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Lab experience with seafood control at the undergraduate level: Cephalopods as a case study

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Abstract

The correct labeling of seafood is important to protect nature and the rights of consumers. Given the certainty that the resources of the sea are not inexhaustible, only strict regulations and the implementation of sustainable fishing systems and reliable and traceable marketing systems can help ensure the long-term sustainability of fishery resources. Detecting mislabeling and seafood fraud is a useful resource for improving students' motivation and developing active learning methodologies in higher education. In the present study, we have proposed to the students a lab exercise consisting of exploring 25 different commercial cephalopod products from three major European supermarkets by using DNA barcoding and analyzing the results under the framework of EU and Spanish regulations. The problem is connected with the last theme (traceability) of the Conservation Genetics and Breeding course with the aim of providing students with a practical research lab experience about a real problem before going deeper into more theoretical contents. In this way, they can use the knowledge and the skills they acquired previously to better comprehend and think critically about the problem. Findings from students' answers to a survey revealed that the use of this approach generates useful information for communities, increases curiosity and feelings of benefit, and leads to high levels of satisfaction with lab practices compared with those in other courses. In conclusion, lab exercises focused on seafood control, in addition to being viable, can be used as a tool in classes to improve students' commitment to higher education.

Keywords: barcoding; cephalopods; lab experiences; seafood control; university

File S1 Lab practices materials

File S2 Survey-Eng

File S3 Student List 2017 COMGE

1 INTRODUCTION

Fish and seafood are important sources of nutrition and health for citizens worldwide. The proteins and micronutrients of these foods are essential for achieving healthy diets. Unfortunately, the long-term sustainability of these foods is currently under serious threat, which is a challenge that must be met by the fishery and aquaculture sectors. The most recent report from the Food and Agriculture Organization (United Nations) on the state of world fisheries and aquaculture explicitly states that industries and markets must be a force that drives seafood sustainability.[1] Seafood should have certification schemes aimed at informing stakeholders along the seafood value chain as to whether a fishery is sustainably managed.

Correct labeling is important for protecting nature and the health and wallets of consumers. The most effective labeling must use the one fish, one name rule.[2] Scientific names are typically required, but these names are occasionally dropped in favor of the market name. Using scientific names improves the traceability of seafood, making it easier for governments to improve food safety, protect endangered species, enforce fisheries laws, and curb seafood fraud. In the European Union (EU) and in Spain at the national level, the authorities have established different regulations about labeling fishery products, aquaculture, and seafood. Correct labeling of unprocessed and prepacked fresh products must include, among others, the commercial designation and scientific names of the species processed, fishing gear categories, production methods, and catch areas.[3] However, for other processed fishery and aquaculture products, such as canned, composite products and breaded products, only information on allergens is mandatory.[3]

The hot topics of mislabeling and seafood fraud have proven to be useful resources for improving students' motivation and developing active learning methodologies in higher education.[4–6] Motivation can be achieved when students learn and simultaneously solve social problems that are relevant to their communities by participating in school projects.[7–9] Traceability studies on commercial cephalopod products could be an interesting case study for students, since they place in a real-world context the outputs of lab research procedures.

The cephalopod products available in European and Spanish markets are a complex case in terms of correct labeling. The increased globalization of markets and open borders permits species from many sources to be available to the consumer, thus complicating the identification and labeling of products containing fish and other seafood.[10] DNA testing of cephalopod food products and ingredients is an effective strategy to identify and authenticate species, detect contaminants or adulterants, and verify label claims. This strategy can also support traceability regulations and prevent food fraud and health problems.[11–15] Previous studies have reported over 20% of ambiguity and attempts to mislead consumers by using incorrect labels when buying products labeled with the commercial name Galician octopus, which is used to legally protect this high value-added product in national and international markets.[15] Moreover, comparisons between molecular and label analyses by Armani et al.[12] suggested that 48.5% of the products presented discrepancies between labeling and molecular identification. Both studies also indicated health issues for consumers.[12, 15]

