Lumain et al. / The Philippine Journal of Fisheries 32(1): 149 - 159
DOI: 10.31398/tpjf/32.1.2024-0096

RESEARCH ARTICLE

Cell Viability and Coelomocyte Characterization of Crown-of-Thorns Starfish (*Acanthaster planci* L.) Collected from Southern Leyte, Philippines

John Philip Lou M. Lumain[1]*, Arvin C. Balala[2], Jerome Jack O. Napala[2], Ryan P. Tomol[2], Jovie Marie P. Alfante[2], Gladys Ludevese-Pascual[2]

[1] *Department of Veterinary Paraclinical Sciences, Faculty of Veterinary Medicine Visayas State University, Visca, Baybay City, Leyte, 6521, Philippines*
[2] *Bioresource Production Technology Center (BioProTeC), Southern Leyte State University-Bontoc, San Ramon, Bontoc, Southern Leyte, 6604, Philippines*

ABSTRACT

The current work is the first effort to quantify cell viability and profiling of cell morphotypes found in the coelomic fluid of the Crown-of-Thorns starfish (COTS) (*Acanthaster planci* L.), collected in the territorial waters of Southern Leyte, Philippines. For the cell viability, based on the trypan blue exclusion test on the varying time interval for counting, at fresh preparation (0 minutes), the counted live cells are 137 (mean: 34.25 ± 6.45) with coelomocyte viability of 6.8 ± 10.26 x 10^5 cells ml; as the time increases, the viable cells from the prepared fractions decrease. This study reveals that the coelomic fluid of COTS contains diverse coelomocyte morphotypes, including spherule, vibratile, fusiform, crystal, progenitor, and phagocyte cells, all of which have distinct cellular sizes and morphologies. These cell types are also present during physiological changes in COTS. These findings offer valuable insights into the physiology and cellular component of COTS, potentially serving as a foundation in developing control strategies, including the discovery of novel and effective compounds to prevent or eradicate outbreaks. Based on the results of this study, the authors point to the need for in-depth systematic profiling and characterization of the COTS coelomocytes using more advanced microscopical techniques (i.e., fluorescence and transmission electron microscopy); percentage determination of each coelomocyte per ml of coelomic fractions depending on the COTS maturity, sex, and status – healthy, stressed, injured, or during spawning seasons; cell viability per coelomocyte type, the impact of seasonal variation to the coelomocytes; and comparison of the coelomocytes of other endemic Asteroid echinoderms.

*Corresponding Author: *philip.lumain@vsu.edu.ph*
Received: *September 13, 2024*
Accepted*: February 28, 2025*

Keywords: *Acanthaster planci, cell viability, coelomocytes, Crown-of-Thorns Starfish, morphotypes*

1. INTRODUCTION

The Crown-of-Thorns starfish (*Acanthaster planci* L.), referred to as COTS, is a specialist corallivore that preys on coral polyps. It is a member of the Phylum Echinodermata, specifically under the class Asteroidea. Members of this class are known for their regenerative abilities. Outbreaks of this coral-eating starfish are currently one of the biggest threats to the coral reefs in the tropics (Grand et al. 2014). In the study of Ludevese-Pascual et al. (2022), they reported five municipalities in the Sogod Bay region, Southern Leyte, Philippines, with an outbreak status of COTS, with a maximum of 20 COTS in 100 m^2 in one of the study area, there has been evident aggregations during summer months leading to rapid coral colony collapse. Outbreaks have also been recorded in other territorial waters, such as Cebu (Nicolas et al. 2024), specifically in Malapascua Island (Kensington 2019), Lutoban – Zamboanguita (Marine Conservation Philippines 2023), Boracay (Aguirre 2020), and El Nido – Palawan (Municipality of El Nido, Province of Palawan 2009), to name a few. The reports could be an understatement of the silent outbreak of COTS, looming in the dark abyss of the ocean and impacting marine ecosystems.

The presence of abundant coelomic fluid in their extensive body cavities supports populations of free cells known as coelomocytes (Chia and Xing 1996; Dai et al. 2016; Kensington 2019). Coelomocytes are the generic collection of cellular morphotypes. These cells are attributed to the animal's immune response, phagocytic digestion of foreign microorganisms, and wound healing-clotting activity (Andrade et al. 2021; Dai et al. 2016; Smith 1981; Stabili et al. 2018).

Numerous ideas have attempted to explain the formation of echinoderm coelomocytes. They may be divided into two categories based on whether they relate to a cytopoietic organ or tissue or indicate free cell self-replication. Based on the findings, they infer that the coelomic epithelium is the sole source of coelomocytes in *Asterias rubens.* This implies that any organ lined with this epithelium can be cytopoietic (Bossche and Jangoux, 1976). While the primary functions of coelomocytes are recognized, the quantity, types, and precise physiological contributions of each morphotype remain unknown. Indeed, due to the variety of morphological descriptions provided in the literature, the use of multiple names for comparable cellular morphotypes, different experimental handling techniques, and the diversity of characterization methods, these circulating cells are exposed to diverse categorization systems and hence cannot yet be systematically recognized (Andrade et al. 2021).

Various available literature has compiled the different coelomocyte morphotypes in other Asteroid echinoderm species (Table 1 and Figure 1). *Asterias rubens* is the most common species being studied; Pinsino et al. (2007) identified four coelomocytes, namely, phagocytic cells (petaloid (Figure 1A) and filopodial forms (Figure 1B), amoebocytes, vibratile cells, and hemocytes. Gorshkov et al. (2009) classified

Table 1. Overview of the different coelomocyte classifications of different Asteroid echinoderms.

