Philippine Journal of Science
151 (6A): 2275-2283, December 2022
ISSN 0031 - 7683
Date Received: 10 Nov 2021

Comparison of Glycogen Content from Three Philippine Mussels

Mitchell Mark B. Marmita[1], **Leni G. Yap-Dejeto**[1]*, **Paulo Ranji A. Hipe**[1], **Rhea Mae S. Dañal**[1], **Eugie V. Cinco**[1], **Rolly G. Fuentes**[1], and **Victor Marco Emmanuel N. Ferriols**[2]

[1]Division of Natural Sciences and Mathematics,
University of the Philippines Visayas Tacloban College, Leyte, Philippines
[2]Institute of Aquaculture, College of Fisheries and Ocean Sciences,
University of the Philippines Visayas, Miagao, Iloilo, Philippines

This study was conducted to measure and compare the glycogen content in three Philippine mussels of the family Mytilidae – *Perna viridis*, *Mytella strigata*, and *Modiolus philippinarum*. *P. viridis*, *M. strigata*, and *M. philippinarum* were collected from the provinces of Samar, Cavite, and Iloilo, respectively. *P. viridis*, locally known as "tahong," is cultured in the Philippines. *M. strigata*, known as "charru" or black mussel, has a distinct brown to black shell coloration that varies with age and is currently considered as an invasive species in the Philippines. Lastly, *M. philippinarum* mostly inhabits intertidal mudflats, that form extensive aggregates. These mussels are edible and are a well-known cheap source of animal protein. Extraction of glycogen involved mussel meat preparation, the addition of hydrolyzing agents, and precipitation. Results showed glycogen content varies in different species from 5.5–3.0% yield. This study is a contribution to the lack of recorded biological information regarding Philippine mussels in terms of biochemical content such as glycogen. This information can later be used to assess the mussels' health, biological, and habitat ecological status.

Keywords: extraction, glycogen, *Modiolus philippinarum*, *Mytella strigata*, *Perna viridis*

INTRODUCTION

Glycogen is a glucose polysaccharide that is found in most animals. It is characterized as a white amorphous powder, soluble in water, and readily hydolyze's mineral acids to produce glucose residues. Along with body fat, it is an important and quickly mobilized source of stored glucose (Chan 2015). Studies suggest that large amounts of polysaccharides such as glycogen are accumulated in bivalves. The percentage of accumulated glycogen in bivalves could vary from different species. It is further influenced by internal factors, such as growth and sexual maturation. There are also external factors such as food availability, season, and temperature. Glycogen content in these animals can be influenced by varying seasons. In a study conducted with Manila clams (*Ruditapes philippinarum*), glycogen accumulation is more prominent in the spring-summer season than in the autumn-winter season. In mussels, stored energy in the form of glycogen is accumulated in the mantle tissue during the resting period while subsequently being utilized during the reproductive state (Bayne *et al*. 1982). Glycogen is always present in these organisms which can be readily extracted from tissues and content varies depending on the physiological state (Vakily 1989).

*Corresponding author: lgyapdejeto@up.edu.ph

Glycogen has been utilized in industries such as biotechnology, cosmetics, and functional foods. In the United States of America, glycogen is a commercial commodity applied in the extraction of nucleotides, such as RNA. These are extracted from oysters and from blue mussels *Mytilus edulis* and are sold commercially at USD 117–129 at 1 mL 20 mg (Merck 2011). A business feasibility study estimates a supplier to earn an annual of PHP 1.0 million (USD 20,618) in year 1 up to PHP 2.5 million (USD 51,545) in year 10, or an aggregate of revenues of PHP 16.6 million (USD 342,262) (Cagara *et al.* 2021).

The Asian green mussel *Perna viridis*, locally known as "tahong," is in the same family, Mytilidae, as the blue mussel *Mytilus edulis* (Gofas 2010). As described by Siddall (1980), both *Perna* and *Mytilus* have two dysodont teeth, pitted residual ridges, and smooth shells. And, aside from the color of the valves, morphological variations in the shells are evident. *Mytilus* has anterior adductors, whereas *Perna* has none; there is only one retractor scar for *Mytilus*, whereas *Perna* has two; *Mytilus* has no primary lateral teeth, whereas *Perna* has 10–18 (Siddall 1980). In addition, *P. viridis* was classified by the Global Invasive Species Database in 2015 to have a high invasion potential based on the weighted sum of the commercial ship and recreational vessel movements and discharge of ballasts from regions affected by the presence of *P. viridis* around the world.

