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As a multi-disciplinary journal, The Palawan Scientist aims to publish high-quality and original research in the fields of agriculture, fisheries and aquatic sciences, environment, education, engineering, mathematics, sociology, and related disciplines (including arts and humanities).

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COVER PHOTOS

The attractive prices for spiny lobster puerulus led to the widespread establishment of settlement traps along the eastern coast of Palawan. However, the study of Mecha et al. showed that the collection of spiny lobster puerulus is not profitable in one of the two study sites. Photos by Ian Lester U. De Jesus

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EDITORIAL

Prominence in research has always been associated with Western Philippines University. In its humble beginnings as a state college, it had been actively involved in research in agriculture and fisheries in strong collaboration with other research institutions. Research outputs were then published in a local journal, "The Palawan Agriculturist," with reviewers from within the college.

The conversion into a comprehensive university, coupled with the expansion of its programs, research became multi-disciplinary, and research outputs further advanced. The local journal had been reinstituted as "The Palawan Scientist" to accommodate publications from fields other than agriculture and fisheries.

Twenty years after its maiden publication, The Palawan Scientist had significantly evolved with significant milestones added to its feat. From the first externally reviewed volume in 2014, the Palawan Scientist received the CHED Journal Incentive Program award in 2016 and was listed in the ASEAN Citation Index and the Emerging Sources Citation Index of Clarivate Analytics the following year. Early this year, the Andrew Gonzalez Philippine Citation Index accepted The Palawan Scientist as a full member.

The international recognition and indexing of The Palawan Scientist benefit the faculty as their publications can be credited to their research performance, possibly improving their citation ratings if other researchers cite their papers. Papers published by the faculty will also enhance the research output of the university, as reported to the oversight agencies, notably the Results-Based Performance Management System and SUC Leveling.

The Palawan Scientist, as a reputable research journal, invites faculty from WPU and other HEIs and researchers from other institutions to publish their outputs in the journal. As envisioned, the peer-reviewed journal serves as a platform for disseminating and sharing relevant research information in various fields in Palawan, nationwide, and even worldwide. Hopefully, these published research outputs will lead to development initiatives that will uplift the communities and the marginalized sectors of society.

As its first editor-in-chief, I commend the editorial board for transforming the journal towards excellence and recognition. As we move forward, let us be wary of the challenges, opportunities, and threats of the advancements in digital technology and artificial intelligence to safeguard and promote intellectual property and the integrity of research outputs.

Padayon!

Noel L. Gauran
Professor
Western Philippines University

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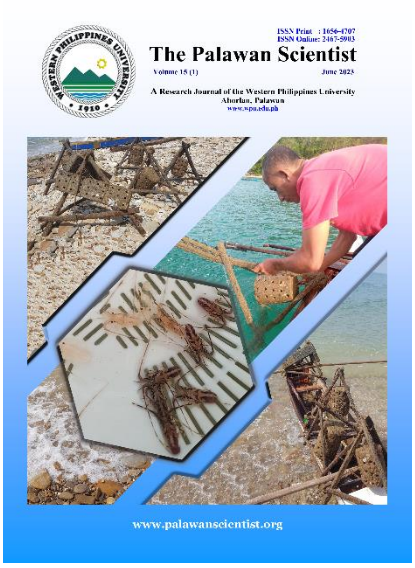

Influence of different population densities of *Portulaca oleracea* L. on growth and yield of transplanted onion *Allium cepa* L.

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ABSTRACT

Portulaca oleracea is one of the weeds of onion in the Philippines. Its negative impact on growth and yield, however, has not been quantified. An experiment was conducted to determine the influence of different density levels of *P. oleracea* on growth and yield of transplanted onions. The weed was allowed to grow with the onion at 0, 5, 10, and 15 plants 176 cm^{-2} from the day of planting until harvest. The experiment was arranged in randomized complete block design with four replications. Growth and yield of transplanted onions were influenced by different densities of *P. oleracea*. The fresh weight of bulbs was reduced by 32.7, 51.3, and 73.6%; the dry weight by 39.6, 59.8, and 71.9% when the weed competed at 5, 10, and 15 plants 176 cm^{-2}, respectively. Correlation and regression analyses showed that the population density of *P. oleracea* were strongly, negatively correlated as well as it influenced by 98.54 and 93.45%, respectively, on the fresh and dry weight of transplanted onion. This study confirmed that *P. oleracea* is truly a weed of transplanted onion implying that it could potentially reduce yield if left unmanaged throughout the crop's growing cycle. Thus, it must be managed effectively whenever seen infesting transplanted onions in the country. Although the results were obtained only under screenhouse conditions, the findings suggest the need to develop a holistic weed control strategy against the weed.

Keywords: additive design, common purslane, crop-weed competition, olasiman, Portulacaceae

INTRODUCTION

Bulb onion *Allium cepa* L. is one of the major rice-based crops (rice-onion, rice-corn, and other rice-vegetable cropping systems) in the Philippines (PhilRice 2007). In 2021, the bulb onion was planted on 19.3 thousand ha with a production volume of 218 thousand metric tons valued at PHP11,501,200,000 (PSA 2022). These figures are expected to further rise to meet the demands for culinary purposes.

Weeds are one of the groups of pests that need to be considered when growing onions. This is because weeds, if left unmanaged, will reduce yield of onions. In fact, in a field study in Nueva Ecija, weeds left uncontrolled reduced the yield of red and white onions by as much as 78 and 97%, respectively (Baltazar et al. 1998a). In another study, the yield of red onion reduced by 79% when major weeds such as *Cyperus rotundus* L., *Echinochloa colona* (L.) Link., and *Trianthema portulacastrum* L. were left to grow and compete in the field (Baltazar et al. 1998b). Thus, it is important that weeds must be managed to ensure the quantity and quality of yield as well higher income from the planting of onions.

Portulaca oleracea L., also known as "common purslane, pigweed, or olasiman", is a C_4 plant that proliferates both by seeds and vegetative stem cuttings (Merrill 1912; Galinato et al. 1999; Ferrari et al. 2020). One mature plant of it could produce a mean of 10,000 seeds plant^{-1} at a mean weight of 0.07 mg seed^{-1} (Galinato et al. 1999). Many of its seeds germinate at the soil surface, particularly when exposed at 30/20°C alternating day/night temperatures with 12/12 h light/dark conditions; no germination at all for those buried at a soil depth of 2 cm (Chauhan and Johnson 2009). Its cut stems with nodes, on the other hand, are the only ones that produce new leaves; those with attached leaves produced the newest leaves (Proctor et al. 2011). In addition, its cut stems with nodes had >70% survival; those from the internodes had 0% survival under field conditions. *Portulaca oleracea* is one of the common weeds of rainfed rice and rice-based crops in the country like corn, tomato, eggplant, string beans, and yam (Fabro and Barcial 2015; Gonzales 2017; Galinato et al. 1999; Donayre et al. 2018). It is also one of the common weeds infesting many bulb onion fields (Baltazar et al. 1999; Casimero 2000; PhilRice 2007). Its negative impact on the growth and yield of direct-seeded onion has been reported in other countries. For example, it reduced the yield of direct-seeded onion by 9, 68.3, and 83.5% when allowed to compete from 0 to 40 days after planting; 53, 81, and 93% when allowed to compete from the day planting until harvest (Adams 1977). Under Philippine conditions, however, its impact has not yet been quantified in either direct-seeded or transplanted onion. Hence, this paper aimed to determine the influence of the different population density of *P. oleracea* on the growth and yield of transplanted onion.