Teaching approaches that imply the integration of real-life research activities into the teaching and learning process during higher education have been proven successful. This integration gives students the opportunity to explore an authentic research problem while applying prior knowledge and learning new concepts. This approach entails the pursuance of a set of inquiry-related procedures, such as finding information, formulating hypotheses, planning methodologies, collecting and analyzing data, and understanding the results. Active and reflecting learning promotes undergraduate students' critical thinking, problem solving, and communication skills.[16–18]

As a particular case of active learning, research-based learning (RBL) strategies have already been applied in many high-level universities within a wide variety of fields, such as the social sciences, engineering, medicine,

and geography.[16, 19] In particular, in the biology education context, they are an effective pedagogical strategy to promote student achievement and learning quality.[20–22] Knutson et al.[23] reported how students achieved high levels of critical biochemical laboratory skills and critical thinking while increasing their confidence and motivation when working in a biochemical research-based experience. Nevertheless, it must be pointed out that selecting an adequate teaching/learning strategy is highly dependent on the context. Students who have already been trained in this type of active learning strategy in previous courses can go into a deeper involvement than students using it for the first time. Levy and Petrulis[24] stated that "There is strong theoretical justification and empirical evidence in favour of providing more tightly structured and guided forms of IBL (identifying research-based learning) at less advanced levels of study."

In the present study, we proposed to the students an activity consisting of exploring 25 different commercial cephalopod products from three major European supermarkets by using DNA barcoding and analyzing the results under the framework of EU and Spanish regulations. The aim is to introduce an active learning strategy in which students are actively or experientially involved in the learning process doing things and thinking about the things they are doing throughout an IBL approach to engage students in the task but minimize the risk of dropping out. We want to assess the motivation and engagement of students researching a real problem in laboratory classes, and we have a secondary goal (as an output of the analyses conducted by students) of updating the data on the levels of mislabeling and food fraud in commercial cephalopod products in our area.

2 MATERIALS AND METHODS

2.1 Population and sample

The population of this educational study consists of students pursuing a degree in biology at the University of Oviedo, who composed the sample obtained by an intentional nonrandom sampling of the students taking the Conservation Genetics and Breeding (COMGE) course in the Department of Functional Biology in the academic year 2016–2017. All the students enrolled in the course participated in the experiment; the sample size was n = 29 (16 women, 13 men). Students provided signed consent to participate in the present study and gave permission to use their results in future publications/reports. The research procedures for Project No. 100/16 (Project Grupin14-093) were approved by the Principality of Asturias's Ethics Committee on July 15, 2016. COMGE is an optional course in the third year of the degree. Students enrolled in COMGE have taken a compulsory course about genetics in their second year.

2.2 Laboratory practices<<Query: Please check the hierarchy of head levels. Ans: it is correct>>

2.2.1 Day 0

As it was pointed out earlier, students have a background on general genetics. The problem they have to deal with is connected with the last theme of the COMGE course (traceability); thus, the approach used here aims to provide students with a practical research lab experience about a real problem before going deeper into more theoretical content, so that they can use the knowledge and the skills they have acquired before developing a better comprehension of the problem. Students have also participated in choosing the real problem to be solved during the lab classes. In the previous theoretical lessons, the professor proposed a set of problems (e.g., genetically modified organism [GMO] detection, mislabeling in cephalopods, and fraud in protected geographical indications), and they voted following their preferences/interest. Hence, the problem of mislabeling was chosen. The research problem and objectives were then defined, and the students received some needed instructions about the techniques they will have to use (Annex 1). The manuals of the commercial kits they should use were also uploaded to the online intranet website of the course. We must emphasize that students had not used these two kits earlier; therefore, they had to study the manuals and learn to perform

the procedures by themselves. The students were organized into two groups, and experiments were conducted in pairs during five independent sessions of 2 hr each from 9:00–11:00 hr and from 11:00–13:00 hr. The first five sessions were held at the genetics lab, and the last two sessions also included computer support in order to ease the analysis and the writing of the results.