The "charru" mussel or black mussel *Mytella strigata* (former: *Mytella charruana*) belongs also to the family Mytilidae. The charru mussel is not a native species in the Philippines and in 2014, *Mytella strigata* was reported to disrupt wild spats of mussels and other bivalve species in the country (Rice *et al*. 2016; Vallejo *et al*. 2017). *M. strigata* has a distinct brown to black coloration of their shell, which varies with age and distribution. The internal anatomy of *M. strigata* is quite like all other mytilid species having not many distinct features in the orientation of its internal organs (Mediodia *et al.* 2017).

Mytella strigata or the "charru" mussel was first reported in Cavite in the northern part of Manila Bay. Cavite operates as a naval base and a docking zone for international vessels. The introduction of the "charru" mussel was thought to be from these international vessels through ballast water discharges. From this, *M. strigata* was able to rapidly disperse to nearby areas in Bulacan, Bataan, and Pangasinan because of the mussel's ability to adapt readily and colonize a wide range of habitats (Rice *et al*. 2016). There is a competition for space, especially on floating substrates for local mussels and other bivalve species because of the adaptive capabilities of the "charru" mussel. This should be a priority in management for bioinvasion control. *M. strigata* may as well compete for space and food with *Perna viridis*, which is known to be actively cultured in the Philippines, as well as with other native bivalve species (Rocha *et al*. 2010).

The Philippine horse mussel or brown mussel *Modiolus philippinarum* belongs to the same family as *P. viridis* and *M. strigata,* in the family Mytilidae. Most of the *Modiolus* species are distributed along the Indo-Pacific region and most live on intertidal mudflats, where they can form extensive aggregates. Iloilo is one of the places in the Philippines where *M. philippinarum* can be found. There are two areas in Iloilo that are well-known for the harvest of the species: Banate Bay and Dumangas. The brown mussel is edible and is a well-known cheap source of animal protein in the area. The mussel *M. philippinarum* reaches sexual maturity in three months and is known to spawn all year round. Aside from being harvested for food, these are used as feed for high-value culture species, such as mud crabs (Napata and Andalecio 2011).

Studies on the glycogen content of mussels in the Philippines are lacking. Aside from potential economic utilization, these kinds of information can later be used to assess the biological and ecological status of the mussels and their habitats, respectively. Thus, this study aimed to measure and compare the glycogen content in the mussel meat of the three Philippine mussels: the Asian green mussel (*Perna viridis*), "charru" or black mussel (*Mytella strigata*), and brown mussel (*Modiolus philippinarum*). The results can be used as baseline data for the glycogen content of these three species.

MATERIALS AND METHODS

Sample Collection

Mytella strigata and *Modiolus philippinarum* were collected in the wild in September 2019 from Manila Bay and in November 2019 from Banate Bay, Iloilo, respectively. *Perna viridis* were gathered from mussel farms of Villareal Bay, Samar in January 2020. The mussels obtained were in their adult-sized stage. These were identified based on their morphology with detailed descriptions in the introduction (*e.g.* primarily smooth, oval, and green valves for *P. viridis*; swollen, oval, and brown valves for *M. philippinarum*; among other characteristics). *M. strigatta* are similar in shape to *P. viridis* except for their smaller-sized, black shells. Voucher specimens are kept in the University of the Philippines Visayas Tacloban College (Tacloban City) laboratory. The mussels were shucked and preserved in a cooler box with ice packs and then transported to the same laboratory. Upon arrival, the mussels were stored in the –18 to –20 ºC freezer. The frozen mussel meat was then thawed for the following isolation method.

Isolation of Glycogen

A total of 10 g of thawed *P. viridis*, *M. strigata*, and *M. philippinarum* were homogenized and added with distilled water. The samples were hydrolyzed in a boiling water bath for 30 min. After, the samples were cooled down. The broth from the samples was then decanted into pre-weighed centrifuge tubes, discarding the meat solids. Four volumes of 95% ethanol were added, and the samples were refrigerated and left to stand for at least 24 h to allow the crude glycogen extract to precipitate from the mixture. After visible precipitates were observed, the samples were centrifuged for 5 min. The supernatant was discarded, and the precipitate was purified using trichloroacetic acid (TCA) and then allowed to stand for 1 h. The mixture was centrifuged for 5 min at 3,000 rpm. Then, the precipitate was removed, and the supernatant was retained. The glycogen solution was recovered by adding 95% ethanol and was centrifuged for another 5 min at 3,000 rpm. The precipitate was retained, and the supernatant was discarded and dried by adding absolute ethanol. The final precipitate was weighed, and the percent yield was calculated.