METHODS

Location and Materials

The study was conducted at the experimental area of the Crop Protection Department, College of Agriculture, Central Luzon State University (CLSU), Science City of Munoz, Nueva Ecija from November 2018 to April 2019. The greenhouse, where the experiment was conducted, was made of steel frames covered with fine nets in all the sides as well as the roofing. It was also built in an open space away from trees and buildings. The soil (Maligaya soil series) used as a medium for planting was collected from the same field area and location. To avoid the growth of other plants, collected soil samples were pulverized, placed inside a polypropylene plastic bag at 2 kg bag^{-1}, and sterilized by mixing with water and heating for 8 h in a huge cylindrical metal drum. After sterilization, the soil sample in each bag was allowed to cool and then transferred later into plastic containers.

Red Pinoy was used as the test onion variety. It was prepared by planting 4-5 seeds hole^{-1} on a seedling tray previously filled with sterilized soil. All germinating seeds were allowed to grow until 40 days under saturated and full sunlight conditions. Mature seeds of *P. oleracea*, collected from onion fields of Sto. Domingo, Nueva Ecija, were also seeded at 10 seeds hole^{-1} on a seedling tray with sterilized soil. One-week-old growing seedlings were thinned and maintained at 2-3 seedlings hole^{-1}. Seedlings were allowed to grow until 40 days under moist and full sunlight conditions.

Experimental Design

Cylindrical plastic container (area=176 cm^2, depth=10 cm), filled with 10 kg of sterilized moist soil, was used as the experimental unit of the study. Each center of the container was transplanted with one 40-day-old seedling of bulb onion as shown in Figure 1 (Islam et al. 2009; Casimero 2000). Following the procedure of Chauhan and Johnson (2009) in growing seeds, 40-day-old seedlings (3 leaf-stage) of *P. oleracea* were planted in each container at 0, 5, 10, and 15 plants container $^{-1}$ or plants 176 cm^{-2}. The planting distances between the crop and weed were 10, 5-10, and 2.5-5 cm for 5, 10, and 15 plants 176 cm^{-2}, respectively. On the other hand, the distances between each weed were 10, 5, and 2.5-5 cm in the same order of plants 176 cm^{-2}. Each experimental unit with transplanted bulb onion and *P. oleracea* at different densities were arranged in randomized complete block design with four replications. All plants inside each experimental unit were grown until the crop's maturity. All plants were nourished with synthetic fertilizers at 67-21-21 kg of N, P_2O_5, and K_2O ha^{-1} recommended rates. Water was also supplied in each box and maintained at saturation level whenever necessary. An additive design of the crop-weed competition, where the density of onion was held constant and that of *P. oleracea* was kept increased, was utilized to determine the outcome of *P. oleracea* - onion competition (Swanton et al. 2015).

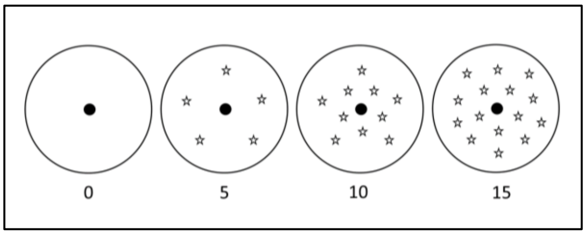

Figure 1. Position of transplanted onion (circles) relative to different densities of *Portulaca oleracea* (stars) inside the experimental unit.

Data Collection

The growth and development of bulb onion were measured in terms of height; number, length, and weight of leaves; length of bulb and roots; and fresh and dry-weight of bulbs. Using a meter stick, the height of bulb onion was determined by measuring it from the base to the tip of the tallest leaf of each plant 60 days after planting (DAP). Using a ruler, the average length of the leaves was also determined by measuring the length of the three leaves close to the base of each plant. The number of leaves per plant was manually counted while the root and bulb lengths as well as the bulb-widths were measured using either a ruler or Vernier caliper (0-150 mm, Mituyo, Japan). Using a sharpened knife, the bulb of each onion was separated from the shoots (leaves) during the harvesting time. The fresh weights of leaves and bulbs were then separately recorded using a digital weighing balance (A&D Electronic Balance FX-3000). To determine the dry weights, the leaves and bulbs were placed separately inside a paper bag. Each bag was then placed inside an oven for drying within 48 h at 70°C. After drying, the leaves and bulbs were weighed using a digital weighing balance. Percentage reductions on growth variables and bulb weight (Y) of onion were calculated using the equation below:

$$Y = \frac{(Yo - Y1)}{Yo}\,(100)$$

where, Y_0 as the mean value at 0 plants 176 cm^{-2} and Y1 as the mean values at 5, 10, and 15 plants 176 cm^{-2}, respectively.

Statistical Analysis

All the data were subjected to ANOVA using the Statistical Tool for Agricultural Research (STAR v. 2.0.1) of International Rice Research Institute. The treatment means, on the other hand, were compared using the Tukey's HSD at 5% level of significance. A Pearson-product moment correlation coefficient was also computed to determine the strength and direction of relationship between the population density of *P. oleracea* and the fresh and dry bulb weights of transplanted onion. In addition, a simple linear model (Y = bx + a) was also fitted to the population density of *P. oleracea* and the fresh and dry bulb weights of transplanted onion to create a prediction model.

RESULTS

Influence on Growth Parameters

Values of the height and number of leaves of onion were high when *P. oleracea* was absent; lowest when the weed was present at 15 plants 176 cm^{-2} (Table 1). No significant difference was observed in height at 5, 10, and 15 plants 176 cm^{-2}. But there was significant difference between the height of onions at 0 and 15 plants 176 cm^{-2}. The number of leaves had no significant difference at 0 and 5 plants 176 cm^{-2}. Similarly, no difference was observed at 5, 10, and 15 plants 176 cm^{-2}. There were significant differences, however, at 0 plants 176 cm^{-2} compared to 10 and 15 plants 176 cm^{-2}. The length of leaves of onion also measured high in the absence of the weed. But the measurement was not significantly different when the weed was present at 5 and 10 plants 176 cm^{-2}. The length of leaves at 5 and 10 plants 176 cm^{-2} was not significantly different; however, it was significantly different at 15 plants 176 cm^{-2}. The dry weight of the leaves of onion was also highest when the weed was absent. The dry weight, however, was not significantly different when the weed was present at three densities.

From 0 plants 176 cm^{-2}, the height of onion was reduced by 16.8, 29.9, and 40.1%; the number of leaves by 39.1, 60.9, and 71.7%; the length of leaves by 17.6, 31.4, and 42.1%; and the dry weight of leaves by 30.4, 37.7, and 55.9%, when the weed was allowed to compete at 5, 10, and 15 plants 176 cm^{-2}, respectively (Table 2).

Table 1. Mean (± se) height, number, length, and dry weight of leaves of transplanted onion as influenced by different population densities of *Portulaca oleracea*. In each column, means with the same letters are not significantly different at 5% level.