2.2.2 Day 1

A total of 25 different ultrafrozen commercial cephalopod products (Table 1, Figure 1) were purchased (€104.3) from the Functional Biology Department of the University of Oviedo at three major supermarkets (coded in this work as **E**, **H**, and **C**). All the supermarkets were localized in northern Spain, Asturias, but had a wide and solid Spanish/European implantation that was localized in other EU countries. The product labels were photographed, and the data were recorded in Excel files by students. For the present study, following the proposed rule of "effective labeling" (one fish, one name) according to Golden and Warner,[25] analyses of labels and classification as "complete" were considered only when the scientific names, production methods, and catch areas were included for all the products. At least three different pieces of each product were considered (81 samples) for genetic analyses in the laboratory practices. One commercial cephalopod product was assigned to each of the student pairs. DNA was extracted from the samples with the QIAamp DNA Mini Kit DNA Kit—Qiagen according to the manufacturer's protocol. In brief, the kit description specifies that it is designed for efficient purification of genomic DNA from different types of tissues. Fresh and frozen samples that have been preserved in alcohol can be used with this kit. This procedure uses a cationic detergent in conjunction with the selective DNA binding of the HiBind matrix. Samples are homogenized followed by a rapid alcohol precipitation step, DNA is further purified, and all salts, proteins, and other contaminants are removed. High-quality genomic DNA is suitable for downstream applications such as endonuclease digestion, thermal cycle amplification, and hybridization techniques.

Table 1 Label contents and species authentications using gen COI-based DNA barcoding of 25 cephalopod commercial products from three different supermarkets localized in northern Spain, Asturias (in red bold products where labels did not include scientific names of the species used to produce the seafood commercial products and where species mix, or substitutions, were detected) (* indicates genetic assignments to *Sepia recurvirostra* or *Sepia aculeata* occurred with same% of identity after blast in genetic databases)