Qualitative Analysis for Carbohydrates

Various qualitative tests for the presence of carbohydrates were performed – including colorimetric tests such as Molisch's, iodine, and Benedict's tests. Molisch's test was done by mixing two drops of Molisch's reagent (5% 1-naphthol in alcohol) with about 1 mL of the test solution. Afterward, while inclining the test tube, about 1 mL of concentrated sulfuric acid was added along the sides of the tube. A positive result for this test is observed when there is a purple ring at the interphase between the sulfuric acid and the test solution. Such a result indicates the presence of carbohydrates. The iodine test was performed by adding two drops of iodine solution to about 1 mL of the test solution. The appearance of a blue to black color confirms the presence of starch in the test solution. The Benedict's test was conducted by adding 2 mL of Benedict's reagent into five drops of the glycogen solutions. Next, the solution was heated into a boiling water bath for 5 min. The solutions were then allowed to cool and any color changes were noted.

Fourier-transform Infrared Spectroscopy (FTIR) Analysis

The standard and samples were prepared and then sent to the Material Science and Nanotechnology Laboratory, Regional Research Center–University of the Philippines Visayas (RRC-UPV), Miag-ao, Iloilo for the Fourier-transform infrared spectroscopy (FTIR, Thermo Scientific) analysis.

Quantitative Analysis of Glycogen Using Anthrone Reagent

Glycogen samples and the oyster standard glycogen (Sigma-Aldrich) with 75% purity were constituted to make 0.1 mg/mL solution in distilled water. An aliquot of 1 mL of the solution was added with a 4-mL anthrone reagent. The absorbance of each sample was read at 620 nm. The percent purity of the extracted glycogen was then computed in relation to the standard glycogen.

RESULTS

Glycogen appeared as a white solid at the end of extraction. *P. viridis* had the highest percent yield with 5.5% extracted unpurified glycogen from the three mussel species. *M. philippinarum* gave a 4.1% percent glycogen yield, whereas *M. strigata* yielded 3% of its mussel flesh body weight (Table 1).

Qualitative results have shown positive results for the Molisch's and iodine test for the glycogen extracts from *P. viridis*, *M. philippinarum*, and *M. strigata* (Table 1). All the glycogen samples tested positive in Molisch's test, which showed the appearance of a purple ring in between the solutions inside each test tube. Positive results were also obtained from the iodine test, which showed a dark brown color reaction when the iodine solution was added to the glycogen samples. Further, all samples showed no color changes in Benedict's test, which suggest no reaction occurred in the sample. The FTIR analyses of extracted glycogen from the three-mussel species showed similar signals of transmittance (Figures 1A–D). At 1,000–1,100 cm^{-} peaks of glycogen from mussel samples were documented. Twin peaks of transmittance were observed

Table 1. Percentage yield and qualitative analysis of glycogen extracted from the three Philippines mussel species.

Species	Percent yield* (%)	Molisch's test	Iodine test	Benedict test
1.*Perna viridis*	5.5 ± 0.5	+	+	–
2. *Mytella strigata*	4.1 ± 0.6	+	+	–
3. *Modiolus philippinarum*	3.0 ± 0.2	+	+	–

*Amount of solids produced after extraction per fresh weight of the Philippine mussel species