***Portulaca oleracea* (plants 176 cm^{-2})**	**Growth parameters**			
	Height of $plant^{-1}$ (cm)	**No. of leaves $plant^{-1}$**	**Length of leaves $plant^{-1}$**	**Dry weight of leaves $plant^{-1}$ (g)**
0	40.6 (±3.2)^{a}	6 (±0.5)^{a}	35.8 (±2.5)^{a}	3.1 (±0.3)^{a}
5	33.8 (±4.3)^{ab}	5 (±0.5)^{ab}	29.5 (±4.5)^{ab}	2.2 (±0.4)^{a}
10	28.4 (±3.4)^{ab}	4 (±0.5)^{b}	24.5 (±2.8)^{ab}	2.0 (±0.4)^{a}
15	24.3 (±2.2)^{b}	4 (±0.3)^{b}	20.4 (±1.7)^{b}	1.5 (±0.5)^{a}

Table 2. Percentage reductions on height and number, length, and dry weight of leaves of onion as influenced by different population densities of *Portulaca oleracea*.

***Portulaca oleracea* (plants 176 cm^{-2})**	**Reductions (%)**			
	Height of $plant^{-1}$ (cm)	**No. of leaves $plant^{-1}$**	**Length of leaves $plant^{-1}$**	**Dry weight of leaves $plant^{-1}$ (g)**
5	16.8	39.1	17.6	30.4
10	29.9	60.9	31.4	37.7
15	40.1	71.7	42.1	55.9

Measurements in bulbs and roots of onions were high when the weed was absent (Table 3). Bulb and root lengths at 0 plants 176 cm^{-2}, however, had no significant differences when the weed was present at 5, 10, and 15 plants 176 cm^{-2}. Likewise, bulb diameter at 0 plants was not different at 5 and 10 plants except at 15 plants 176 cm^{-2}. From 0 plants 176 cm^{-2}, bulb length reduced by 3.4, 8.1, and 9.4%; bulb diameter by 21.8, 28.8, and 51.8%; and root length by 6.7, 14, and 23.1% when the weed was at 5, 10, and 15 plants 176 cm^{-2}, respectively (Table 4).

Influence on Yield

Fresh and dry weights of bulbs were also high when *P. oleracea* was absent (Figure 2). The weight of bulbs at 0 plants 176 cm^{-2}, however, did not differ at 5 and 10 plants 176 cm^{-2} except at 15 plants 176 cm^{-2}. From 0 plants 176 cm^{-2}, the fresh weight of bulbs reduced by 32.7, 51.3, and 73.6%; and the dry weight of bulbs by 39.6, 59.8, and 71.9%; when the weed was allowed to compete at 5, 10, and 15 plants 176 cm^{-2}, respectively (Table 5).

Correlation and Regression

The population density of *P. oleracea* were strongly negatively correlated to the fresh (R = -0.993) and dry bulb weights (R = -0.967) of transplanted onion. The simple linear regression analysis also showed that 98.54 and 93.45% of fresh and dry bulb weights of transplanted onion, respectively, were influenced by the population density of *P. oleracea*. Further analysis also showed that the population density of the weed was a significant predictor and contributor to both the fresh and dry bulb weights of the crop at 5% level of significance (P = 0.033). The final predictive models for the fresh weight of bulb was y = -1.672(*P. oleracea* population density) + 33.69 while for the dry weight of bulb was y = -0.2146(*P. oleracea* population density) + 4.212 (Figure 3).

Table 3. Mean (± se) bulb length, bulb diameter, and root length of transplanted onion as influenced by different population densities of *Portulaca oleracea*. In each column, means with the same letters are not significantly different at 5% level of significance.

***Portulaca oleracea* (plants 176 cm^{-2})**	**Growth Parameters**		
	Bulb length plant^{-1} (cm)	**Bulb diameter plant^{-1} (cm)**	**Root length plant^{-1} (cm)**
0	8.0 (±0.6)^{a}	4.0 (±0.4)^{a}	5.5 (±0.5)^{a}
5	7.7 (±0.4)^{a}	3.1 (±0.5)^{ab}	5.1 (±0.8)^{a}
10	7.4 (±0.5)^{a}	2.9 (±0.3)^{ab}	4.7 (±0.5)^{a}
15	7.3 (±0.4)^{a}	1.9 (±0.5)^{b}	4.2 (±0.5)^{a}

Table 4. Percentage reductions on bulb length, bulb diameter, and root length of transplanted onion as influenced by different population densities of *Portulaca oleracea*.

***Portulaca oleracea* (plants 176 cm^{-2})**	**Reductions (%)**		
	Bulb length plant^{-1} (cm)	**Bulb diameter plant^{-1} (cm)**	**Root length plant^{-1} (cm)**
5	3.4	21.8	6.7
10	8.1	28.8	14.0
15	9.4	51.8	23.1

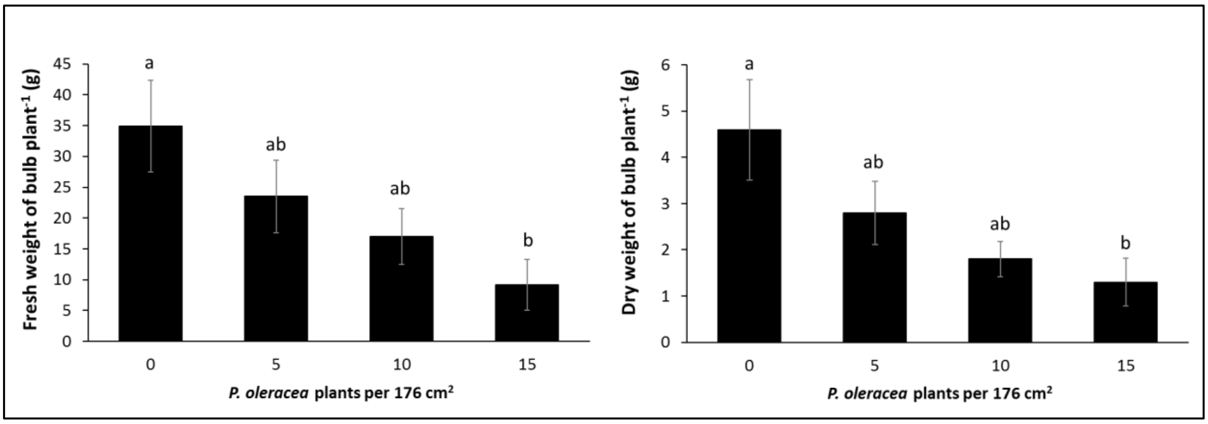

Figure 2. Mean (± se) weight (g) of fresh and dry bulb onion as influenced by different population densities of *Portulaca oleracea* (mean with the same letters are not significantly different at 5% level of significance.

Table 5. Percentage reductions on fresh and dry weights of bulb of transplanted onion as influenced by different population densities of *Portulaca oleracea*.

Portulaca oleracea **(plants 176 cm^{-2})**	**Reductions (%)**	
	Fresh weight of bulb plant^{-1} (g)	**Dry weight of bulb plant^{-1} (g)**
5	32.7	39.6
10	51.3	59.8
15	73.6	71.9

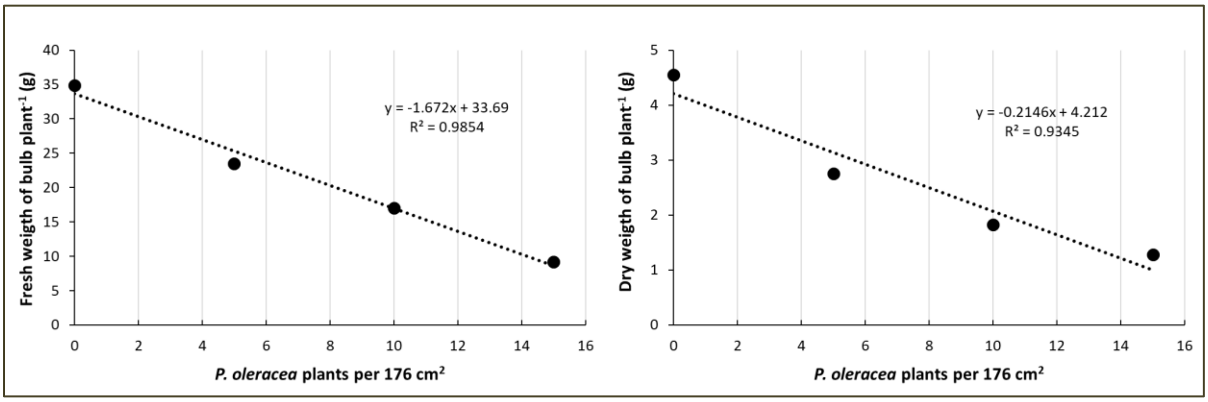

Figure 3. Correlation and simple linear regression between population density of *Portulaca oleracea* and the fresh and dry weights of transplanted onion.

