Perspectives

Descriptions of Centrifugation Manipulations in the Literature Illustrate the Need for Better Laboratory Training of Biologists

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Abstract: Students in environmental sciences, ecology, or wildlife management are often reluctant to acquire training in basic laboratory techniques. To advocate the importance of this training, we reviewed literature in the fields of biological and environmental science for one frequently used technique—centrifugation—and evaluated whether centrifugation parameters are properly expressed. Centrifugation is used to extract and purify different types of biomolecules for further characterization or quantification. The repeatability of the procedure depends on the proper identification of parameters defining the gravitational force applied to a solution, for example the duration, distance from central axis, and angular velocity. Correctly expressing rotation velocity—the "speed"—is therefore crucial to ensuring repeatability and the possibility of using the same protocols in different laboratories. When scrutinizing the materials and methods sections of publications in ecology, zoology, botany, or general biology journals, we noticed that velocity is expressed in different ways and essential information is often missing. We sampled 2000 articles in different fields of biological sciences that recorded centrifugation as a technique in the materials and methods section. We found centrifugation velocity to be properly expressed in gravitational force "g" in only 47.8% of the papers. The score dropped to 40.4% in journals specialized in ecology. We use this analysis to advocate for a minimum of training in the techniques of biochemistry that are of common use in environmental sciences. Better training would allow higher reproducibility of results in scientific publications.

Keywords: Centrifugation, Laboratory training, Analytical biochemistry, Ecology, Wildlife management, Biology program

Introduction

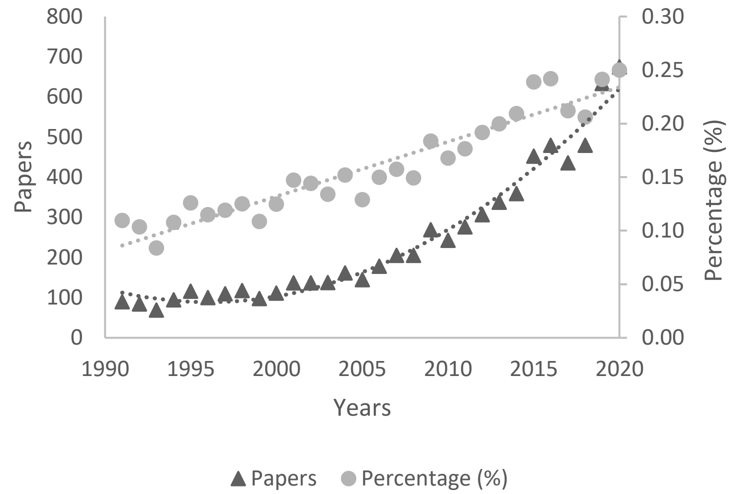
While ecosystems face major treats (global changes, pollution, plastic wastes, invasive species, loss of biodiversity and habitats), environmental scientists are increasingly required to objectively evaluate the scale of deterioration, to suggest strategy to alleviate their impact and to adapt to new environment. Environmental sciences are multi-disciplinary and may require skills from different disciplines (for example chemistry and ecology in eco-toxicological studies). By tradition, training in colleges and universities has been compartmentalized according to these disciplines often leading to highly specialized professions. For example, in biology, students interested in environmental sciences are often oriented to specialize in ecology, wildlife management or conservation biology. More importantly, these students might have a narrow perception of environmental sciences and do not realize the importance of transdisciplinary training. After teaching for close to three decades in a bachelor program of biological sciences to students mostly interested in ecology, marine biology or wildlife management, it is clear for us that they usually do not realize the importance of other disciplines like chemistry, biochemistry, genetics and the relevance of a minimum of literacy and competence in these disciplines.

Ecological sciences have been using basic biochemical techniques for many decades. For example, proximal analysis or protein content analysis has been routinely performed to evaluate the physiological conditions of individuals or wild populations (e.g., Dutil et al., 2008). With the development of molecular ecology and physiology conservation, the use of biochemical or molecular techniques in ecology, zoology, botany, and their allied sciences has significantly progressed in the last 30 years. As an example, the number and percentage of articles containing "enzymatic activit*" as a topic in these fields of study have increased steadily between 1991 and 2020 (Fig. 1).

A good knowledge of laboratory techniques is therefore essential in the training of biologists or ecologists outside the fields that traditionally use these techniques (e.g., biochemistry, clinical laboratory science). Nevertheless, this training in undergraduate or graduate programs might be inadequate or even absent. To illustrate this, we surveyed the literature for reports of centrifugation speed. Centrifugation is a common and simple technique for the separation, preparation, or purification of cells, structures, or biomolecules, and this technique can be useful in a wide range of biological sciences and applications. We therefore used this technique and the proper expression of its parameters as a proxy of the knowledge and proper training of researchers.