		Labels' data					**COI gene genetic analyse<<Query: Please check the edits made to Table 1. Ans: I considered necessary to change what were initially referred as IN RED to BOLD..and what were in bold to UNDERLINED since for the print version colors are not available.>>s**
Super market	**Samples code**	**Product name**	**FAO region**	**Common name**	**Scientific name**	**N samples/*N* attempted**	**Species detected**
E	ES01	Sepia limpia ultracongelada	Océano Indico Oriental FAO 51	Sepia	*Sepia pharaonis*	**2/3**	***Sepia recurvirostra-aculeata**/Illex argentinus***
E	ET02	Tubo de pota argentina ultracongelada	Océano Atlántico SudOeste	Pota argentina	*I. argentinus*	2/3	*I. argentinus*
E	ET03	Rabas precocinadas empanadas ultracongeladas	**x**	**Pota**	***X***	**0/30/3 underlined instead of bold**	
E	ET04	Anillas a la Romana precocida empanizada ultracongelada	**x**	**Pota**	***X***	3/3	*I. argentinus*
E	ET05	Delicias (anillas) a la Romana empanizadas precocidas y ultracongeladas	**x**	**Pota**	***X***	4/4	*Dosidicus gigas*
E	ET06	Empanadas (rabas) empanizadas precocidas y ultracongeladas	**x**	**Potón**	***X***	3/3	*D. gigas*
C	CS01	Sepia limpia cruda ultracongelada	**Océano Indico Este y Oeste**	**Sepia**	***Sepia* spp**	**4/4**	***S. pharaonis/Sepia prashadi/Sepia ramani***
C	CT02	Rodajas de tentáculos de potón del pacifico cocido ultracongelado	Pacifico Sudoriental FAO 87	Potón	*D. gigas*	4/4	*D. gigas*
C	CT03	Anillas de pota argentina ultracongelada	Oceano Atlantico Sur Oeste	Pota argentina	*I. argentinus*	3**(2)/3**	***S. recurvirostra-aculeata***/I. argentinus*
C	CK04	Puntilla de calamar cruda limpia ultracongelada	**Oceano Pacifico Noroeste FAO 61**	**Calamar**	***Loligo* spp**	**2/3**	***I. argentinus/D. gigas***
C	CR05	Chipirones troceados ultracongelados	**Oceano Atlantico Sur Oeste**	**Chipirones**	***Loligo* spp**	**1/3**	***Doryteuthis gahi/Loligo gah*i**
C	CK06	Calamar limpio ultracongelado	**Oceano Atlantico Centro Este**	**Calamar**	***Loligo* spp (such as *loligo duvauceli, loligo gahi*)**	2/3	*Uroteuthis duvaucelii*
C	CS07	Sepia limpia ultracongelada	**Oceano Indico Oeste**	**Sepia**	***Sepia* spp**	**2/3**	***Sepia lycidas/Sepia inermis***
C	CT08	Rejos de potón del pacifico cocidas, troceadas, congeladas	Oceano Pacifico Sudoriental FAO 87	Potón del Pacifico	*D. gigas*	2/3	*D. gigas*
H	HK01	Calamar limpio ultracongelado	**Océano Atlantico Centro-Este FAO 34**	**Calamar**	***Loligo* spp *(loligo duvauceli,loligo gahi…)***	**0/30/3 underlined instead of bold**	
H	HP02	Pulpo crudo ultracongelado	Océano Atlantico Centro-Este FAO 34	Pulpo	*Octopus vulgaris*	**2/3**	***Octopus maya***
H	HT03	Rejos cocidos de pota ultracongelados	**Oceano Pacifico Sud Este**	**Pota**	***Dosidicus* spp**	4/4	*D. gigas*
H	HT04	Anillas de pota argentina ultracongelada	Océano Atlantico Sud Oeste	Pota argentina	*I. argentinus*	4/4	*I. argentinus*
H	HK05	Calamar patagónico ultracongelado	Oceano Atlantico Sud Oeste FAO 41	Calamar patagónico	*Loligo patagonico*	**0/30/3 underline this instead of bold**	
H	HT06	Anillas de pota ultracongelada	**Oceano Atlantico Sud Oeste (Argentina)**	**Pota**	***Illex* spp**	2/3	*I. argentinus*
H	HK07	Puntilla de calamar crudo ultracongelado	**Oceano Nor Oeste FAO 61**	**Calamar**	***Loligo* spp**	**0/30/3 undelrined this instead of bold**	
H	HS08	Sepia limpia cruda ultracongelada	**Oceano Indico Este y Oeste**	**Sepia**	***Sepia* spp**	**3/3**	***S. pharaonis/Sepia elegans***
H	HP09	Tentáculos de pulpo cocidos ultracongelados	**x**	**Pulpo**	***X***	3/3	*O. vulgaris*
H	HT10	Rabas empanizadas precocidas ultracongeladas	**x**	**Potón**	***X***	3/3	*D. gigas*
H	HT11	Aros (anillos) a la romana precocidos ultracongelados	**x**	**Pota**	***X***	4/4	*D. gigas*
3 supermarkets	25 products	**Labeling not showing scientific names**				59/81	**Species mix/substitutions**
		E: 4/6 = 67%, **C:** 5/8 = 63%, **H:** 8/11 = 73%					**E:** 2/14 = 14%,
		Total: 17/25 = 68%					**C:** 11/20 = 55%,
							H: 5/25 = 20%
							Total: 18/59 = 31%

Note: In bold are the cCommercial products without PCR amplifications are underlined.

Abbreviation: COI, cytochrome oxidase subunit I.