DISCUSSION

Influence on Growth and Yield

Similar to other plants, the leaves are the primary organ of onion for processing its food by way of photosynthesis. It plays a significant role in relation to yield, particularly during the early bulb developmental stages of many onions. In a study by Siliquini et al. (2015), they reported that onions having 0% defoliation (complete leaf area) at the beginning and later part of the bulb formation had the highest bulb weights produced. But when the leaf areas of onions were intentionally defoliated by 40 and 60% at the beginning of bulb formation, the bulb weights were reduced by 29 and 43% while 4 and 21%, respectively, when defoliation was done at 30 days after the bulb formation. In this study, leaves of transplanted onion was highest when *P. oleracea* was absent. However, when the weed was present, the number, length, and weight of leaves were reduced. Thus, it is no wonder why the height and bulb weights of transplanted onion were severely reduced particularly at 15 plants 176 cm^{-2}. It only implicates that allowing *P. oleracea* to grow and compete until the maturity stage will negatively affect the growth and yield of transplanted onion.

Portulaca oleracea negatively affected the size and weight of bulb of transplanted onion. In fact, the regression analysis showed that its population density had strong and negative correlation to the fresh and dry weights of the crop suggesting that further competition of the weed at higher density and failure to control the weed throughout the crop's growing cycle will definitely result to the reduction on crop's yield. In the United States of America, Doohan and Felix (2012) also reported that *P. oleracea*, together with *Amaranthus blitoides* S. Wats., reduced the yield of the green onion by 22.2 and 32% in 2006 and 2007 field experiments, respectively. Adams (1977), on the other hand, had varying results in their field experiment involving different densities of *P. oleracea* under Michigan State University Muck Farm conditions. They reported that the presence of the weed at 58 plants m^{-2} from day 0 to 30 days after planting (DAP) did not reduce the yield of direct-seeded onion. From 0 to 40 DAP, however, the weed reduced the yield by 9%. At 153 plants m^{-2}, the presence of the weed from 0 to 20 DAP still did not affect the yield; instead, it reduced the yield by 57.6 and 68.3% when present from 0 to 30 and 0 to 40 DAP, respectively. At 463 m^{2}, the weed did not also significantly affect the yield when present from 0 to 10 DAP. It reduced the yield by 56, 71.2, and 83.5%, on the other hand, when present from 0 to 20, 0 to 30, and 0 to 40 DAP, respectively. When the weed was allowed to compete for the entire growing season, the yield of direct-seeded onion was reduced by 9.9-53% at all densities. Similar to *P. oleracea*, Morla et al. (2022) also reported that *Fimbristylis miliacea* (L.) Vahl had a negative influence on the growth and yield of transplanted and direct-seeded onions. From 5 densities, shoot biomass of *F. miliacea* increased by 1.3-2.7 folds in transplanted onion; 1.6-13 folds in direct-seeded onion as density increased by 15-25. Bulb weights of transplanted onion were reduced by

11, 17.4, 22.1, and 38.7%; direct-seeded onion by 86.4, 89.6, 88.8, and 88.1% at 10, 15, 20, and 25 *F. miliacea* densities, in that order. Bulb weights of direct-seeded onion suffered the greatest reductions due to weed competition.

Correlation and regression are statistical techniques that are commonly used to investigate the relationships between two variables (Bewick et al. 2003). Correlation quantifies the strength of the relationship between a pair of variables while the regression expresses the relationship or predicts the outcome of relationships by way of an equation. In this study, the population density of *P. oleracea* were strongly, negatively correlated to the fresh and dry bulb weights of transplanted onion. The simple linear regression analysis also showed that the population density of the weed was a significant predictor and contributor to both the fresh and dry bulb weights of the crop as had been shown in Figure 3. The correlation analysis indicated that the population density of *P. oleracea* was highly involved to the reductions on yield of transplanted onion. The regression analysis, on the other hand, suggest that the simple linear model can be used to predict the outcome of the competition between the population density of *P. oleracea* and the transplanted onion. In the study of Morla et al. (2022) on *F. miliacea* against onion, they also reported that the density and shoot biomass of the weed were strongly negatively correlated to the bulb weights of transplanted onion ($r = -0.987, -0.995$) and direct-seeded onion ($r = -0.986, -0.999$). They added that the regression analysis also showed that 97.49 and 99.95% of bulb weights of transplanted onion, and 97.25 and 99.95% of direct-seeded onion were attributed to the density and shoot biomass of *F. miliacea*. They then confirmed that *F. miliacea* is truly a weed of bulb onion and could reduce yield if left uncontrolled throughout the crop's growing season.

Knowledge of weed biology and ecology helps decide what, how, and when to implement control measures effectively. In this study, *P. oleracea* significantly reduced the growth and yield of transplanted onion implying that control must be executed whenever the weed grows and competes in the field. Although the findings were obtained only under screenhouse conditions, the results imply the need of developing a holistic weed control strategy against the weed. In managing weeds of onion, PhilRice (2007) recommends implementing thorough land preparation, rice straw mulching, rice hull burning, hand weeding, and herbicide application.

This study confirms that *P. oleracea* is a weed of transplanted onion and can potentially reduce the yield, especially if left unmanaged at population density of 5-15 plants 176 cm^{-2} from the time of planting until the maturity stage. Thus, it must be managed whenever observed infesting any field of transplanted onion. To find out more about its ecology and management, it is recommended that further *P. oleracea*-onion competition studies must be conducted, particularly under the field conditions; determine its critical period of control in both transplanted and direct-seeded onion; and evaluate different weed control strategies to develop a holistic management system that is effective, economical, and environment-friendly.

FUNDING

This study was personally funded by the first author.

ETHICAL CONSIDERATIONS

Since this study was conducted under greenhouse conditions, the experimental units of each treatment were specifically arranged in a randomized complete design to address the gradients of solar radiation coming from sunrise to sunset.

DECLARATION OF COMPETING INTEREST

The authors declare that there is no competing interests to any authors.