Asteroid echinoderm species	Coelomocyte morphotypes classification	Method	References
Acanthaster planci	• Large hyaline cells • Small hyaline cells • Colorless spherule cells • Spherule cells • Fusiform cells • Phagocytes	Light microscopy and Scanning Electron Microscope	Grand et al. (2014)
Asterias rubens	• Phagocytic cells - Petaloid forms (Fig. 1A) - Filopodial forms (Fig. 1B) • Amoebocytes • Vibratile cells • Hemocytes	Light microscopy	Pinsino et al. (2007)
	• Immature coelomocyte (Fig. 1C) • Mature coelomocyte (Fig. 1D)	Light microscopy	Gorshkov et al. (2009)
	• Lymphocyte-like cells • Agranular amoebocytes • Granular amoebocytes	Light microscopy	Kudryavstev et al. (2016)
Marthasterias glacialis	• Spherule cells • Vibratile cells • Amoebocytes • Phagocytes	Light microscopy	Franco et al. (2011)
	• P1 morphotypes (P1L and P1S) (Fig. 1E) • P2 morphotypes (petaloid & filopodial sub-populations and phagocyte/ immunocytes) (Fig. 1F)	Fluorescence microscopy	Andrade et al. (2021)
Echinaster sepositus	• Colorless spherula cells (Fig. 1G) • Amoebocytes (Fig. 1H) • Vibratile cells (Fig. 1I)	Light microscopy	Stabili et al. (2018)

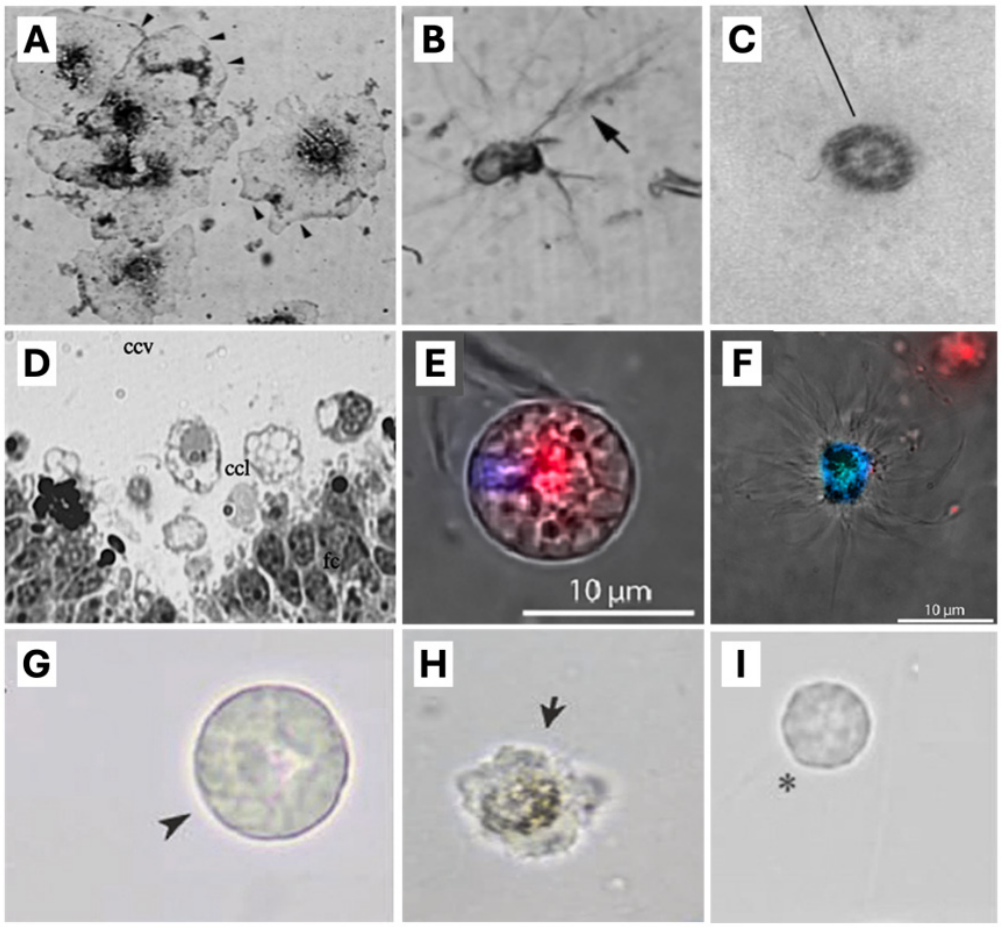

Figure 1. A photomicrograph of the different coelomocyte morphotype classifications from different Asteroid echinoderm species. *Asterias rubens:* A. phagocytic cells – petaloid forms, B. phagocytic cells – filopodial form (Pinsino et al. 2007); C. immature cells, D. mature cells (Gorshkov et al. 2009); *Marthasterias glacialis:* E. P1 morphotypes, F. P2 morphotypes (Andrade et al. 2021); *Echinaster sepositus:* G. colorless spherula cells, H. ameobocytes, I. vibratile cells (Stabili et al. 2018).

coelomocytes based on their ultrastructural analysis; they indicated that the first form is the immature cells (Figure 1C), which are formed as coelomic epithelium flagellar cells, then develop into young coelomocyte with flagellum; after a while it will lose its flagellum (late stage), transforming to the second form – mature coelomocytes (Figure 1D) (characterized with various amounts of lysosomes and phagocytes). The same animal was employed in the study of Kudryavtsev et al. (2016), wherein they identified the coelomocytes into three types: lymphocyte-like cells, agranular amoebocytes, and granular amoebocytes. *Marthasterias glacialis* is the other species used by Franco et al. (2011) and Andrade et al. (2021). Franco et al. (2011) identified four coelomocytes (spherule cells, vibratile cells, amoebocytes, and phagocytes), while Andrade et al. (2021) classified the detected coelomocytes into two: P1 morphotypes (P1L and P1S) (Figure 1E) which is classified as coelomocytes with prominent nucleus with minimal cytoplasmic structures, and P2 morphotypes (petaloid and filopodial sub-populations and phagocyte/immunocytes) (Figure 1F). For *Echinaster sepositus,* three coelomocytes have been identified by Stabili et al. (2018): the colorless spherula cells (Figure 1G), ameobocytes (Figure 1H), and vibratile cells (Figure 1I). Only one article has documented the morphotypes of *Acanthaster planci; based* on the study of Grand et al. (2014), COTS has large hyaline cells, small hyaline cells, colorless spherule cells, red spherule cells, fusiform cells, and phagocytes.