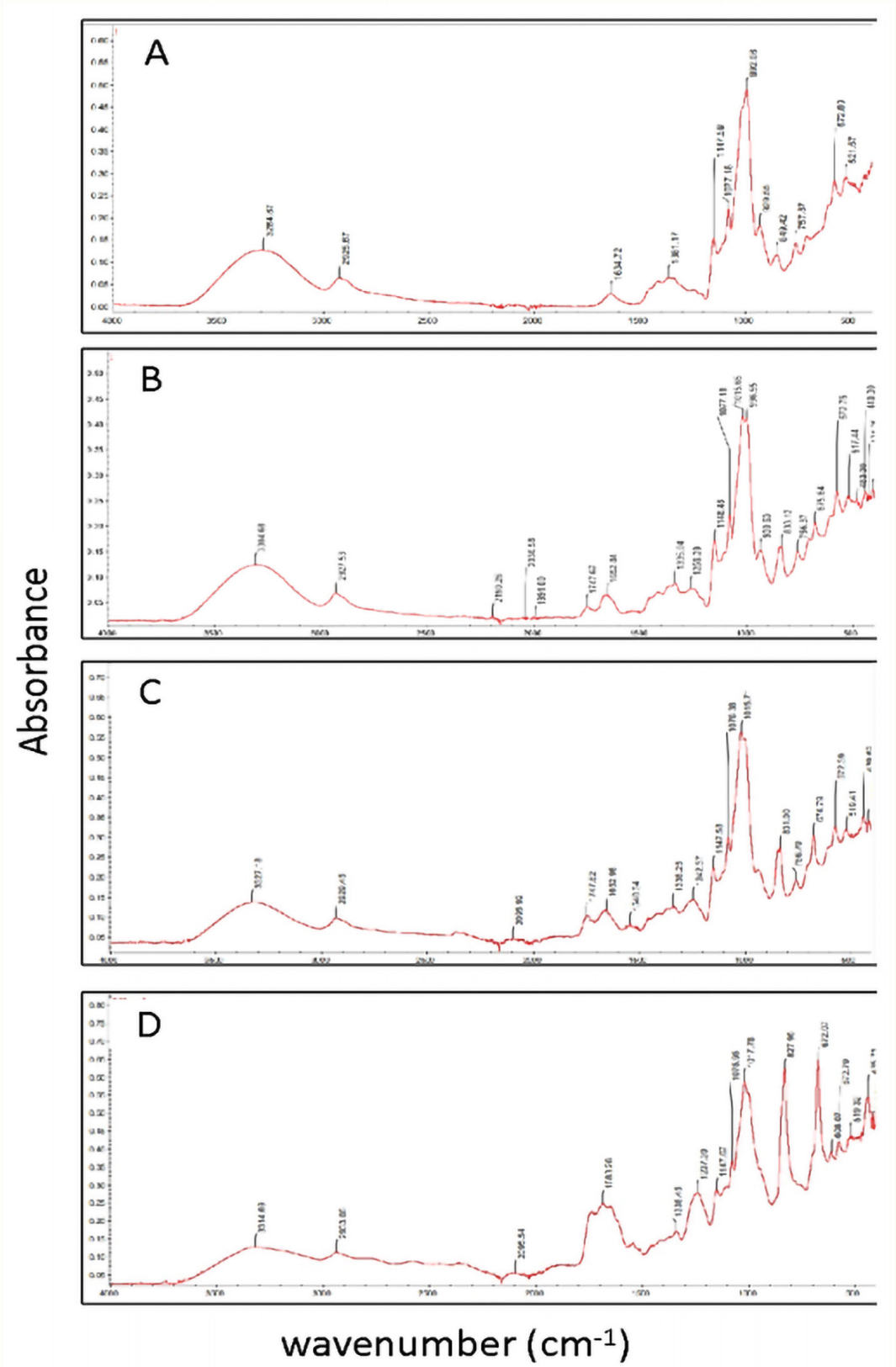


Figure 1. FTIR analysis of glycogen standard from mussel [A], *Perna viridis* [B], *Modiolus philippinarum* [C], and *Mytella strigata* [D].

at 996 and 1,016 cm^{-1} for both green and brown mussels. Around 996 and 1018 cm^{-1} for black mussel were also observed. The peak for standard glycogen (Sigma-Aldrich) with 75% purity was observed at 992 cm^{-1} with a slight twin peak at around 1016 cm^{-1}, too.

The percent purity of the extracted glycogen from the three Philippine mussel species was estimated by comparing their absorbance after reacting with anthrone reagent with standard glycogen, which has 75% purity (Figure 2). Results showed that the glycogen extract of *P. viridis* has a purity of 57.3 ± 6.1%, *M. philippinarum* was calculated to have 17.2 ± 0.7% purity, and *M. strigata* was found to be 14.3 ± 12.2% glycogen.

DISCUSSION

The white glycogen solid underwent qualitative and quantitative analysis to determine the purity and to confirm the identity of glycogen extracted from the three Philippine mussels. Molisch, iodine, and Benedict's tests were used as qualitative tests for carbohydrates. Molisch's test is a general test for carbohydrates. The color formed is due to the reaction of alpha-naphthol with furfural and/or its derivatives formed by the dehydration of sugars by concentrated sulfuric acid (Elzagheid 2018). The purpose to conduct the different qualitative tests was to confirm that what we have extracted is [1] a carbohydrate (Molisch's test); [2] it is glycogen (iodine test), and [3] it is not a reducing sugar (Benedict's test).