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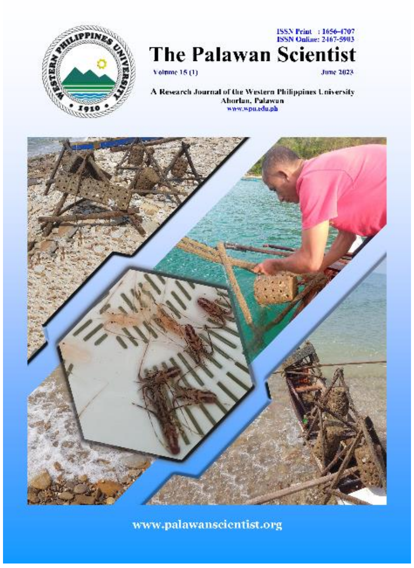
Original Article



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A checklist of damselfishes (Pomacentridae) from Palawan, Philippines

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ABSTRACT

This study was conducted to generate information on the number of damselfish species in Palawan, the most common tropical reef-associated fishes in the world. Photos of reef-associated fishes taken in conjunction with other reef surveys in 10 localities between 2019 and 2021 were used and evaluated for the presence of damselfishes. A total of 4,038 photos of reef-associated fishes were compiled and analyzed. Only 60 species of damselfishes were identified wherein Puerco Island in the municipality of Roxas had the highest number (14 genera and 32 species) while Hartman's Beach in Puerto Princesa City had the lowest (3 genera and 8 species). Three of the identified damselfishes were potentially new records in Palawan: *Amblypomacentrus clarus* Allen & Adrim, 2000, *Dischistodus darwiniensis* (Whitley, 1928); and *Pomacentrus aurifrons* Allen, 2004. The current data make a total of 137 damselfish species already documented in Palawan. Continued photo-video documentation is encouraged to increase understanding on the species richness of damselfishes and other reef-associated fauna.

Keywords: biodiversity, marine fishes, marine water, species checklist, species richness

INTRODUCTION

Damselfishes (family Pomacentridae) are one of the most diverse and widespread family of marine fishes found throughout the tropical oceans (Bellwood and Wainwright 2002; Allen et al. 2003). According to Parenti (2021), there are 423 valid species of damselfishes in the world and only 202 species are found in tropical Pacific (Allen et al. 2003).

Although the majority of damselfishes were categories as major fishes (English et al. 1997), they have varied ecological and economic importance such promoting the abundance of preferred algae for the settlement of depleted corals through a variety of 'farming' activities (Jones et al. 2006), which also serve as refuge for juvenile benthic and demersal plankton (Lobel 1980). Chase et al. (2020) reported that coral colonies with damselfishes accumulated much less sediment up to 10-fold with higher chlorophyll and protein concentrations compared to colonies without fishes. Some damselfishes (*Abudefduf sexfasciatus, A. vaigensis, A. zonatus, Dischistodus perspillatus* and *Hemiglyphidodon plagiometapon*) are also consumed as food (Gonzales

2013), while the colorful species are in high demand in the aquarium industry (Bruckner 2005; Muyot et al. 2019). The global aquarium fish trade consists of over 1,400 species of reef fishes that constitute over 50% of the global volume in which damselfish and anemonefish are included (Bruckner 2005).

The fish surveys and documentations of damselfishes resulted to the increasing number of species and expanding distribution range. For example, Allen and Wright (2003) reported a new species *Pomacentrus rodriguesensis* from Rodrigues Island, Indian Ocean in 2003. Pyle et al. (2008) described five new species (*Chromis abyssus*, *C. circumaurea*, *C. degruyi*, *C. brevirostris* and *C. earina*) of damselfish from deep coral reefs in the tropical Western Pacific in 2008. In the same year, Allen et al. (2008) also described a new species *(Amphiprion barberi)* of anemonefish fish, from coral reefs of Fiji, Tonga, and Samoa. The following year, two new pomacentrids (*Chromis albicauda* and *Chromis unipa*) from Indonesian seas were described by Allen and Erdmann (2009). In 2010, another species (*Amphiprion pacificus*) was discovered from Wallis Island and Tonga in the Western Pacific and in the reefs of Fiji and Samoa (Allen et al. 2010). In the Philippines, several species have also been described. For example, *Pomacentrus cheraphilus* was described based on 19 specimens collected at Brunei and northern Palawan, Philippines (Allen et al. 2011). A shallow inhabiting species *(Altrichthys alelia)* was also described from specimens collected off Busuanga Island, Palawan Province, Philippines (Bernardi et al. 2017), while Arango et al. (2019) described three new species of Chromis (*Chromis gunting*, *C. hangganan*, and *C. bowesi*) from mesophotic coral ecosystems of Batangas, Lubang, Puerto Galera, and Verde Island.

The expanded distribution range of a few damselfish species were also reported thus increasing the number of species listed for a particular locality. For example, *Pomacentrus caeruleopunctatus*, previously restricted to the Seychelles Islands, Madagascar, and Tanzania, to the Mascarene Archipelago has been recorded from Reunion Island (Boujorn et al. 2019). Four new records of damselfish species have also been reported for the first time in the reefs of Saint Martin's Island in the northern Bay of Bengal, Bangladesh (Islam and Habib 2020).

In the province of Palawan, Philippines, the information about damselfishes mostly form part of reef fish assessment studies (Gonzales 2013; Balisco and Dolorosa 2019), however, there is no information as to the total number of species found in the province. This study is the first to provide a list of damselfish species known to occur in the reefs of Palawan, Philippines.

METHODS

Study Sites and Photo-documentation

The day scuba diving activities were conducted in shallow reef areas (2-10 m deep) in the municipalities of El Nido, Taytay, Roxas, Narra, San Vicente, Araceli, and in Puerto Princesa City (PPC) between 2019 and 2021 (Figure 1; Table 1). Opportunistic photo-documentation of reef-associated fishes were carried out during fish visual census and in conjunction with other scuba diving activities such as in-situ induced spawning and restocking of giant clams. A total of 4,038 photos of reef-associated fishes were compiled and evaluated for the presence of damselfishes.

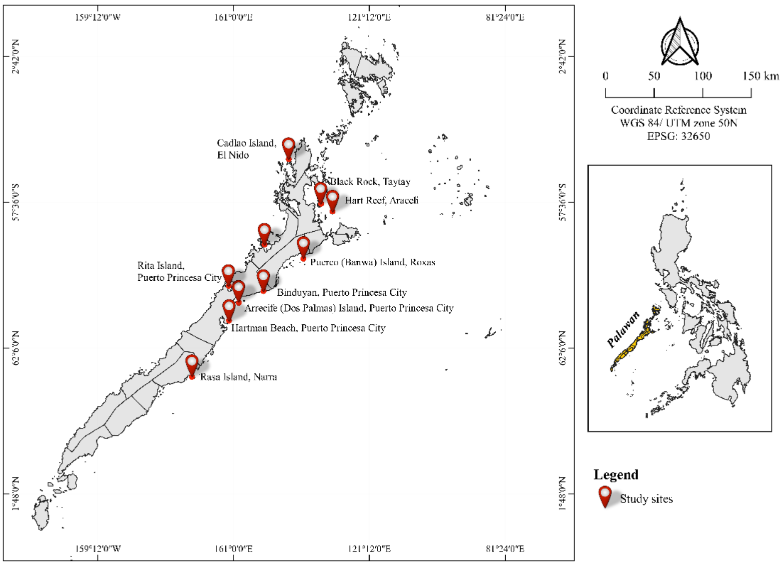

Figure 1. Map of Palawan showing the ten study sites.

Table 1. Number of dives, number of divers, and dive duration spent at each study site for the documentation of reef-associated fishes.