Given the variety of reported morphologies, it is now widely acknowledged that the forms and properties of coelomocytes differ among the five echinoderm groups. An agreement on the categorization of intra-class (i.e., Asteroidea)

coelomocytes has yet to be obtained, most likely due to the inherent variety of morphotypes, low taxonomic sampling, and as previously indicated, diversity in experimental techniques (Andrade et al. 2021). This lack of standardized terminology for Asteroid echinoderm coelomic fluid cells limits our ability to draw comparisons between cells in different echinoderm classes.

Since a few published articles discuss the coelomocyte features, cell viability, and cell morphology, specifically COTS collected from Southern Leyte, through this research, we aim to contribute to a diverse knowledge of the cellular components in the coelomic fluid of this representative Asteroid echinoderm species. The results of this study will serve as baseline information on the coelomocyte cytological diversity and cellular physiological changes of the Crown-of-Thorns Starfish. The data can be used to create key strategies for developing effective control methods, such as discovering novel and potentially effective compounds that can alter coelomocyte physiological properties to prevent or eradicate the COTS population. This is important, especially in the territorial waters of Southern Leyte, Philippines, where COTS outbreaks are rampant and destructive.

2. MATERIALS AND METHODS

2.1 COTS collection and maintenance

Prior to sample collection, our team gathered information from locals regarding COTS sightings within their area (Figure 2A). Approval from the specific Local Government Units (LGUs) was obtained before the collection. About eight (8) adult COTS were collected through the Scuba method (Figure 2B) at the territorial waters of Libagon (10.3374°N,125.0719°E) and Sogod (10.4329°N,124.9948°E), Southern Leyte. The animals were transferred to the Bioresource Production Technology Center (BioProTeC), Southern Leyte State University (SLSU) – Bontoc Campus. They were housed in open-circuit tanks with recirculating saltwater at 15°Celsius and 33% salinity. To control the impact of abiotic variables (e.g., changes in salinity or temperature), all specimens were kept in the same condition during the experimental process (Andrade et al. 2021) (Figure 3A). Our team also participated in the COTS extraction efforts by the different LGUs.

2.2 COTS preparation and collection of coelomic fluid

Before collecting the coelomic fluid, the COTS was placed in its aboral to let the arms settle flat. To estimate the animal's age, the longest ventral diameter was measured. Each of the samples was weighed on a tared kitchen scale. The number of undamaged, whole, regenerating areas was counted. This is to check if the body injury acquired by COTS can directly influence the number and types of coelomocytes detected.

Due to the anatomy of the COTS, they cannot be held for collection. Thus, they were placed in a flat container filled with 300-500 mL of salt water and using an 18-gauge intravenous (IV) catheter (the needle part), the aboral side of the selected undamaged

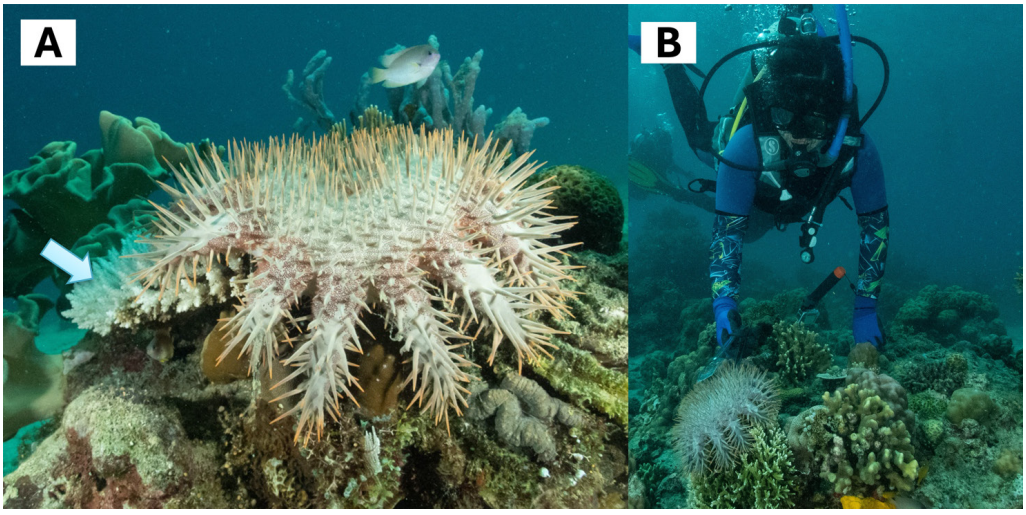

Figure 2. A. Actual Crown-of-Thorns starfish feeding on a coral with the evident white feeding scars (pointed by the light blue arrow); B. Collection of COTS through Scuba method, using metal thongs to remove it from the corals (Actual photo: SLSU-BioProTeC)

