	74	4	8
	2.1	20	40	22*	44	7	14	2	4	1	2
-	2.2	24*	48	1	2	20	40	2	4	3	6
Control	2.3	2	4	5	10	40*	80	1	2	2	4
-	2.4	5	10	25*	50	6	12	4	8	10	20
-	2.5	3	6	8	16	9	18	23*	46	7	14

Note. P: Percentage

Table 6 Texts on derivatives

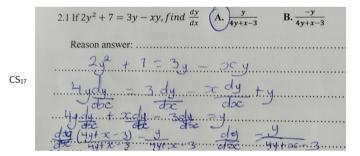


Figure 3. One excerpt in the control group (Source: Field study, Control Participant 17)

In **Table 6**, items 2.1-2.5 showed that 96% of the experimental group correctly answered item 2.1, while 2 (4%) missed it. For the control group, 22 (44%) correctly answered item 2.1, and 28 (56%) missed it.

In Figure 3, 84% of the experimental group correctly answered it whereas 16% missed it. In the control group, 52% missed it.

The difficulties observed with the control group's notational order, writing  $\frac{d}{dx}\left(\frac{u}{v}\right) = \frac{u\frac{dv}{dx}-v\frac{du}{dx}}{v^2}$  instead of  $\frac{d}{dx}\left(\frac{u}{v}\right) = \frac{v\frac{du}{dx}-u\frac{dv}{dx}}{v^2}$ , And carelessness with negative signs.

In **Figure 4**, 30% of the control group failed to recognize the function of a product. They simply differentiated it as though it was a function of a function, and this led to incorrect decisions.

In **Table 7**, 92% of the experimental group answered item 16A correctly, and only 8% got it wrong. In the control group, 54% correctly answered the item, 34% had it wrong and the remaining 12% left the item unanswered. In items 16B, 16C, and 16D; 86%, 78%, and 54% in the experimental group answered them correctly. Careful examinations of their work show that the difficulties were taking 'function value as 'limit value', incorrect computation, and sloppy handwriting.

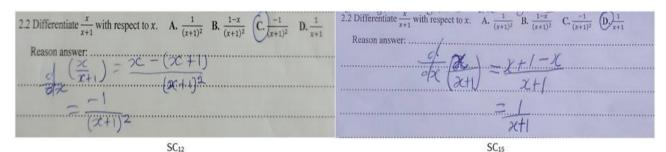


Figure 4. Two excerpts in the control group-2 (Source: Field study, Control Participants 12 & 15)

#### Table 7. Texts on item 16

		Experime	ental group		Control group						
Co	rrect	Incorrect		Non-response		Correct		Incorrect		Non-response	
Ν	P (%)	Ν	P (%)	Ν	P (%)	Ν	P (%)	Ν	P (%)	Ν	P (%)
46	92	4	8	0	0	27	54	17	34	6	12
43	86	7	14	0	0	23	46	20	40	7	14
39	78	9	18	2	4	21	42	18	36	11	22
28	56	16	32	6	12	19	38	16	32	15	30
	N 46 43 39	Correct           N         P (%)           46         92           43         86           39         78	Correct         Inco           N         P (%)         N           46         92         4           43         86         7           39         78         9	Correct         Incorrect           N         P (%)         N         P (%)           46         92         4         8           43         86         7         14           39         78         9         18	N         P (%)         N         P (%)         N           46         92         4         8         0           43         86         7         14         0           39         78         9         18         2	Correct         Incorrect         Non-response           N         P (%)         N         P (%)         N         P (%)           46         92         4         8         0         0           43         86         7         14         0         0           39         78         9         18         2         4	Correct         Incorrect         Non-response         Co           N         P (%)         N         P (%)         N         P (%)         N           46         92         4         8         0         0         27           43         86         7         14         0         0         23           39         78         9         18         2         4         21	Correct         Incorrect         Non-response         Correct           N         P (%)         N         P (%)         N         P (%)           46         92         4         8         0         0         27         54           43         86         7         14         0         0         23         46           39         78         9         18         2         4         21         42	Correct         Incorrect         Non-response         Correct         Incorrect           N         P (%)         N         P (%)         N         P (%)         N         P (%)         N           46         92         4         8         0         0         27         54         17           43         86         7         14         0         0         23         46         20           39         78         9         18         2         4         21         42         18	Correct         Incorrect         Non-response         Correct         Incorrect           N         P (%)         N         P (	Correct         Incorrect         Non-response         Correct         Incorrect         Non-response           N         P (%)         N           46         92         4         8         0         0         27         54         17         34         6           43         86         7         14         0         0         23         46         20         40         7           39         78         9         18         2         4         21         42         18         36         11