Date of Survey	Sites	Number of dives	Number of divers	Diving duration (h)	Total dive duration (h)
05 October 2020	Cadlao Island, El Nido	1	2	2	13
09 December 2020		1	3	3	
10 May 2019	Hart Reef, Araceli	1	2	2	4
10 April 2019	Black Rock, Taytay	1	2	2	12
11 May 2019		1	2	2	
12 May 2019		1	2	2	
30 November 2018	Puerco Island, Roxas	1	3	3	90
24 August 2019		2	3	6	
25 August 2019		2	3	6	
26 August 2019		1	3	3	
26 April 2019	Port Barton, San Vicente	2	3	6	72
27 April 2019		2	3	6	
25 September 2019	Binduyan, Puerto Princesa City	2	4	8	130
25 November 2019		1	4	4	
12 December 2020		1	5	5	
14 December 2020		1	5	5	
23 September 2020		1	4	4	
04 May 2019	Rita Island, Puerto Princesa City	1	3	3	31
09 October 2019		1	3	3	
12 November 2019		1	3	3	
15 August 2021		1	2	2	
17 December 2020	Arrecife Island, Puerto Princesa City	1	2	2	4
17 November 2019	Hartman Beach, Puerto Princesa City	1	2	2	8
23 November 2019		1	2	2	
13 January 2018	Rasa Island, Narra	2	2	4	52
02 May 2019		2	3	6	
TOTAL	**10**	**33**	**74**	**96**	**416**

Identification

The work of Allen et al. (2003) was used in identifying the species. Unidentified photo of damselfishes was posted on a Facebook page ID please (Marine Creature Identification) (https://www.facebook.com/groups/396180553763159) for initial identification and was validated using the reef fishes field guide Allen et al. (2003). Taxonomic classification of all identified damselfishes was updated base from World Register of Marine Species (WORMS; http://www.marinespecies.org) and Fish Base Worlds Wide Web electronic publication (http://fishbase.org). An offline database serves as current repository of the photographs.

RESULTS

Among the 10 sites, Puerco Island had the highest number of species (32) belonging to 14 genera. This was followed by Binduyan, PPC with 30 species belonging to 9 genera, while the Hartman Beach in PPC had the lowest species (8) belonging to 3 genera recorded (Figure 2; Table 2).

In total, 60 species of damselfishes were recorded (Table 2), three of which were potential new record in Palawan: A*mblypomacentrus clarus* Allen & Adrim, 2000 cf.; *Dischistodus darwinensis* (Whitley, 1928) cf.; and *Pomacentrus aurifrons* Allen, 2004, cf. (Figure 3; Table 3).

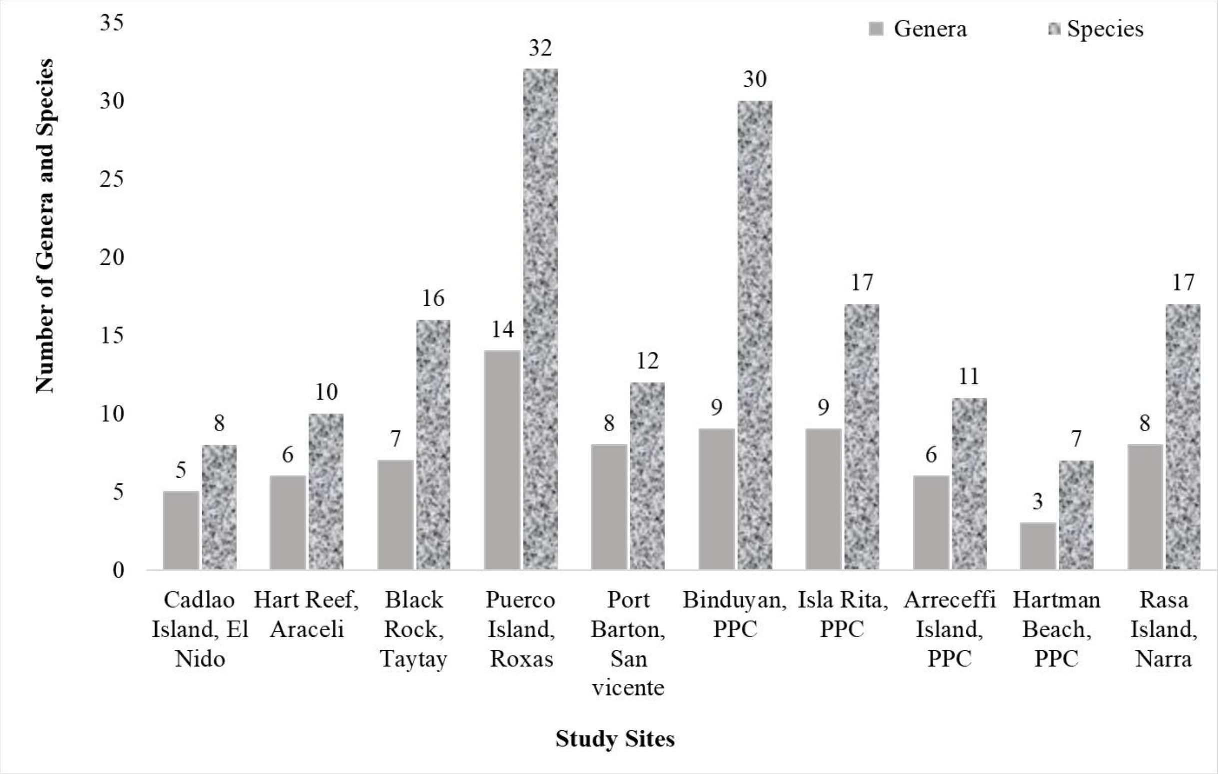

Figure 2. Number of genera and species of damselfishes been recorded in Palawan.

Table 2. Damselfish species encountered during the survey in various localities in Palawan. Note: (✓) Indicates the present species in the area. CIEN – Cadlao Island, El Nido; HRA – Harts Reef, Araceli; BRT – Black Rock, Taytay; PIR – Puerco Island, Roxas; PBSV – Port Barton, San Vicente; BPPC – Binduyan, Puerto Princesa City; RIPPC – Rita Island, Puerto Princesa City; AIPPC – Arrecife Island, Puerto Princesa City; HBPPC – Hartman Beach, Puerto Princesa City; RIN – Rasa Island, Narra.

No.	Scientific Name	CIEN	HRA	BRT	PIR	PBSV	BPPC	RIPPC	AIPPC	HBPPC	RIN
1	*Abudefduf bengalensis* (Bloch, 1787)							✓			
2	*Abudefduf lorenzi* Hensley & Allen, 1977							✓			
3	*Abudefduf sexfasciatus* (Lacepède, 1801)	✓	✓		✓	✓		✓	✓		
4	*Abudefduf vaigiensis* (Quoy & Gaimard, 1825)				✓		✓			✓	✓
5	*Acanthochromis polyacanthus* (Bleeker, 1855)			✓	✓			✓			✓
6	*Amblyglyphidodon aureus* (Cuvier, 1830)					✓		✓			✓
7	*Amblyglyphidodon batunai* Allen, 1995				✓			✓			
8	*Amblyglyphidodon curacao* Bloch, 1787)	✓		✓	✓	✓	✓	✓	✓		✓
9	*Amblyglyphidodon leucogaster* (Bleeker, 1847)						✓				
10	*Amblypomacentrus clarus* Allen & Adrim, 2000				✓						
11	*Amphiprion clarkii* (Bennett, 1830)					✓	✓	✓			✓
12	*Amphiprion frenatus* Brevoort, 1856		✓				✓	✓			
13	*Amphiprion ocellaris* Cuvier, 1830						✓	✓	✓		
14	*Amphiprion perideraion* Bleeker, 1855						✓				