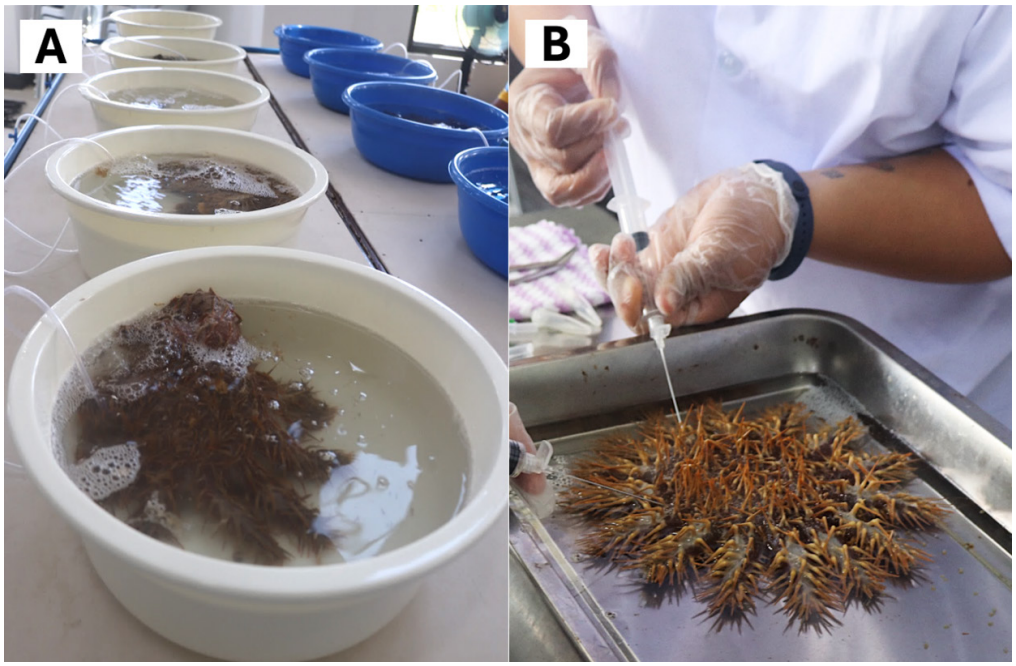

Figure 3. A. Collected Crown-of-Thorns starfish were placed in open circuit tanks with recirculating saltwater; B. Actual collection of coelomic fluid from the selected intact arm using the needle part of the 18-gauge IV catheter and 10mL syringe barrel (Actual photo: SLSU-BioProTeC)

arm was pierced no further than 1 cm from the distal tip of the arm to avoid damage to the internal organs resulting in the contamination of the coelomic fluid (Figure 3B). The collected coelomic fluid was placed in a clean, labeled centrifuge tube. About 10 mL of coelomic fluid was collected per COTS.

After the collection of the animal data and sample, the COTS carcass was properly buried in the sand 20 meters away from the shore. The seawater from the filtered tanks was also discarded onto the dry sand. It was ensured that the water was not thrown back into the sea since it may contain COTS gonads (Kensington 2019; Ludevese-Pascual et al. 2022).

2.3 Cell viability using trypan blue exclusion test

The trypan blue exclusion test was used to determine the cell viability of the COTS coelomic fluid fractions. This method is based on the principle that living cells possess intact cell membranes and thus exclude the dye, and dead cells absorb it (Louis and Siegel 2011; Stabili et al. 2018; Strober 1997). An aliquot of 1 mL of coelomic fluid from each sample was poured into 1 mL of ice-cooled filtered seawater (FSW) with 30 mM Ethylenediaminetetraacetic acid (EDTA). About 90 µl of the coelomic fluid solution was collected and placed in a separate tube; here, the solution was added with 10 µl of 0.8% trypan blue in FSW-EDTA and mixed thoroughly (Stabili et al. 2018).

About 10µl of the suspension was gently introduced into the hemocytometer until the entire counting chamber was full (Louis and Siegel 2011). It was examined using the Motic BA210 Elite Microscope Series. The number of viable and non-viable coelomocytes was counted per chamber with the following time intervals: 0 minutes, 15 minutes, 25 minutes, and 35 minutes.

2.4 Cell staining

A portion (~2 mL) of the fresh coelomic fluid was centrifuged at 400 xg for 2 minutes. The supernatant was decanted and set aside while the pelleted coelomocytes were reconstituted with 100 µl of FSW. About 50 µl of the suspension was placed in a clean glass slide for smear preparation. Two smear preparations were prepared per sample and were stained individually using Hema-Quick stain (Stabili et al., 2018; Xing et al., 2008).

2.5 Microscopy and morphometrics

Stained samples was identified under light microscope, according to size, shape, wall characteristics, nucleus morphology/ contents,

and presence of cell extensions (i.e., flagellum). Microscopy images were acquired using a Motic BA210 Elite Microscope Series equipped with a Moticam (software: Motic Images Plus ML version 3.0 using a 100X/1.25/S-Oil (WD 0.15mm)) (Andrade et al. 2021). Calibrated ocular micrometer and ImageJ (ver 1.8.0 172) software was used for the measurement of the coelomocyte morphotypes. Scale bars (μm) was set using using the ImageJ software (Ferreira and Rasband 2012).

2.6 Data management and analysis

The data gathered from the study was managed using Microsoft Excel 2016, where data cleaning was carried out. Cleaned data were then imported into the statistical software JASP (version 0.16.2), where means, standard deviations, and differences were calculated.

3. RESULTS

3.1 Cell viability

Regardless of the COTS' sex, the trypan blue exclusion test for cell viability showed that as the counting time interval increased, the number of viable cells from the prepared fractions decreased. At fresh preparation (0 minutes), the counted live cells are 137 (mean: 34.25 ± 6.45) with coelomocyte viability of 6.8 ± 10.26 x 10^5 cells ml (Table 2). Dead cells can be differentiated from live ones since they absorb the trypan blue stain.