In structure, glycogen is similar to amylopectin. The difference is that glycogen is more branched with 8–12 glucose units in between branches, and these branches are also shorter. The iodine test is a test for the detection of polysaccharides, such as starch and glycogen. In this case, glycogen is tested, a polymer of glucose with alpha (1–4) links, branched on the alpha (1–6) at approximately every 8–12 residues (Chan 2015). The positive result is indicated as the formation of the blue-black color in starch or brown-black color in glycogen after the addition of iodine solution. The formation of the glycogen-iodine complex occurs in the polysaccharide to iodine reaction. Helical coils form with the iodine atoms inserted into these helices. Since glycogen has shorter glucose branches, the helices are shorter, and less iodine binds than that of starch. Therefore, the color reaction of the glycogen-iodine complex of brown-black is less intense than the starch-iodine complex of blue-black (Nayak *et al*. 2015).

Benedict's test is a chemical test for simple sugars and for identifying the presence of reducing sugars in a given solution. The positive result for this test is shown as the solution changes to orange or brick red color due to the copper ions in Benedict's solution being reduced in the presence of reducing sugars (Morell-Garcia *et al*. 2014). Polysaccharides – which do not have an aldehyde or a reducing sugar end, such as the glycogen – are not considered reducing sugars, which was confirmed by the negative results of Benedict's test.

FTIR analysis, or transmission or reflectance spectroscopy, is a qualitative test that describes molecules based on their

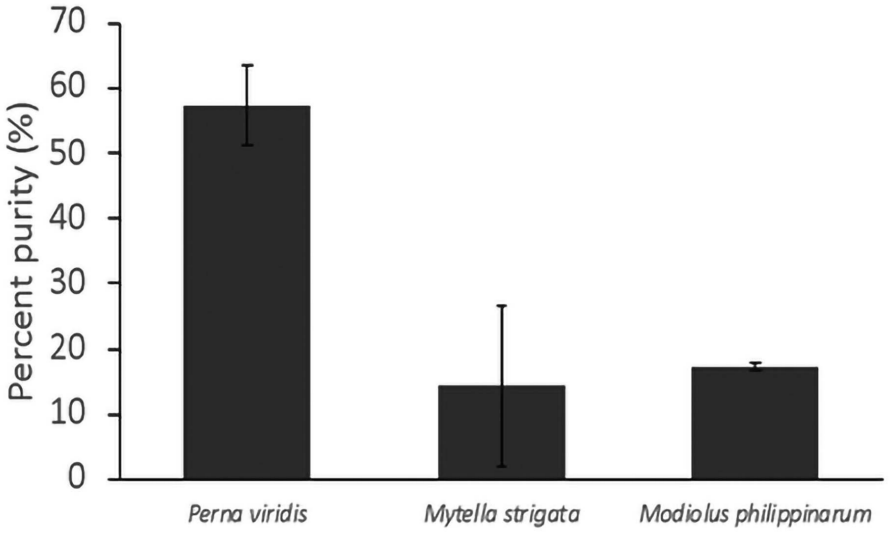


Figure 2. Percent purity of the glycogen extacted from the meat of *Perna viridis*, *Mytella strigata*, and *Modiolus philippinarum*. Data are presented as mean ± SD (n = 2). Purity of glycogen was determined by determining the absorbance after reaction with anthrone and compared with standard mussel glycogen with 75% purity.

transmittance or reflectance from infrared light, which in turn characterizes the geometry of these molecules. The oyster glycogen standard shows the characteristic peaks at 3285 cm^{-1} (O-H stretching) and 2926 cm^{-1} (C-H stretching). The bands for O-H stretching and C-H bending were observed at 1635 and 1361 cm^{-1}, respectively. Peaks in the fingerprint region show the C-O stretching band at 1148 cm^{-1}, whereas O-H deformation band at 992 cm^{-1} and at around 1016 cm^{-1}. These peaks were comparable to the published values for glycogen (Adhikary *et al.* 2007). According to Adhikary *et al.* (2007), the twin peaks may have been caused by stretching vibrations of CH_2—O—CH_2. Following these observations, the unknown white solid extracted from the three Philippine mussel samples was confirmed to be glycogen based on the comparison of their respective FTIR spectra with the standard glycogen. Furthermore, no signals were observed in all glycogen extracts of the three species of Philippine mussel for nitrogen groups – such as amide, amine, and nitrile – which are basic structures for protein and carboxylic groups. Any signal characteristic of lipid structures was also not detected, indicating the absence of these organic compounds and implying the purity of the glycogen extracts.