A quick consultation of different textbooks on biochemistry or laboratory techniques revealed that the easiest way to express centrifugation velocity to ensure reliability is in "g" (gravity) or RCF (relative centrifugal force) rather than in revolutions per minute (rpm). The force of sedimentation depends not only on the velocity of rotation, but also on the distance from the rotation's axis (rotation radius). Therefore, knowing the rpm with no information on rotation radius (or rotor) cannot be repeated in laboratories equipped with different centrifuges. On the other hand, gravity can be repeated on any centrifuge when we control the velocity and know the distance of the solution or homogenate from the central axis (Basha, 2020; Boyer, 2012; Farrell & Taylor, 2006; Gallagher, 2012; Holtzhauer, 2006; Juban & Barkley, 1996; Kamoun, 1999; Katoch, 2011; Lo, 2019;

Figure 1. Publications with "enzymatic activit*" as a topic in peer-reviewed journals in the following Web of Science categories: Ecology, Behavioral sciences, Zoology, Marine biology and Freshwater biology, Limnology, Oceanography, Fisheries, Forestry, Plant sciences, Mycology, Environmental sciences, and Environmental studies (Web of Science, 2021)



Percentage (%): the proportion of the total number of publications in these fields that have "enzymatic activit*" in the keywords or abstracts.

Ohlendieck & Harding 2018; Robyt & White, 1987; Rosenberg, 2005; Wilson & Walker, 2010).

Most modern centrifuges can be programmed to directly select the correct gravity "g" If this is not possible, a simple nomogram may be used to determine the relative centrifugal field (Boyer, 2012; Burtis & Ashwood, 1999; Gallagher, 2012; Hofmann & Clokie, 2018; Kaneko et al., 1997; Ohlendieck & Harding, 2018; Rosenberg, 2005). The equation

$$RCF = 1.118 \times 10^{-5} \times r \times n^2$$

gives equivalent results, where 1.118×10^{-5} is an empirical factor, r is the horizontal distance (i.e., the radius in centimeters) from the center to the bottom of the tube in the rotor cavity or bucket, and n is the rotation velocity of the rotor in revolutions per minute (rpm) (Basha, 2020; Bermes & Young, 1999).

We observed that the improper utilization of velocity is particularly frequent in publications in ecology, zoology, and related sciences. Owen (2011) observed the same trend in the materials and methods sections of ornithological publications. In this paper, we examined the expression of centrifugation velocity (g vs. rpm) in peer-reviewed papers appearing in publications related to ecology, aquatic science, environmental science, botany, and zoology. Our objective is not only to promote proper descriptions and terminology for centrifugation protocols to make the appropriate expression of velocity, but more importantly to highlight the importance of good training in basic laboratory techniques for biologists in any field.

Methods

Using the Google Scholar, Web of Science, and Scopus databases, we sampled 2000 peer-reviewed articles published between 2000 and 2018 in which the centrifugation technique was mentioned in the methodology. Papers came from journals in five categories (Table 1). We used the expression centrifug* with a category name to find references. We were then noting the way that centrifugation procedures were expressed and the units that were used in the section "Material and methods". For example, RPM, g, etc. (Table 2)

For comparison, we sampled 200 papers published between 2000 and 2018 from a biochemical journal. We chose Comparative Biochemistry and Physiology A, B, C, or D because the field of comparative physiology and biochemistry involves scientists with training in both biological and biochemical sciences. This makes these journals a good control group for comparison with biologists mostly involved in environmental sciences.

To check if the article by Owen (2011) published in the Journal of Field Ornithology had an impact on the way of expressing centrifugation velocity in studies in ornithology, we selected publications in this field of zoology from our database. We compared 119 papers before (2000–2011; N=74) and after (2012–2018; N=45) it was published. We also validated whether centrifugation step descriptions were more rigorous based on the reputation of the journal. We compared publications based on their impact factors as a proxy of their importance and how centrifugation was described in the material and methods sections.

We used exact binomial tests (Dorai-Raj, 2014) to compare the differences between categories, the conditions of centrifugation before and after the Owen's paper (2011) and according to the journal impact factors. Statistical analysis performed with R version 4.1.0 (R Core Team 2021)

Results and Discussion

Table 2 illustrates the strong inconsistency in the way that velocity is expressed in all fields covered by this study except in comparative biochemistry and physiology. More than 52% of the sampled references from the different journals showed an inappropriate description (rpm or missing value of velocity). The phenomenon is particularly and significantly marked in ecological ($p<0.001$), environmental ($p=0.014$), and zoological sciences ($p=0.009$), where appropriate units were used only 40%

Table 1: Categories used for the search.