3.3 Coelomocyte characterization

With light microscopy observation of the different coelomic fractions stained with Hema-Quick, it was seen that COTS has a diverse array of cell morphotypes. Following the keys to the diagnosis of echinoderm coelomocytes by Chia and Xing (1996) and in comparison to the outlined coelomocyte morphotype classification outlined in Table 1 and Figure 1, about seven coelomocyte types were identified, of which spherule cells (mean size: 10 μm) (Figure 4A) were the most common. These cells are circular, devoid of cell extensions or pseudopodia, and the cytoplasm is filled with cell inclusions. Next are the vibratile cells (mean cell size: 8 μm; flagellum length: 25 μm) (Figure 4B); these morphotypes have a circular cell body with a long flagellum (f) protruding from the basal granule (g). In fresh preparation, it was observed that these cells exhibit rapid movement with the help of their long flagellum. Fusiform cells (mean size: 6 μm) (Figure 4C), the third morphotype detected, appears to have a "spindle-shape" with a divided nucleus and two pseudopodia (p) on both ends. The fourth morphotype detected is the crystal cells (mean size: 5 μm) (Figure 4D). It has a hexagonal shape with a central circular nucleus. The fifth morphotype is the progenitor cells (mean size: 8 μm) (Figure 4E). It has an ovoid or spherical shape with a prominent nucleus dominating the cell body. The sixth and seventh morphotypes are derivatives of the phagocyte cells, with Figure 4F (mean size: 12 μm) as the petaloid (pe) and Figure 4G (mean size: 25 μm) as the filiform. The petaloid form is characterized by petal-like pseudopodia that extend from the central body. Meanwhile, the filopodial form has slender spikes/ pseudopodia (p) that radiate from the central body.

4. DISCUSSION

Based on the result of the cell viability, it implies that COTS coelomic fraction preparation for activity observation (i.e., antimicrobial activity, lysozyme, toxicity assay, antioxidant, and hemolytic profiling) should be carried out at most 15 minutes from the time of collection. Some coelomocytes (i.e., vibratile cells) tend to have shorter livability and can be detected only in fresh preparation (up to two minutes from the time of collection).

COTS are members of the echinoderm class, known as coelomic animals. Their body cavities are filled with different cellular morphotypes called

Table 2. Viability of the **A. planci** cells in different time intervals.

Time (Minutes)	Average Live Cells Counted (N*)	Mean ± SD	Range (Lower – Upper)	Confidence Level (95%)	Cell Viability (x 10^5 cells ml)
0	137	34.25 ± 6.45	23.99 – 44.51	10.26	6.8
15	131	32.75 ± 7.50	20.82 – 44.68	11.93	6.5
25	48	12 ± 4.76	4.42 – 19.58	7.58	2.4
35	0	0	0	0	0

N: number of cells counted in Hema-Quick stain

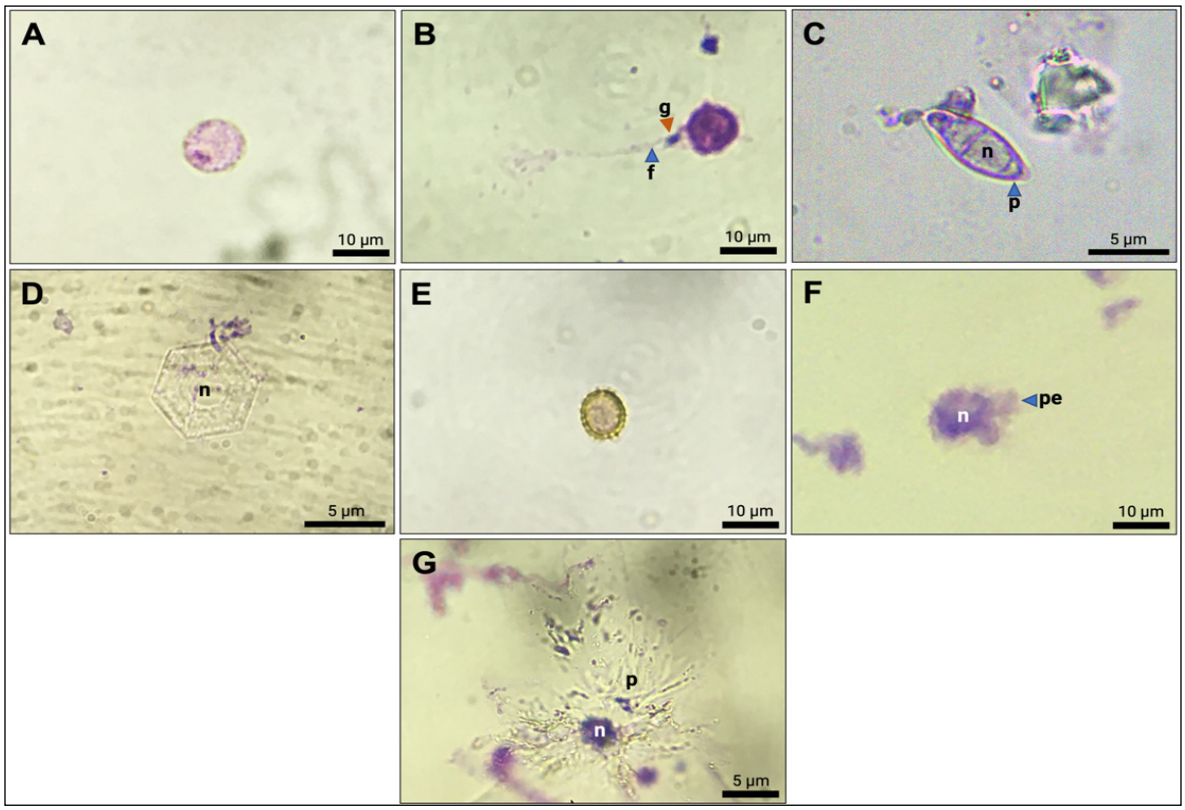

Figure 4. A photomicrograph of different coelomocyte morphotypes from sampled COTS stained using Hema-Quick staining technique: (A) spherule cell, (B) vibratile cell, (C) fusiform cell, (D) crystal cell, (E) progenitor cell, (F) phagocyte cell-petaloid form, and (G) phagocyte cell-filiform. Viewed under 1000X. Labels – g: granule; f: flagellum; n: nucleus; pe: petal form; p: pseudopodia

coelomocytes (Andrade et al. 2021; Chia and Xing 1996). Coelomocytes are believed to carry an array of functions ranging from immunity, nutrient transport, waste excretion, regeneration, wound healing, and clotting (Chia and Xing 1996; Kudryavtsev et al. 2016; Stabili et al. 2018; Xing et al. 2008). Bossche and Jangoux (1976) indicated that the coelomocytes of Asteroid echinoderms originated from the coelomic epithelium and that all organs lined by these are considered cytopoietic.