P. viridis have a wide range of food selection from a variety of phytoplankton and zooplankton. Sexual maturity is reached at 2–3 mo old and can spawn all year round with peak spawning in January–February and September–October (Layugan *et al*. 2018). Based on this data, the gonadal stage of the *P. viridis* samples collected from E. Samar must probably have been in their spawning stage. Detailed biology and ecology of *P. viridis* in Eastern Samar can be found in an article by Toralde *et al*. (2021).

Studies regarding *M. philippinarum* biology and ecology are limited. However, Ozawa in 2001 conducted a study on the reproductive cycle and spawning of *M. philippinarum*, revealing that species have peak gametogenesis and spawning from the months of July–September. In addition, a close relative of the brown mussel *Modiolus modulaides* has been studied and observed to have a selective diet with a preference of phytoplankton – including *Thalasionema* spp., *Pleurosigma* spp., *Rhizosolenia* spp., *Diploneis* spp., and *Detonula* spp. (Uba and Monteclaro 2020).

M. strigata is also observed to have peak spawning from the months of July–September. Nutritional stress may result in sex change in *Mytella*, with the preference of turning into females if food is plentiful and if food is scarce, individuals may stay or turn into males. *Mytella* individuals may maintain as females as long as food is highly available, lengthening their spawning periods (Stenyakina *et al*. 2010).

The environmental conditions of the sites would affect the production of glycogen. Mussel glycogen is dependent on the site and type of microalgae ingested (Pleissner *et al*. 2012). The different sites in this study were chosen based on the availability of the mussel species in the area. There would be slight variations in temperature for the sites as the Philippines is a tropical country. For example, in Villareal Bay – where *P. viridis* samples were collected – temperatures ranged from 29–30.5 °C (Ravelo *et al*. 2022). In Manila Bay, where *M. strigata* were gathered, this ranged from 17.7–30.1 °C (Borja *et al*. 2019). Nutrients, chlorophyll or organic matter will indicate food availability. But Villareal Bay, where *P. viridis* were harvested from, had the lowest nutrient and chlorophyll levels or organic matter compared to Manila Bay and Banate Bay. Villareal Bay was reported to have Chlorophyll *a* (Chl-*a*) at 0.2 µg/L; nitrate at 0.04-0.08 µM; phosphates at 0.01 µM; and total suspended solids (TSS) at .05-0.12 mg/L (Ravelo *et al*. 2022). Manila Bay was recorded to have the following values; the range of Chl-*a* was at 0.016-10.3 µg/L; nitrate ranges at 0.393-0.408 µM and phosphate at 0.0386-0.0945 µM (Borja *et al*. 2019). Banate Bay, where *M. philippinarum* was collected, was reported to be turbid with TSS at 4.7–45.8 mg/L, particulate organic carbon at 21.8–95.3 µM, and particulate nitrogen at 3.5–12.5 µM (Yamamoto *et al*. 2019). It would be an interesting study to compare the different levels of glycogen of one species grown in different sites.

The percent purity values of the glycogen extracted from the different mussel species were not similar, with *P. viridis* having the highest purity at 57% and *M. strigata* with the lowest purity at 14%. This means that the purifying agent that we used, TCA, was most effective in denaturing proteins, nucleotides, and other organic compounds of *P. viridis*. Other acids must be used for the other species to increase the purity of their glycogen extracts.

Comparing glycogen yield to other bivalves, the yield of *P. viridis* glycogen at 5.5% is higher than that of the blue mussel *Mytilus edulis* at 4.15% yield (Fernandez *et al*. 2015). It was also observed that glycogen content on *M. edulis* varies in different seasons. It remains high in spring and early-summer and declines in autumn and early-winter (Zwaan and Zandee 1972). More recent studies on *M. edulis* glycogen content show that since this is affected by temperature, this could be an indicator of climate change (Clements *et al*. 2018; Matoo *et al*. 2021). As the sampling and extraction of glycogen were not year-round in this study, this provides a baseline glycogen content of these three Philippine mussels. Similar studies in varying conditions should be done in the future.

CONCLUSION

Perna viridis showed the highest percent yield of glycogen at 5.5%. This is followed by *Mytella strigata* at 4.1%, whereas *Modiolus philippinarum* had the lowest yield at 3% The different environmental conditions the mussels were in and the biological conditions of the mussels themselves would most probably have contributed to these differences in glycogen content. Further studies to determine which factors greatly influence glycogen content in these three mussels are necessary.