Categories
Aquatic Sciences, including water research, limnology, and marine sciences
Botany and mycology
Ecology, including behavioral ecology, wildlife management, and conservation biology
Environmental sciences, including ecotoxicology and pollution research
Zoology

of the time, while this proportion reached in a very highly significant way with 83% in Comparative Biochemistry and Physiology publications (p<0.001). Our results appear to show critical training gaps in the different fields of biology, which can lead to poor repeatability of methodological protocols. The reviewers and editors of these peer-reviewed journals should therefore pay more attention to the use of appropriate units.

Velocity is still often expressed in rpm with no mention of the rotor, potentially rendering centrifugation procedures among laboratories difficult to reproduce. On many occasions, speeds were vaguely defined like "centrifuged at high speed, low speed, maximum speed or highest level" or even gentle, mild or brief centrifugation. (Ahyong & O'Meally, 2004; Aroca et al., 2006; Collins et al., 2008; Cureton et al., 2011; Morris et al., 2002;Cleveland et al., 2010; Dietz et al., 2013; Helm et al., 2008; Mills & Sebens, 2004; Ziegler and Wittwer, 2005; Straubinger-Gansberger et al., 2014); Bellstedt et al., 2010; Potts et al., 2003;(Fujishige et al., 2000; La Terza et al., 2004; Weir et al., 2012). In some publications, velocity is expressed with unusual units, or no units at all: for example, pm (Shoresh & Harman, 2010), tpm (Heulin et al., 2008), rps (Li et al., 2011), 12 000 with no speed unit (Kori-Siakpere et al., 2006), and 1 g (Pride, 2005) among others. Without the centrifuge and rotor models, or without proper expression and units of the velocity, it is just impossible to precisely repeat the experimental conditions.

Some authors have also noted the same lack of uniformity in their field of research. For example, Pendleton (2006) published a paper (study of pollen contained in honey) on the standardization of centrifugation speeds by using RCF or g. This study gave many examples of papers where speed was expressed in rpm without the size of the rotor and argued for the correct use of the centrifugation unit to obtain reproducible results. In a letter to the editor, Ata et al. (2016) promoted the use g force instead RPM in medicine, arguing that gravitational force is standard while RPM does not represent the same force in different centrifuge models. The same inconsistency had already been noticed in ornithology. Owen (2011) deplored the use of rpm in articles specialized in ornithology. In her paper, he proposed that blood needs to be generally centrifuged for 5–20 minutes at 10000–15000 g. To validate whether Owen (2011) had any impact on methods using this technique, we sampled our dataset for publications in which they mentioned blood centrifugation in ornithological journals before and after its publication.

The use of g as a unit doubled—to a still-low 40%—between 2012 and 2018 (Table 3) and this increase is highly significant (p<0.001). The recommended speed of10000 to 15000 g to obtain plasma or serum in birds has almost never been respected. The way that centrifugation velocity has been expressed in the ornithological literature has improved, but it remains below 50% of proper expression.

We evaluated to what extent the impact factor of the journal in which the studies were published could influence the rigor of defining proper protocol procedures (Table 4). In journals for which the impact factor is unknown or low (< 1), recording of centrifugation details is particularly inappropriate (70.7% and 60.5%, respectively). These both levels were very highly significantly lower than the average (p < 0.001). However, for journals with impact factors higher than 1, the results appear to be the same with approximately 50% having an

Table 2: Units of centrifugation velocity used in publications separated by field of study.

Category	N	g	Rpm and g	rpm	Missing	Inconsistent	No unit	Appropriate	Inappropriate
Aquatic sciences	386	54.1	2.8	32.1	8.8	2.1	0.0	57.0	43.0
Botany and mycology	349	56.4	1.7	28.9	11.7	1.1	0.0	58.2	41.8
Ecology	515	38.4	1.9	36.9	22.1	0.6	0.0	40.4	59.6
Environmental sciences	349	41.8	1.4	44,7	8.3	3.7	0.0	43.3	56.7
Zoology	401	41.4	2.0	37.9	16.2	2.2	0.2	43.4	56.6
Total	2000	45.8	2.0	36.15	14.1	1.85	0.05	47.8	52.2
Comp. Biochem. Phys	200	80.5	2.5	9.5	5.0	2.5	0.0	83.0	17.0

N: number of publications; g, rpm and g, rpm: percentages of publications specifying centrifugation in these units; Missing: no mention of centrifugation speed; Inconsistent: methods with several centrifugation steps where speeds are indicated in g or rpm without uniformity; No unit: centrifugation speed indicated but no unit specified; Appropriate: centrifugation method described correctly (i.e., g or rpm+g); Inappropriate: centrifugation method described incorrectly (i.e., rpm, Missing, Inconsistent, No unit). Values are expressed as a percentage

Table 3: Conditions of centrifugation of avian blood in ornithological journals before and after the publication of Owen (2011).