Grand et al. (2014) reported six coelomocyte morphotype classifications of *Acanthaster planci*. Still, these morphotypes were observed after administering Oxbile injections and antibiotic treatment, thus elaborating immune response, ultimately altering the normal appearance/ physiology of the coelomocyte morphotypes. These changes make comparing our harvested coelomocyte morphotypes from the study of Grand et al. (2014) difficult. Considering this, we used Grand et al. (2014) and other morphotype characteristics indicated in the reference review to curate our classification based on the structure and characteristics of the detected coelomocytes.

Based on our observation, the spherule cells (Figure 2A) were the most common. In other published articles, spherule cells are identified as counterparts of mast cells in higher vertebrate animals. Spherule cells have two forms: colorless spherule cells and red spherule cells. The colorless spherule cells undergo despherulation or the breakdown of the cell membrane and release of granules to aid in wound healing activity and ultimately appear red (Chia and Xing 1996). Grand et al. (2014) reported the presence of spherule cells in their sampled *Acanthaster.*

Vibratile cells are relatively small, with a prominent flagellum-like extension. In fresh preparation, these cells are rapidly moving, thus making them difficult to identify. With their active movement, some works of literature have considered them protozoans (Hetzel 1963). Despite their function being yet to be determined, reports indicated that vibratile cells assist in the circulation of coelomic fluid within the body cavity through their vigorous movements (Chia and Xing, 1996; Xing et al. 2008). Bertheussen and Seljellid (1978) reported that vibratile cells discharged mucoid substances and became gel-

like soon after release. These authors suggest that vibratile cells have the same functionality as platelets of higher life forms. Based on the observation of Grand et al. (2014) and our findings, there could be a high chance that vibratile cells are the large hyaline cells as Grand et al. (2014) classified in their research.

Fusiform cells have a pear or "spindle-shape" with two pseudopodia at both ends. Grand et al. (2014) reported the presence of this morphotype in their sampled *Acanthaster*. During our examination, the fusiform and vibratile cells are not detected in every sample. Crystal cells appear in various shapes, including cuboid, styloid, and hexagonal (most common). It possesses a single nucleus with a thin layer of hyaline cytoplasm. Eliseikina and Magarlamov (2002) suggest that the primary function of crystal cells is to participate in osmoregulation.

In other literature, progenitor cells characterized by a dominant nucleus and surrounded by a hyaline cytoplasm are termed lymphocytes (Chia and Xing 1996; Xing et al. 2008). Fontaine and Hall (1981) reported that these cells also represent the stem cells of specific coelomocytes, such as spherule and phagocyte cells, during the transitional form. They can exhibit three forms: a circular form devoid of pseudopodia, a central body with two at opposite poles, and a triangular central body equipped at its corners with a filiform pseudopodium extension (Xing et al. 2008). Based on the characteristics of this morphotype and with the description by Grand et al. (2014), we can assume that the progenitor cells are small hyaline cells.

The phagocyte cells are the second most abundant cells in every coelomic fraction preparation. In other literature, it is known as "amoebocytes/ amebocytes" (Xing et al. 2008). Other research outputs that analyze the cell composition of Asteroid echinoderms indicated that the phagocytic amoebocytes have the dominant cell population (Kaneshiro and Karp 1980; Kozlova et al. 2006; Pinsino et al. 2007). The cell cytoplasm contains vesicles, vacuoles, and a prominent nucleus. These cells elaborate on two distinct forms, the petaloid and filiform, and they are differentiated based on the changes in the cell pseudopodia and cytoplasm redistribution. The petaloid form has a petal-like or bladder-like pseudopodium extension from its central body. At the same time, filiform has an elongated or spine-like pseudopodium that branches in different directions. It is indicated that phagocytes spontaneously transform from petaloid to filiform. Activation from petaloid to filiform is dynamic, based on the readjustment of the actin cytoskeleton (Gorshkov et al. 2009). The phagocyte population adapts to external and internal changes, with a higher number during trauma, injury, and predation experienced by Asteroid echinoderms. The spine-like pseudopodium or projection increases the cell surface, making it easy to isolate and collect/ phagocytize pathogenic microorganisms or foreign particles (Hillier and Vacquier 2003; Shabelnikov et al. 2019).

Additionally, the aggregation of the filiform phagocyte could create a "plug," to prevent coelomic fluid loss, and encourage wound clotting (Chia and Xing 1996; Shabelnikov et al. 2019; Xing et al. 2008). Surprisingly, out of the eight COTS, three have apparent missing or recently healed arms, and the phagocyte cells tend to be more numerous in comparison to those COTS with no missing or healed arms. This indicates that after rapid injury, the loss of the arm may lead to an increase in the fusiform and vibratile cells that heal the injury.

With the evident influence of global warming, the temperature of the sea tends to be higher, making it a suitable environment for COTS spawning, resulting in alarming outbreaks. Southern Leyte is experiencing this overwhelming phenomenon where this starfish ravages coral reefs, leading to fewer coral fish and other marine populations. The Local Government Unit of Southern Leyte collaborates with academic institutions (Southern Leyte State University and Visayas State University) to further assess and study COTS population replenishment. The Southern Leyte fisherfolks were also informed about the situation and were encouraged to harvest COTS and bury them away from the shore. They are also tasked to note areas where COTS aggregation is evident. Various volunteers (i.e., Dap-ag Boys) and research organizations (i.e., Southern Leyte Coral Reef Conservation Project) also participate in the said endeavor (Ludevese-Pascual et al. 2022).