No.	Scientific Name	CIEN	HRA	BRT	PIR	PBSV	BPPC	RIPPC	AIPPC	HBPPC	RIN
15	*Amphiprion polymnus* (Linnaeus, 1758)						✓				
16	*Amphiprion sandaracinos* Allen, 1972				✓	✓					✓
17	*Chromis atripectoriales* Welander & Schultz, 1951	✓									
18	*Chromis retrofasciata* Weber, 1913						✓				
19	*Chromis ternatensis* (Bleeker, 1856)						✓				✓
20	*Chromis viridis* (Cuvier, 1830)		✓		✓		✓				✓
21	*Chromis weberi* Fowler & Bean, 1928				✓		✓				
22	*Chromis xanthura* (Bleeker, 1854)						✓				
23	*Chrysiptera parasema* (Bleeker, 1877)				✓	✓	✓	✓			
24	*Chrysiptera oxycephala* (Fowler, 1918)							✓			
25	*Chrysiptera rex* (Snyder, 1909)			✓							
26	*Chrysiptera rollandi* (Whitley, 1961)						✓				
27	*Dascyllus auranus* (Linnaeus, 1758)	✓					✓				
28	*Dascyllus melanurus* Bleeker, 1854				✓					✓	
29	*Dascyllus reticulatus* (Richardson, 1846)	✓		✓	✓		✓		✓	✓	✓
30	*Dascyllus trimaculatus* (Rüppell, 1829)	✓	✓		✓		✓		✓		✓
31	*Dischistodus chrysopoecilus* (Schlegel & Müller, 1839)				✓					✓	
32	*Dischistodus darwiniensis* (Whitley, 1928)				✓						
33	*Dischistodus perspicillatus* (Cuvier, 1830)				✓			✓			
34	*Dischistodus prosopotaena* (Bleeker, 1852)				✓			✓			
35	*Hemiglyphidodon plagiometapon* (Bleeker, 1852)				✓			✓			
36	*Neoglyphidodon melas* (Cuvier, 1830)			✓	✓	✓	✓				
37	*Neoglyphidodon nigroris* (Cuvier, 1830)		✓	✓	✓	✓					
38	*Neoglyphidodon oxyodon* (Bleeker, 1858)				✓						
39	*Neopomacentrus filamentosus* (MacLeay, 1882)				✓	✓					
40	*Plectroglyphidodon lacrymatus* (Quoy & Gaimard, 1825)			✓	✓		✓		✓		✓
41	*Pomacentrus adelus* Allen, 1991										
42	*Pomacentrus alexanderae* Evermann & Seale, 1907			✓	✓	✓	✓	✓	✓		✓
43	*Pomacentrus amboinensis* Bleeker, 1868						✓				
44	*Pomacentrus armillatus* Allen, 1993			✓	✓		✓		✓		
45	*Pomacentrus aurifrons* Allen, 2004			✓	✓						
46	*Pomacentrus auriventris* Allen, 1991								✓		
47	*Pomacentrus bankanensis* Bleeker, 1854		✓		✓						
48	*Pomacentrus brachialis* Cuvier, 1830		✓				✓				
49	*Pomacentrus burroughi* Fowler, 1918										

No.	Scientific Name	CIEN	HRA	BRT	PIR	PBSV	BPPC	RIPPC	AIPPC	HBPPC	RIN
50	*Pomacentrus chrysurus* Cuvier, 1830									✓	
51	*Pomacentrus coelestis* Jordan & Starks, 1901	✓		✓	✓		✓		✓	✓	✓
52	*Pomacentrus grammorhynchus* Fowler, 1918				✓				✓		
53	*Pomacentrus geminospilus* Allen, 1993						✓				
54	*Pomacentrus lepidogenys* Fowler & Bean, 1928		✓	✓			✓				✓
55	*Pomacentrus moluccensis* Bleeker, 1853	✓	✓	✓	✓	✓	✓			✓	✓
56	*Pomacentrus philippinus* Evermann & Seale, 1907			✓			✓				✓
57	*Pomacentrus stigma* Fowler & Bean, 1928		✓	✓	✓						✓
58	*Pomacentrus tripunctatus* Cuvier, 1830									✓	
59	*Pomacentrus vaiuli* Jordan & Seale, 1906						✓				
60	*Premnas biaculeatus* (Bloch, 1790)				✓	✓		✓			
Total		**8**	**10**	**16**	**32**	**12**	**30**	**17**	**11**	**8**	**17**

Figure 3. Underwater photographs of three potential new records of damselfishes in Palawan. A) *Amblypomacentrus clarus* Allen & Adrim, 2000; B) *Dischistodus darwiniensis* (Whitley, 1928); and C) *Pomacentrus aurifrons* Allen, 2004.

Table 3. Distribution of damselfish species in the Philippines and in this study. (*) asterisk as indicated in numbers 20, 111, and 150 were the new records for Palawan.

No.	Scientific Name	Tropical Pacific (Allen et al. 2003)	Northern Palawan (Allen et al. 2011)	Taytay, Palawan (Gonzales et al. 2014)	EL Nido, Palawan (Allen et al. 2015)	Busuanga Island, Palawan (Bernardi et al. 2017)	Western Sulu Sea (Balisco and Dolorosa 2019)	Tubbataha Reefs Natural Park (Unpublished data)	This Study
1	*Abudefduf bengalensis* (Bloch, 1787)	✓					✓		✓
2	*Abudefduf lorenzi* Hensley & Allen, 1977	✓					✓		✓
3	*Abudefduf notatus* (Day, 1870)	✓							
4	*Abudefduf septemfasciatus* (Cuvier, 1830)	✓					✓		
5	*Abudefduf sexfasciatus* (Lacepède, 1801)	✓					✓	✓	✓
6	*Abudefduf sordidus* (Forsskål, 1775)	✓					✓		
7	*Abudefduf vaigiensis* (Quoy & Gaimard, 1825)	✓					✓	✓	✓

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8	*Abudefduf whitleyi* Allen & Robertson, 1974	✓							
9	*Acanthochromis polyacanthus* (Bleeker, 1855)	✓		✓			✓	✓	✓
10	*Altrichthys curatus* Allen, 1999	✓							
11	*Altrichthys alelia* Bernardi, Longo & Quiros, 2017					✓			
12	*Altrichthys azurelineatus* (Fowler & Bean, 1928)	✓							
13	*Amblyglyphidodon aureus* (Cuvier, 1830)	✓		✓			✓	✓	✓
14	*Amblyglyphidodon batunai* Allen, 1995	✓					✓		✓
15	*Amblyglyphidodon curacao* (Bloch, 1787)	✓		✓			✓	✓	✓
16	*Amblyglyphidodon leucogaster* (Bleeker, 1847)	✓		✓			✓	✓	✓
17	*Amblyglyphidodon orbicularis* (Hombron & Jacquinot, 1853)	✓							
18	*Amblyglyphidodon ternatensis* (Bleeker, 1853)	✓					✓		
19	*Amblypomacentrus breviceps* (Schlegel & Müller, 1839)	✓					✓	✓	
20	***Amblypomacentrus clarus* Allen & Adrim, 2000	✓							✓
21	*Amphiprion akallopisos* Bleeker, 1853	✓							
22	*Amphiprion akindynos* Allen, 1972	✓						✓	
23	*Amphiprion barberi* Allen, Drew & Kaufman, 2008	✓							
24	*Amphiprion Chrysopterus* Cuvier, 1830	✓						✓	
25	*Amphiprion clarkii* (Bennett, 1830)	✓		✓			✓	✓	✓
26	*Amphiprion ephippium* (Bloch, 1790)	✓							
27	*Amphiprion frenatus* Brevoort, 1856	✓					✓	✓	✓
28	*Amphiprion latezonatus* Waite, 1900	✓							
29	*Amphiprion leucokranos* Allen, 1973	✓							
30	*Amphiprion mccullochi* Whitley, 1929	✓							
31	*Amphiprion melanopus* Bleeker, 1852	✓					✓	✓	
32	*Amphiprion ocellaris* Cuvier, 1830	✓					✓	✓	✓
33	*Amphiprion pacificus* Allen, Drew & Fenner, 2010	✓							
34	*Amphiprion percula* (Lacepède, 1802)	✓							
35	*Amphiprion perideraion* Bleeker, 1855	✓					✓	✓	✓
36	*Amphiprion polymnus* (Linnaeus, 1758)	✓					✓		✓