ACKNOWLEDGMENTS

The authors acknowledge RRC-UPV for the laboratory services required in the study. The authors are grateful to all the research staff from UPV who have assisted in the laboratory analyses – namely, Jessebel Valera-Gadot, Marj Gem Bunda, Susci Ann Sobrevega, Dr. Jade Pahila, Marizka Juliano, and Nizzy Hope Cartago. We extend our gratitude to Wency Dagayloan, Alessandra Malacura, and Fatima Mae Folio for the help in the initial extractions of glycogen; Richard Cagara for the feasibility study; and Rodrigo Dejeto for the interpretation of the FTIR images. The authors also give thanks to the fisherfolk from Villareal Samar, Banate Iloilo, and Bacoor Cavite for supplying the well-needed mussel species. This study was funded by IARRD, DOST-PCAARRD through the Mussel Biotechnology Program.

REFERENCES

ADHIKARY P, TIWARI KN, SINGH RP. 2007. Synthesis, characterization, and flocculation characteristics of polyacrylamide-grafted glycogen. Journal of Applied Polymer Science 103(2): 773–778.

BAYNE BL, BUBEL A, GABBOTT PA, LIVINGSTONE DR, LOWE DM, MOORE MN. 1982. Glycogen utilisation and gametogenesis in *Mytilus edulis* L. Marine Biology Letters 3(2): 98–105.

BORJA V, FURIO E, GATDULA N, IWATAKI M. 2019. Occurrence of harmful algal blooms caused by various phytoplankton species in the last three decades in Manila Bay, Philippines. Philippine Journal of Natural Sciences 24: 80–90.

CAGARA R, MARMITA MM, HIPE P, R DANAL, L YAP-DEJETO. 2021. Glycogen Processing Project: a Report on Business Feasibility [Manuscript in Preparation]. University of the Philippines Visayas Tacloban College.

CHAN DL. 2015. Refeeding syndrome in small animals. Nutritional Management of Hospitalized Small Animals. p. 159–164.

CLEMENTS JC, HICKS C, TREMBLAY R, COMEAU LA. 2018. Elevated seawater temperature, not pCO_2, negatively affects post-spawning adult mussels (*Mytilus edulis*) under food limitation. Conservation Physiology 6(1): cox078.

ELZAGHEID MI. 2018. Laboratory activities to introduce carbohydrates qualitative analysis to college students. World Journal of Chemical Education 6(2): 82–86.

FERNÁNDEZ A, GRIENKE U, SOLER-VILA A, GUIHÉNEUF F, STENGEL DB, TASDEMIR D. 2015. Seasonal and geographical variations in the biochemical composition of the blue mussel (*Mytilus edulis L.*) from Ireland. Food Chemistry 177(1): 43–52.

GLOBAL INVASIVE SPECIES DATABASE. 2015. Species profile *Perna viridis*. Retrieved on 24 Jul 2019 from http://www.iucngisd.org/gisd/species.php?sc=731

GOFAS S. 2010. *Perna* Philipsson, 1788. In: World Marine Mollusca database. Bouchet P, Gofas S, Rosenberg G ed. Accessed through World Register of Marine Species.

LAYUGAN EA, TABASIN JP, ALEJOS MS, PIDOY LE. 2018. Growth Performance of Green Mussel *Perna viridis* Transplanted in Buguey Lagoon, Philippines. Acta Scientific Agriculture 2: 43–47

MATOO OB, LANNIG G, BOCK C, SOKOLOVA IM. 2021. Temperature but not ocean acidification affects energy metabolism and enzyme activities in the blue mussel, *Mytilus edulis*. Ecology and Evolution 11(7): 3366–3379.

MEDIODIA PD, SANTANDER-DE LEON SM, ANASCO NC, BAYLON CC. 2017. Shell Morphology and Anatomy of the Philippine Charru Mussel *Mytella charruana* (d'Orbigny 1842). Asian Fisheries Science 30(2017):185–194.

MERCK. 2011. Glycogen from oyster. Retrieved from https://www.sigmaaldrich.com/PH/en/product/sial/50573

MORELL-GARCIA D, BAUÇA JM, BARCELÓ A, PEREZ-ESTEBAN G, VILA M. 2014. Usefulness of Benedict's test for the screening of galactosemia. Clinical Biochemistry 47(9): 857–859.

NAPATA RP, ANDALECIO MN. 2011. Exploitation and management of brown mussel (*Modiolus philippinarum*) resources in Iloilo, Philippines. Philippine Journal of Social Sciences Human 16(2): 22–34.