Note. P: Percentage

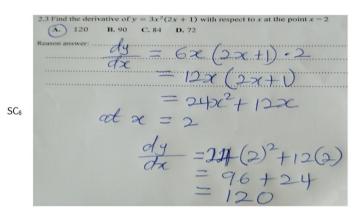
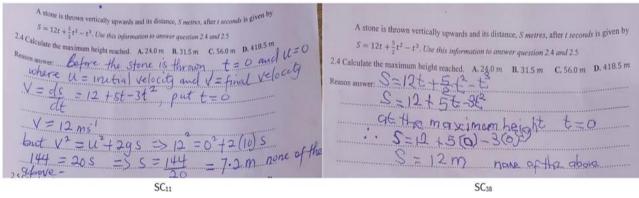
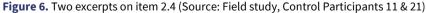


Figure 5. One excerpt on item 'A' (Source: Field study, Control Participant 4)





In **Figure 5**, 78% in the experimental group provided correct responses, and only 50% in the control group responded correctly. The item highlighted conceptual and procedural errors as the main difficulties.

In Figure 6, the difficulties were associated with derivatives in the experimental group.

In Figure 7, the difficulties were recorded;

- 1. Confusion with notations like  $f^{-\prime}(x)$  instead of f'(x).
- 2. Failure to realize that at the maximum concentration, f'(x) = 0.
- 3. Difficulties factorizing quadratic equation.

Figure 8 shows two excerpts.

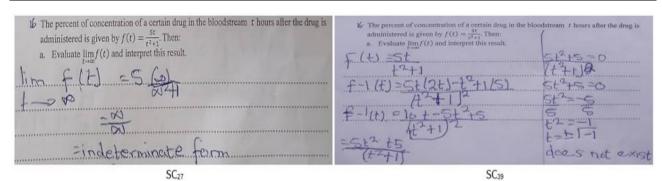


Figure 7. Two excerpts on item 16 (Source: Field study, Control Participants 21 & 39)

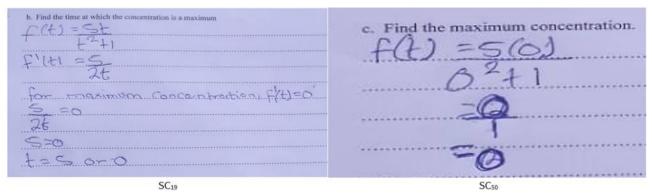


Figure 8. Two excerpts-1 (Source: Field study, Control Participants 29 & 50)

# Table 8. Texts on integrals

ltomo			Α	В		С			D	Non-respondents	
Items		Ν	P (%)	Ν	P (%)	Ν	P (%)	Ν	P (%)	Ν	P (%)
	3.1	40*	80	5	10	3	6	2	4	0	0
Experimental	3.2	3	6	2	4	43*	86	2	4	0	0
-	3.3	1	2	1	2	2	4	46*	92	0	0
	3.1	25*	50	4	8	5	10	9	18	7	14
Control	3.2	5	10	6	12	20*	40	4	8	15	30
-	3.3	1	2	4	8	5	10	24*	48	16	32

Note. P: Percentage

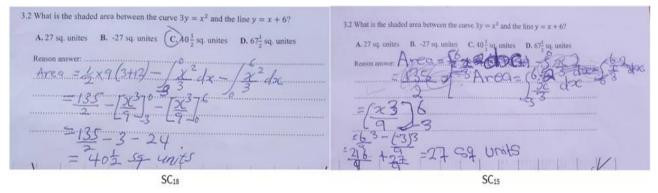


Figure 9. Two excerpts-2 (Source: Field study, Control Participants 13 & 15)

# **Texts on Integrals**

Items 3.1-3.3 were closed-ended to evaluate knowledge of integrals.