Figure 1 Commercial presentations for some of the 25 cephalopod commercial products under study in this work (sample codes: HK01, HT03, ET05, ET03, CT08, and HP02). Supermarket names and commercial trademarks have been covered

2.2.3 Day 2

On the second day, students conducted species identification. Polymerase chain reaction (PCR) amplification of the cytochrome oxidase subunit I (COI) gene fragment was performed using the following primer pair: jgLCO1490 TITCIACIAAYCAYAARGAYATTGG and jgHCO2198: TAIACYTCIGGRTGICCRAARAAYCA.[26] The final PCR volume was 20 µl, including Green GoTaq® Buffer 1X, 2.5 mM $MgCl_2$, 0.25 mM dNTPs, 10 pmol of each primer, 2 µl of template DNA, and 0.65 U of DNA Taq polymerase (Promega). The following PCR conditions were used: 95°C for 5 min, followed by 35 cycles at 95°C for 60 s, 48°C for 60 s and 72°C for 60 s, and a final elongation step at 72°C for 5 min.

2.2.4 Day 3

The third day was used to check PCR results and to prepare sequencing products. The PCR amplicons were purified by using the ExoSAP-IT™ PCR Product Cleanup Reagent (Applied Biosystems™) and subsequently submitted for sequencing at the sequencing unit of the University of Oviedo by using the classic Sanger protocol.[27]

2.2.5 Day 4

The resulting sequences were edited with the BioEdit Sequence Alignment Editor software.[28] Species assignments were achieved by blasting new sequences against public DNA databases by using the BOLD (barcode of life database)[29] and the NCBI webpage (National Center for Biotechnology Information).[30] The settings used were best matched with a minimum 97% identity and 99% coverage, percentages that are commonly used for species identification and that are most appropriate when using a COI gene.[31]

2.2.6 Day 5

On day 5, we conducted a group discussion and evaluation session. The results were shared within the group so that students can have a global overview not only of their analysis (samples and supermarket) but also of their colleagues' analyses. An academic search of available previous results in scientific journals was used by students to compare their global results with previous reported data. Instructions to write a scientific

report structured in Background, Aims, Material and Methods, Results, and Discussion in pairs were given to students in the last session. Delivering and evaluation of these written reports were scheduled for the next 15 days.

2.3 Survey application and analyses

After the discussion and in order to assess the efficacy of the active learning strategy to increase student motivation, 12 items were answered on a voluntary survey that was applied to all the samples (Annex 2) with a 100% response ratio. The content validity of the survey to measure the motivation of the students was previously assessed by Borrell et al.[6] In this instrument, the students scored various aspects of the practices from 1 (least) to 5 (most appreciated) on a Likert scale. Additionally, one item was used to compare their satisfaction with other practical lessons (item XII, Annex 2) and to determine their motivation related to the practical lessons in which actual samples were analyzed in laboratory classes. For each item, the correlation between the item and complete test, once removed from the latter, was assessed. This correlation is the corrected index of homogeneity (IHC<<Query: Define "IHC" if applicable. Ans: There is not need of this. IHC is the Corrected Index of Homogeneity and it is defined in cite 32>>) following Peters and Van Vorhis.[32] To interpret the approach, this index is typically taken as a reference value of 0.20. Thus, all items that are IHC with values less than 0.20 should be eliminated. To evaluate the reliability of the test, Cronbach's alpha coefficient was calculated. To interpret the alpha coefficient, it is widely accepted that this value must be equal to or greater than 0.70 to show the sufficient reliability.[33] The survey results were compared with the results obtained from the 2015 COMGE practical lessons.[6] All statistical analyses, including the comparison between the groups in terms of the means and distributions, were conducted using nonparametric tests (Mann–Whitney tests) in IBM SPSS statistics.

3 RESULTS

3.1 Evaluation of the lab experience using survey results

In active strategies, students must participate as much as possible. Here, the starting point was selecting the research topic, followed by self-study of technical manuals, sample classifications, DNA extractions, PCRs and band purifications, manual editing and alignment of the sequences, blast databases, and searching scientific journals for previously reported data about fraud in cephalopod markets to produce a scientific report.

The survey results revealed good correlation indices among items since all the items were above an IHC value of 0.20, ranging from 0.36 to 0.71, except for item VI ("objective evaluations are better than practical ones") with IHC = −0.0006. This item was eliminated from any further analyses. Calculation of the Cronbach's alpha coefficient for the survey generated a value of 0.82. No statistically significant differences between students' gender and students' lab group were observed in any of the items.