NAYAK AK, PAL D, SANTRA K. 2015. Screening of polysaccharides from tamarind, fenugreek and jackfruit seeds as pharmaceutical excipients. International Journal of Biological Macromolecules 79(1): 756–760.

OZAWA H. 2001. Reproductive cycle and spawning of *Modiolus philippinarum* (Bivalvia: Mytilidae) in a seagrass bed in Kin Bay, Okinawa Island, Southern Japan. Journal of the Malacological Society of Japan 60(3): 173–181.

PLEISSNER D, LUNDGREEN K, RIISGÅRD HU, ERIKSEN N. 2012. Biomass Composition of Blue Mussels, *Mytilus edulis*, is Affected by Living Site and Species of Ingested Microalgae. ISRN Zoology 2012(1–3).

RAVELO SF, YAP-DEJETO LG, SILARAS MLS, AMPARADO MLL, OCAMPO JA, ABRIA EG, ALBINA MB. 2022. A Snapshot on the Distribution of Coastal Phytoplankton Communities in Five HAB-Affected Bays in Eastern Visayas, Philippines. Frontiers in Marine Science, Vol. 9.

RICE MA, RAWSON PD, SALINAS AD, ROSARIO WR. 2016. Identification and salinity tolerance of the Western hemisphere mussel *Mytella charruana* (D'Orbigny, 1842) in the Philippines. Journal of Shellfish Research 35(4): 865–873.

ROCHA RM, CANGUSSU LC, BRAGA MP. 2010. Stationary substrates facilitate bioinvasion in Paranaguá Bay in southern Brazil. Brazilian Journal of Oceanography 58(SPE3): 23–28.

SIDDALL SE. 1980. A clarification of the genus *Perna* (Mytilidae). Bulletin of Marine Science 30(4): 858–870.

STENYAKINA A, WALTERS LJ, HOFFMAN EA, CALESTANI C. 2010. Food availability and sex reversal in *Mytella charruana*, an introduced bivalve in the southeastern United States. Molecular Reproduction and Development: Incorporating Gamete Research 77(3): 222–230.

TORALDE CB, SILARAS MLS, GARCIA MMM, YAP-DEJETO LG. 2021. Biology and Ecology of Wild and Cultured Green Mussel, *Perna viridis* in Eastern Visayas, Philippines. Philippine Journal of Natural Science 26: 20–31.

UBA KIN, MONTECLARO HM. 2020. Habitat Characteristics of the Horse Mussel *Modiolus modulaides* (Röding 1798) in Iloilo, Philippines. Philippine Journal of Science 149(3-a): 977–987.

VAKILY JM. 1989. The biology and culture of mussels of the genus *Perna*. Internation Center for Living Aquatic Resources Management 494(1): 8–21.

VALLEJO JR. B, CONEJAR-ESPEDIDO J, MANUBAG L, ARTIAGA KCC, DAMATAC II AM, IMPERIAL ICV, ITONG TAB, FONTANILLA IK, CAO EP. 2017. First record of the Charru mussel *Mytella charruana* d'Orbignyi, 1846 (Bivalvia: Mytilidae) from Manila Bay, Luzon, Philippines. BioInvasions Record 6(1): 49–55.

YAMAMOTO T, MALINGIN MACL, PEPINO MM, YOSHIKAI M, CAMPOS W, MIYAJIMA T, A WATANABE, Y TANAKA, N MORIMOTO, R RAMOS, H PAGKALINAWAN, NADAOKA, K. 2019. Assessment of coastal turbidity improvement potential by terrigenous sediment load reduction and its implications on seagrass inhabitable area in Banate Bay, Central Philippines. Science of The Total Environment 656: 1386–1400.

ZWAAN AD, ZANDEE DI. 1972. Body distribution and seasonal changes in the glycogen content of the common sea mussel *Mytilus edulis*. Comparative Biochemistry and Physiology Part A: Physiology 43(1): 53–58.

APPENDIX

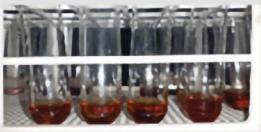
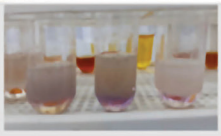
Test	Expected Result	Actual Result
Benedict's Test	Presence of reducing sugars (Yellow to orange ppt)	- 
Iodine's Test	Presence of glycogen (brick red ppt)	+ 
Molisch's Test	Presence of carbohydrates (purple ring)	+ 

Figure I. Color reactions of glycogen extracts from three Philippine mussels using different qualitative tests.