The territorial waters of Southern Leyte are known for their high abundance and diversity of marine life, especially hard corals. Under the Expanded National Integrated Protected Areas System Act (E-NIPAS), RA 11038, in relation to the Coastal and Marine Ecosystems Management Program (CMEMP), Senate Bill 1690 declares Panaon Island as a protected seascape (CMEMP 2019; DENR-BMB 2020). Another bill has been consolidated, covering four municipalities (Liloan, San Francisco, Pintuyan, and San Ricardo) as part of the protected seascape (Oceana 2023). These newly protected biodiversity hotspots are in great peril due to the silent outbreak of COTS within the area, looming in the dark abyss of the ocean and impacting the marine ecosystem.

Our study aims to contribute to understanding the cytological diversity and physiological response of COTS to create novel biological substances that can be administered and thus prevent or control outbreaks.

5. CONCLUSION

The current work is the first effort to quantify cell viability and profiling of cell morphotypes found in the coelomic fluid of the Crown-of-Thorns starfish (COTS) (*A. planci*), found in the territorial waters of Southern Leyte, Philippines. The findings of this study indicate that *A. planci* coelomic fluid is made up of a variety of coelomocytes, including spherule, vibratile, fusiform, crystal, progenitor, and phagocyte cells, all of which have distinct cellular sizes and morphologies and are present in varying relative abundances. Despite the nature of COTS as a specialist corallivore that preys on coral polyps, we believe the current work interests many researchers, especially in understanding the biology of these animals and the novel search for compounds from marine invertebrates.

Based on the results of this study, the authors point to the need for in-depth systematic profiling and characterization of the COTS coelomocytes using a more advanced microscopical techniques (i.e., fluorescence and transmission electron microscopy); percentage determination of each coelomocyte per ml of coelomic fractions depending on the COTS maturity, sex, and status – healthy, stressed, injured, or during spawning seasons; cell viability per coelomocyte type, the impact of seasonal variation to the coelomocytes; and comparison of the coelomocytes of other Asteroid echinoderms endemic to the territorial waters of Southern Leyte Province, other key biodiversity areas and adjacent municipal waters in the Philippines.

ACKNOWLEDGMENT

The authors would like to thank the faculty and staff of the BioProTeC, Southern Leyte State University – Bontoc Campus and the Diagnostic Laboratory, Faculty of Veterinary Medicine – Visayas State University for their help in the conduct of the research, the Local Government Unit of Liloan and Sogod for their support of the project, and the Dap-ag Boys for their help in the collection of the COTS.

This research project received funding from the Department of Science and Technology-National Research Council of the Philippines (DOST-NRCP) through the Science and Technology Experts' Pool (NSTEP) Research Grant.

AUTHOR CONTRIBUTIONS

Lumain JPLM: Conceptualization, Methodology, Data gathering, Writing – Draft preparation, review, and editing. **Balala AC:** Data gathering. **Napala JJO:** Data gathering. **Tomol RP:** Data gathering. **Alfante JMP:** Data gathering. **Pascual GL:** Conceptualization, Methodology, Data gathering, Supervision.

CONFLICTS OF INTEREST

The authors have no competing interest to declare.

ETHICS STATEMENT

The procedures for collecting, processing, and disposing of COTS followed the principles of the Department of Environment and Natural Resources—Biodiversity Management Bureau 2020 (Preliminary guidance for COTS outbreaks). The researchers followed all institutional and national guidelines for the care and use of the said animals. A letter of request was also sent to the specified LGUs for the field collection of COTS. Furthermore, the team helped the LGUs in their ongoing efforts to extract COTS in their territorial waters during the conduct of the study.

REFERENCES

Aguirre JN. 2020. In picturesque Boracay, a crown-of-thorns infestation is eating away a reef: Mongabay; [updated 2020 Jul 23; accessed 2025 Mar 3]. https://news.mongabay.com/2020/07/in-picturesque-boracay-a-crown-of-thorns-infestation-is-eating-away-at-the-reef/

Andrade C, Oliveira B, Guatelli S, Martinez P, Simões B, Bispo C, Ferrario C, Bonasoro F, Rino, J, Sugni M, and others. 2021. Characterization of coelomic fluid cell types in the starfish *Marthasterias glacialis* using a flow cytometry/imaging combined approach. Frontiers in immunology. 12:641664. https://doi.org/10.3389/fimmu.2021.641664

Bertheussen K, Seljelid R. 1978. Echinoid phagocytes in vitro. Experimental cell research. 111(2): 401–412. https://doi.org/10.1016/0014-4827(78)90185-4

Bossche JV, Jangoux M. 1976. Epithelial origin of starfish coelomocytes. Nature. 261(5557):227–228. https://doi.org/10.1038/261227a0

Chia FS, Xing J. 1996. Echinoderm coelomocytes. Zoological Studies-Taipei. 35:231–254. https://zoolstud.sinica.edu.tw/Journals/35.4/231.pdf

[CMEMP] Coastal and Marine Ecosystems Management Program. 2019. DENR-BMB; [updated 2019 Feb 15; accessed 2025 Mar 3]. https://sdg.neda.gov.ph/coastal-and-marine-ecosystems-management-program-cmemp/

Dai Y, Prithiviraj N, Gan J, Zhang XA, Yan J. 2016. Tissue extract fractions from starfish undergoing regeneration promote wound healing and lower jaw blastema regeneration of zebrafish. Scientific reports. 6(1):1–11. https://doi.org/10.1038/srep38693

[DENR-BMB] Department of Environmental and Natural Resources – Biodiversity Management Bureau. 2020. Preliminary guidance for Crown-of-thorns (COTS) outbreaks.