The survey items in the current lab practices revealed means close to or above four (out of five) in the student survey results (Figure 2a). A comparison revealed significant differences in favor of the results of Borrell et al.[6] (in which students were also the samplers) for producing more comments to students' families as well as better understanding and learning perception when working with actual cases (Figure 2a). However, a greater impact on changing habits (i.e., reading labels) and a higher level of satisfaction with the practices was obtained in the current survey (Figure 2a). More than 72% of students considered that their satisfaction with the current COMGE practical lessons was higher compared with other practices in courses included in the degree in biology offered by the University of Oviedo (Figure 2b). According to the survey results, choosing (among different alternatives) a topic of interest was a good starting point for the strategy and increased student's engagement and curiosity. The total cost of the experience (sampling: €104.3; DNA extraction (81 samples): €174.2; PCR procedures and sequencing: €388.8 (€4.8 per sample); total cost per student = €23) did not deviate significantly from other classical practices conducted by the department, for example, species identification using PCR in the second year in the General Genetics course.

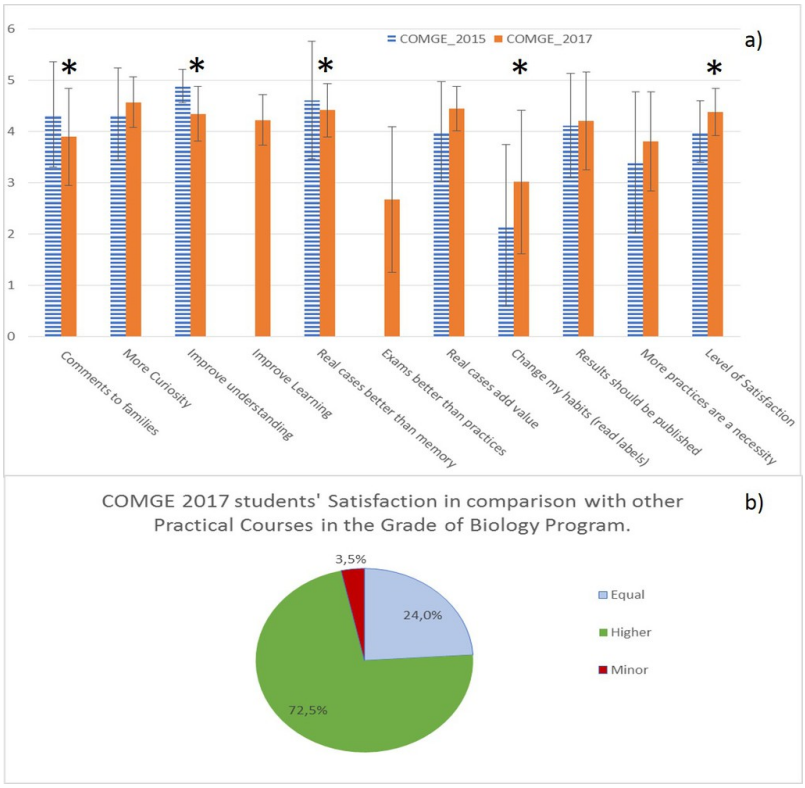

Figure 2 (a) Mean values (±1 SD) and (b) compared level of satisfaction from the results obtained after conducting a survey in the course COMGE at the University of Oviedo in co<<Query: The supplied figure 2 is in Low resolution. Kindly provide us the better version. Please refer to http://media.wiley.com/assets/7323/92/electronic_artwork_guidelines.pdf for the guidelines on how to produce good figures. Ans: The previous figure had 96ppp , this one has 150ppp.
Its a figure done in powerpoint and more resolution is not possible.>>urses 2015 (n=18) and 2017 (n=29) to assess students' motivation after the food control approach used in laboratory practices. *p<.05 after Mann–Whitney nonparametric test

Two other indirect measures indicate successful practices when using this approach: the practice reports, in English and following a scientific report format, trained our students in scientific writing, which is a transversal competence and a learning outcome in the degree in biology. A high mean value for the students' final report evaluations ($\mu_{\text{Course 2017}}$= 84.1%) was obtained. In addition, when the last theory theme, traceability, was covered at the end of the course, we could focus on practical cases recently reported in media and newspapers since the theoretical framework of this subject was already covered by the laboratory practices.