In **Table 8**, 40% of the control group were correct, 30% left the item unanswered and 30% got the item wrong. Options A, B, and D were distractors arrived at from students' failures to sketch the function and compute the area of the curve.

On item 3.3, 92% of the experimental group answered the item correctly, while only 8% had it wrong. However, students in the control group (52%) answered it wrongly.

Figure 9 shows some excerpts.

### Table 9. Responses to item 17

			Experim	ental group		Control group							
Item	Co	Correct		Incorrect		Non-response		Correct		Incorrect		Non-response	
	Ν	P (%)	Ν	P (%)	Ν	P (%)	Ν	P (%)	Ν	P (%)	Ν	P (%)	
17A	42	84	8	16	0	0	20	40	27	54	3	6	
17B	41	82	9	18	0	0	19	38	20	40	11	22	
17C	48	96	2	4	0	0	28	56	16	32	15	30	

Note. P: Percentage

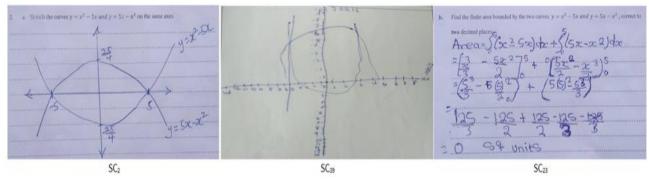


Figure 10. Excerpts of students' responses on graphing (Source: Field study, Control Participants 1, 20 & 29)

Itomo			Α		В		С		D	Non-respondents	
Items		Ν	P (%)	Ν	P (%)	Ν	P (%)	Ν	P (%)	Ν	P (%)
Europeine entel	4.1	2	4	1	2	3	6	44*	88	0	0
Experimental	4.2	1	2	3	6	43*	86	2	4	1	2
Constant	4.1	7	14	9	18	9	18	24*	48	1	2
Control	4.2	12	24	2	4	25*	50	10	20	1	2

Note. P: Percentage

Table 10. Texts on FTC

In **Table 9**, 84% of the experimental group sketched 17A, while only 16% had an incorrect curve. In the control group, 40% sketched it, 54% had incorrect sketches and 6% left it unanswered. In 17B, 82% of the experimental group answered well, while 18% had difficulties. For the control group, only 38% had it correct while 40% had it wrong, and as high as 22% left it unanswered. For item 17C, 96% of the experimental group had correct answers and 4% were incorrect. In the control group, 56% had it correct 32% wrong, and 30% left it unanswered. The difficulties were inappropriate scale, *x* and *yy*-intercepts wrong computations, and units.

Figure 10 shows excerpts of students' responses on graphing.

#### **Students' Text Analysis on FTC**

In Table 10, the experimental group achieved 88% proficiency whereas the control group demonstrated 48%.

# DISCUSSION

The findings indicate that the traditional approach was severely weak. The poor performance was corroborated by Butakor and Dziwornu's (2018) findings of irregular training models in calculus (West Africa Examination Council, 2020). Alam (2020) suggests a blend of interconnected strategies. However, the GSI model brought modelling, practicing, and re-enforcement components to allow students to visualize, think-pair-share, collaborate, and communicate (Ali, 2023; Owusu-Darko et al., 2022).

Undoubtedly, calculus is the nucleus for pursuing STEM programs (Da, 2023). Comparing the groups, the experimental outperformed the control (Tettey et al., 2018) due to the GSI model's enhanced conceptual understanding, visualization, problemsolving, and engagement skills (Bouck et al., 2019) and guided them to model calculus (Hinton & Flores, 2019). Findings recommend remedial lessons, remediations, multiple representations, and information and communication technology tools to augment the model (Lemonidis et al., 2020; Spektar, 2023).

# CONCLUSION

The experimental group outperformed their peers in the control group. The GSI model yielded the high significant differences.

The qualitative texts portrayed ambiguity and procedural and conceptual errors. This was accomplished by the GSI model. It was recommended that the GSI model be employed to detect and reduce errors before teaching and learning basic concepts.

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Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

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