Eliseikina MA, Magarlamov TY. 2002. Coelomocyte morphology in the holothurians *Apostichopus japonicus* (Aspidochirota: Stichopodidae) and *Cucumaria japonica* (Dendrochirota: Cucumariidae). Russian Journal of Marine Biology. 28(3):197–202. https://doi.org/10.1023/A:1016801521216

Ferreira T, Rasband W. 2012. ImageJ user guide. ImageJ/Fiji. 1:155–161. http://imagej.nih.gov/ij/docs/guide

Fontaine AR, Hall BD. 1981. The haemocyte of the holothurian *Eupentacta quinquesemita*: ultrastructure and maturation. Canadian Journal of Zoology. 59(10):1884–1891. https://doi.org/10.1139/z81-256

Franco CF, Santos R, Coelho AV. 2011. Proteome characterization of sea star coelomocytes–the innate immune effector cells of echinoderms. Proteomics. 11(17):3587–3592. https://doi.org/10.1002/pmic.201000745

Grand A, Pratchett M, Rivera-Posada J. 2014. The immune response of *Acanthaster planci* to oxbile injections and antibiotic treatment. Journal of Marine Sciences. 1:769356. https://doi.org/10.1155/2014/769356

Gorshkov AN, Blinova MI, Pinaev GP. 2009. Ultrastructure of coelomic epithelium and coelomocytes of the starfish *Asterias rubens* L. in norm and after wounding. Cell and Tissue Biology. 3:477–490. https://doi.org/10.1134/S1990519X09050113

Hillier BJ, Vacquier VD. 2003. Amassin, an olfactomedin protein, mediates the massive intercellular adhesion of sea urchin coelomocytes. The Journal of cell biology. 160(4):597–604. https://doi.org/10.1083/jcb.200210053

Hetzel HR. 1963. Studies on holothurian coelomocytes. I. A survey of coelomocyte types. The Biological Bulletin. 125(2):289–301. https://www.journals.uchicago.edu/doi/abs/10.2307/1539404

Kaneshiro ES, Karp RD. 1980. The ultrastructe of coelomocytes of the sea star *Dermasterias imbricata*. The Biological Bulletin. 159(2):295–310. https://www.journals.uchicago.edu/doi/abs/10.2307/1541094

Kensington N. 2019. An assessment of the population of crown-of-thorns starfish (*Acanthaster planci* L.) around the island of Malapascua, Republic of the Philippines. Bsc Research Project, The University of Dublin. https://www.peopleandthesea.org/wp-content/uploads/2020/01/NKensignton-COT.pdf

Kozlova AB, Petukhova AO, Pinaev GP. 2006. The analysis of cellular elements in coelomic fluid of the starfish *Asterias rubens* L. in early regeneration time. Tsitologiia. 48:175–183. https://scispace.com/papers/the-analysis-of-cellular-elements-in-coelomic-fluid-during-hxx65v5692

Kudryavtsev IV, D'yachkov IS, Mogilenko DA, Sukhachev AN, Polevshchikov AV. 2016. The functional activity of fractions of coelomocytes of the starfish *Asterias rubens* Linnaeus, 1758. Russian Journal of Marine Biology. 42:158–165. https://doi.org/10.1134/S106307401602005X

Louis KS, Siegel AC. 2011. Cell viability analysis using trypan blue: manual and automated methods. Methods Mol Biol. 740:7–12. https://doi.org/10.1007/978-1-61779-108-6_2

Ludevese-Pascual G, Napala JJO, Tomol RP, Oyong LJL, Alfante JMP, Lumain JPLM. 2022. Crown-of-Thorns starfish (COTS) outbreak in Sogod Bay, Southern Leyte, Philippines. Journal of Ecosystem Science and Eco-Governance. 4(1):24–30. https://doi.org/10.54610/jeseg/4.1.2022.003

Marine Conservation Philippines. 2023. Crown of Thorns in Lutoban, Zamboanguita; [updated 2023 Apr 24; accessed 2025 Mar 3]. https://www.marineconservationphilippines.org/crown-of-thorns-lutoban/

Municipality of El Nido, Province of Palawan. 2009. Crown of Thorns Outbreak: Republic of the Philippines, Municipality of El Nido; [updated 2009 Jul; accessed 2025 Feb 29]. https://elnido.gov.ph/crown-of-thorns-outbreak/

Nicolas J, Pepingco VALO, Cruz-Abeledo CCV. 2024. Five Decades Behind the Limelight: Crown-of-Thorns Sea Star Outbreaks Razing the Already Ailing Philippine Reefs. The Philippine Journal of Fisheries. 31(1):1–16. https://doi.org/10.31398/tpjf/30.2.2022-0032

Oceana. 2023. Marine biodiversity in Southern Leyte's Panaon island gets added boost for protection in the Senate – Press Release; [updated 2023 Feb 1; accessed 2025 Feb 29]. https://ph.oceana.org/press-releases/marine-biodiversity-in-southern-leytes-panaon-island-gets-added-boost-for-protection-in-the-senate/

Pinsino A, Thorndyke MC, Matranga V. 2007. Coelomocytes and post-traumatic response in the common sea star *Asterias rubens*. Cell stress & chaperones. 12(4):331. https://pmc.ncbi.nlm.nih.gov/articles/PMC2134795/

Shabelnikov SV, Bobkov DE, Sharlaimova NS, Petukhova OA. 2019. Injury affects coelomic fluid proteome of the common starfish, *Asterias rubens*. Journal of Experimental Biology. 222(6):jeb198556. https://doi.org/10.1242/jeb.198556

Smith VJ. 1981. The echinoderms. Invertebrate blood cells. 2:513–562. https://cir.nii.ac.jp/crid/1571980074164897792

Stabili L, Acquaviva MI, Cavallo RA, Gerardi C, Narracci M, Pagliara P. 2018. Screening of three echinoderm species as new opportunity for drug discovery: Their bioactivities and antimicrobial properties. Evidence-based complementary and alternative medicine. 2018(1):7891748. https://doi.org/10.1155/2018/7891748

Strober W. 1997. Trypan blue exclusion test of cell viability. Current protocols in immunology. 21(1):A.3B.1–A.3B.2. https://doi.org/10.1002/0471142735.ima03bs21

Xing K, Yang HS, Chen MY. 2008. Morphological and ultrastructural characterization of the coelomocytes in *Apostichopus japonicus*. Aquatic Biology. 2(1):85–92. https://doi.org/10.3354/ab00038