3.2 Molecular results

Reliable sequences were obtained from 59 tissue samples (72%) and are available in the figshare platform, an open access repository in which research outputs are available (DOI: 10.6084/m9.figshare.5705641). Blast procedures enabled the detection of several cases of mislabeling. We also considered cases in which complete scientific names were not available in the labels. Incomplete labeling was found in 68% of the products studied (Table 1), with no evident differences among supermarkets (**E**: 4/6 = 67%; **C**: 5/8 = 63%; **H**: 8/11 = 73%) (Table 1). In 7 of the 25 cases analyzed, the scientific names, FAO fishery area, and production methods were absent (28%), while in the other 10 cases (40%), the genera were the only information available on the labels (Table 1).

Successful molecular identification of the tissue samples under study (59 samples) revealed 18 cases in which species substitutions or a mixture of different species in single commercial cephalopod products were found (31%) (Table 1). The supermarket coded as **C** showed higher percentages of species substitutions/ mistakes (55%) than the other two supermarkets, **E** (14%) and **H** (20%) (Table 1). Analyses of four commercialized products revealed interesting findings with regard to species identification (Table 1):

- The ES01 product was labeled as *Sepia pharaonis*, but molecular analyses assigned this product to *Ilex argentinus* in one of the samples analyzed and to *Sepia recurvirostra-aculeata* in another analyzed sequence.

- The CT03 product was labeled as *I. argentinus*, but molecular analyses assigned the samples to *Sepia* spp. (*recurvirostra-aculeata*) in 2 out of the 3 tissue samples analyzed.

- The CT04 product was labeled as *Loligo* spp., but molecular analyses assigned the samples to *I. argentinus* and *Dosidicus gigas*.

- The HP02 product was labeled as *Octopus vulgaris*, but molecular analyses assigned the two sequences obtained from 2 samples of the product to *Octopus maya*.

4 DISCUSSION

Connecting biology learning processes with highly topical research problems has been proven to be an efficient teaching practice.[6, 9] Additionally, the literature shows how active learning methods increase not only students' complex lab skills but also, and especially, their critical thinking about the processes and about the real problems they deal with.[23]

Motivation is defined as an internal state that maintains learning behavior.[7, 34] Surveys are typically used as valid instruments to measure the motivational orientations and efficacy of different learning strategies of college students.[7] In Borrell et al.,[6] the laboratory practices in COMGE were planned to use “students as samplers” in a citizen science project. Students were advised to bring fresh fish products purchased from local markets by their families for consumption at home with pictures of the fish product labels. In the current study, we slightly changed the sample source and the teaching staff purchased and sampled the markets to avoid sample heterogeneity (dispersed and inconclusive data) and obtain a better picture with more complete and updated results regarding the levels of mislabeling and food fraud in a concise case study (e.g., the commercial cephalopod products in our area). The current study shows that research on a specific problem and associated learning has worked as a motivational strategy to make the students assume the role or researchers by answering a research problem related to their daily life context using advanced knowledge in genetics, using experimental tools in the lab, and making decisions about the research questions initially posed to them in this activity. Moreover, even when the experience has been designed by teachers and thus is a “bounded independence” RBL experience (since it is guided by a professor),[24] it constitutes a very first active experience that has increased students' engagement in learning processes and enhances students' abilities to develop further autonomous RBL (“authoring” learning as defined in Reference 24).**