	N	g	rpm	Missing speed	10000–15000×g	Appropriate	Inappropriate
2000–2011	74	18.92	39.19	41.89	2.70	18.92	81.08
2012–2018	45	40.00	33.33	26.67	8.89	40	60

2000–2011: papers before the publication of Owen (2011); 2012–2018: papers after the publication of Owen (2011)

N: number of papers; g, rpm: percentages of publications specifying centrifugation in these units; Missing: no mention of centrifugation speed; 10000–15000×g: percentage of papers using Owen's recommended centrifugation speed to obtain plasma or serum in birds; Appropriate: centrifugation method specifies g; Inappropriate: centrifugation method specifies rpm or speed is missing.

Table 4: Publications where centrifugation was used classified by journal impact factor.

Impact factor	n	g	rpm and g	rpm	Missing	Insconsistent	0 unit	appropriate	Inappropriate
Unkown	82	29.3	0.0	53.7	13.4	2.4	1.2	29.3	70.7
0-0.99	263	39.2	0.4	42.6	14.4	3.4	0.0	39.5	60.5
1-1.99	479	49.1	2.3	34.7	12.1	1.9	0.0	51.4	48.6
2-2.99	491	45.6	3.7	35.0	14.3	1.4	0.0	49.3	50.7
3-3.99	210	51.9	0.5	34.8	11.9	1.0	0.0	52.4	47.6
4-4.99	228	43.4	1.8	36.8	15.4	2.6	0.0	45.2	54.8
5 and +	247	49.4	2.0	29.1	18.6	0.8	0.0	51.4	48.6

N: number of publications; g, rpm and g, rpm: percentages of publications specifying centrifugation in these units; Missing: no mention of centrifugation speed; Inconsistent: methods with several centrifugation steps where speeds are indicated in g or rpm without uniformity; No unit: centrifugation speed indicated but no unit specified; Appropriate: centrifugation method described correctly (i.e., g or rpm+g); Inappropriate: centrifugation method described incorrectly (i.e., rpm, Missing, Inconsistent, No unit).

Table 5: Conditions of centrifugation from 291 papers in biology education journals.

n	g	rpm and g	rpm	Missing	Inconsistent	Appropriate	Inappropriate
291	38.83	4.12	26.46	23.02	7.56	42.96	57.04

N: number of publications; g, rpm and g, rpm: percentages of publications specifying centrifugation in these units; Missing: no mention of centrifugation speed; Inconsistent: methods with several centrifugation steps where speeds are indicated in g or rpm without uniformity; Appropriate: centrifugation method described correctly (i.e., g or rpm+g); Inappropriate: centrifugation method described incorrectly (i.e., rpm, Missing, Inconsistent).

accurate velocity unit ($p>0.05$) which remains low. We wondered if the inappropriate recording of centrifugation speed was due to the way this technique is taught. To answer this question, we found 291 articles in the laboratory activities section of seven journals dedicated to teaching biology with centrifugation in the methods section (Advances in Physiology Education, American Biology Teacher, Biochemistry and Molecular Biology Education, Bioscene, CBE - Life Science Education/ Cell Biology Education, Journal of Biological Education, and Journal of Microbiology and Biology Education). We were surprised at the results: only 42.95% of speeds were correctly expressed (Table 5).

Conclusion

We report here on the lack in scientific rigor in the expression of centrifugation velocity in publications from different fields of environmental sciences: ecology, zoology, aquatic sciences, botany, and mycology. Our objective was to illustrate the importance and necessity of better basic training in analytical techniques in biochemistry and physiology for biologists involved in these different fields. In addition, proper descriptions of the methods used are of paramount importance to ensure the repeatability of all studies and the associated methodological protocols. The editorial committees and reviewers of peer-reviewed journals in these disciplines should also be more attentive to the accuracy of these descriptions; their difficulties in identifying these pitfalls may also result from inadequate training.

The aim of this study was to highlight the transdisciplinary nature of environmental and life science in general and the importance of good training in basic laboratory techniques for biologists in any field. This demonstration through the survey of good and bad expression of the units of velocity while using a simple and widely use technic (centrifugation), clearly exhibit the importance to develop skills often perceived as outside our defined field of expertise. Considering the future environmental challenges, we advocate that strong and wide basic education in science preclude efficient transdisciplinary communication and approaches, essential to face these challenges.

Conflicts of Interest

There are no conflicts of interest to report.

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