Although the current approach generated fewer comments by students' families than observed by Borrell et al.,[6] the survey findings reinforce that the learning strategy followed in the current study also increased curiosity, perceptions of added value to the practices, and satisfaction. This reinforcement is attained because students were learning research lab techniques and simultaneously working on a project that generated useful information to their communities; thus, they were able to transfer the research results into a context of interest by answering a research question. Moreover, almost all the students believed that this information should be

shared and published (see item 9, Annex 2), since they assumed that correct labeling of seafood is important for protecting nature and the rights of consumers. A correct and certified seafood value chain is a priority to help stakeholders and protect responsible organisms with regard to regulating exploitation levels and to assess sustainability in a fishery. Students have experimented how genetics is essential when working in traceability. Thus, the first main goal for this study can be considered to be satisfactorily attained. Moreover, the active learning strategy seems to be viable in terms of costs. The course includes six ECTS (European Credit Transfer and Accumulation System) credits, and enrollment in the COMGE course costs €16.97 per credit (Official Bulletin of the Principality of Asturias, 151, 30-IX-2018). In this way, the experience cost was approximately 20% of the total cost for students' course requirements.

Regarding the second main goal of our study, which was updating the data on the levels of mislabeling and food fraud in commercial cephalopod products, an acceptable level of success (72%) in obtaining accurate sequencing was obtained in this course by students with no previous experience in molecular analysis on real (commercial) samples. Other courses in biology (e.g., General Genetics, the second course toward the degree in biology), when using strictly educative lab materials, have reported similar percentages of PCR success. For some squid samples (Table 1), it was not possible to obtain PCR bands. The COI gene primer pairs used here[26] are not completely universal.[35] Thus, further attempts using other “universal primers” could improve the production of PCR bands for sequencing. In any case, the student work enabled the detection of high values of incorrect (or not informative and complete) labeling of commercial cephalopod products (68%), including species mix/substitutions (31%) in three major European supermarkets. It is difficult to fully assess the reasons behind the higher percentages of labeling problems found in one of the supermarkets (**C**) compared with the other two supermarkets (**H** and **E**). Information on the details of commercial practices and providers is unfortunately currently unavailable. These percentages are slightly higher than those previously published in other recently conducted cephalopod studies.[12, 13, 15] This result, even if not completely representative of the global cephalopod market (due to the limitations in the sampling), raises serious concerns and prompts wider traceability studies by the responsible regulatory agencies. Moreover, this fact makes students' work as researcher-learners much more valuable from the educational point of view because they have successfully undertaken a complex research task, and they have also obtained interesting research outputs, which highlights the success of our lab research-based strategy.

In the present study, 6 of the 25 products (ET03, ET04, ET05, ET06, HT10, and HT11) were breaded products, and only the common name Pota/Potón was used on all the labels. These results demonstrated that different species share the same common name (and thus do not meet the one fish, one name rule), thereby confusing consumers and regulators about what was captured and processed by the industry. This mislabeling seems to only have a commercial reason and does not contribute to a transparent and accountable seafood supply chain. Indeed, mislabeling could lead to serious increases in seafood fraud and IUUIllegal, unreported, and unregulated <<Query: Define “IUU” if applicable. Ans: Illegal, unreported, and unregulated>> fishing as well as related economic, social, and environmental consequences.[36] Scientific names, not only market names, on commercial seafood products should be a strict requirement for all seafood products available in markets to improve the traceability of seafood, making it easier for governments to improve food safety, protect endangered species, enforce fisheries' laws, and curb seafood fraud.[25] The European labeling regulations for prepacked and non-prepacked products have established the commercial designation and scientific names, production methods, and catch areas as requirements. However, for other processed fishery (and aquaculture) products, such as canned, composite, and breaded products, only information on allergens is mandatory.[3] In light of all the recent results on fraud and mislabeling in seafood,[36] this requirement should be changed.

5 CONCLUSIONS

In summary, the current active learning approach in laboratory practices focusing on seafood control strategy targeting the traceability of cephalopod products shows a positive synergy: it improves the learning process by engaging students in a research-based activity that increases the motivation, curiosity, and academic results of students, and it also generates useful scientific information for stakeholders and regulators.

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