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37	*Amphiprion rubrocinctus* Richardson, 1842	✓							
38	*Amphiprion sandaracinos* Allen, 1972	✓					✓		✓
39	*Amphiprion sebae* Bleeker, 1853	✓						✓	
40	*Cheiloprion labiatus* (Day, 1877)	✓					✓		
41	*Chromis acares* Randall & Swerdloff, 1973	✓						✓	
42	*Chromis agilis* Smith, 1960	✓						✓	
43	*Chromis albicanda*	✓							
44	*Chromis albomaculata* Kamohara, 1960	✓							
45	*Chromis alleni* Randall, Ida & Moyer, 1981	✓							
46	*Chromis alpha* Randall, 1988	✓							
47	*Chromis amboinensis* (Bleeker, 1871)	✓					✓	✓	
48	*Chromis analis* (Cuvier, 1830)	✓					✓	✓	
49	*Chromis atripectoralis* Welander & Schultz, 1951	✓					✓	✓	✓
50	*Chromis atripes* Fowler & Bean, 1928	✓					✓	✓	
51	*Chromis caudalis* Randall, 1988	✓					✓	✓	
52	*Chromis chrysura* (Bliss, 1883)	✓						✓	
53	*Chromis cinerascens* (Cuvier, 1830)	✓							
54	*Chromis delta* Randall, 1988	✓						✓	
55	*Chromis dimidiate* (Klunzinger, 1871)	✓							
56	*Chromis elerae* Fowler & Bean, 1928	✓					✓	✓	
57	*Chromis flavipectoralis* Randall, 1988	✓						✓	
58	*Chromis flavomaculata* Kamohara, 1960	✓					✓		
59	*Chromis fumea* (Tanaka, 1917)	✓						✓	
60	*Chromis iomelas* Jordan & Seale, 1906	✓						✓	
61	*Chromis lepidolepis* Bleeker, 1877	✓					✓	✓	
62	*Chromis leucura* Gilbert, 1905	✓						✓	
63	*Chromis lineata* Fowler & Bean, 1928	✓						✓	
64	*Chromis margaritifer* Fowler, 1946	✓					✓	✓	
65	*Chromis nitida* (Whitley, 1928)	✓						✓	

No.	**Scientific Name**	**Tropical Pacific (Allen et al. 2003)**	**Northern Palawan (Allen et al. 2011)**	**Taytay, Palawan (Gonzales et al. 2014)**	**EL Nido, Palawan (Allen et al. 2015)**	**Busuanga Island, Palawan (Bernardi et al. 2017)**	**Western Sulu Sea (Balisco and Dolorosa 2019)**	**Tubbataha Reefs Natural Park (Unpublished data)**	**This Study**
66	*Chromis notata* (Temminck & Schlegel, 1843)	✓						✓	
67	*Chromis opercularis* (Günther, 1867)	✓					✓	✓	
68	*Chromis ovatiformes* Fowler, 1946	✓						✓	
69	*Chromis retrofasciata* Weber, 1913	✓					✓	✓	✓
70	*Chromis scotochiloptera* Fowler, 1918	✓						✓	
71	*Chromis ternatensis* (Bleeker, 1856)	✓					✓	✓	✓
72	*Chromis vanderbilti* (Fowler, 1941)	✓						✓	
73	*Chromis viridis* (Cuvier, 1830)	✓		✓			✓	✓	✓
74	*Chromis weberi* Fowler & Bean, 1928	✓					✓	✓	✓
75	*Chromis westaustralis* Allen, 1976	✓							
76	*Chromis xanthochira* (Bleeker, 1851)	✓					✓	✓	
77	*Chromis xanthura* (Bleeker, 1854)	✓					✓	✓	✓
78	*Chrysiptera arnazae* Allen, Erdmann & Barber, 2010	✓							
79	*Chrysiptera biocellata* (Quoy & Gaimard, 1825)	✓					✓	✓	
80	*Chrysiptera bleekeri* (Fowler & Bean, 1928)	✓							
81	*Chrysiptera brownriggii* (Bennett, 1828)	✓					✓		
82	*Chrysiptera caeruleolineata (*Allen, 1973)	✓							
83	*Chrysiptera chrysocephala* Manica, Pilcher & Oakley, 2002				✓				
84	*Chrysiptera cyanea* (Quoy & Gaimard, 1825)	✓		✓			✓	✓	
85	*Chrysiptera cymatilis* Allen, 1999	✓							
86	*Chrysiptera flavipinnis (*Allen & Robertson, 1974)	✓							
87	*Chrysiptera glauca* (Cuvier, 1830)	✓						✓	
88	*Chrysiptera hemicyanea* (Weber, 1913)	✓							
89	*Chrysiptera kuiteri* Allen & Rajasuriya, 1995	✓							
90	*Chrysiptera oxycephala* (Fowler, 1918)	✓					✓	✓	✓
91	*Chrysiptera parasema* (Bleeker, 1877)	✓		✓			✓	✓	✓
92	*Chrysiptera rex* (Snyder, 1909)	✓					✓	✓	✓
93	*Chrysiptera rollandi* (Whitley, 1961)	✓					✓	✓	✓
94	*Chrysiptera sinclairi* Allen, 1987	✓							

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95	*Chrysiptera* sp.	✓							
96	*Chrysiptera springeri* (Allen & Lubbock, 1976)	✓						✓	
97	*Chrysiptera starcki* (Allen, 1973)	✓							
98	*Chrysiptera talboti* (Allen, 1975)	✓						✓	
99	*Chrysiptera taupou* (Jordan & Seale, 1906)	✓							
100	*Chrysiptera traceyi* (Woods & Schultz, 1960)	✓						✓	
101	*Chrysiptera tricincta* (Allen & Randall, 1974)	✓		✓					
102	*Chrysiptera unimaculata* (Cuvier, 1830)	✓						✓	
103	*Dascyllus aruanus* (Linnaeus, 1758)	✓					✓	✓	✓
104	*Dascyllus auripinnis* Randall & Randall, 2001	✓							
105	*Dascyllus carneus* Fischer, 1885	✓						✓	
106	*Dascyllus flavicaudus* Randall & Allen, 1977	✓							
107	*Dascyllus melanurus* Bleeker, 1854	✓					✓		✓
108	*Dascyllus reticulatus* (Richardson, 1846)	✓		✓			✓	✓	✓
109	*Dascyllus trimaculatus* (Rüppell, 1829)	✓		✓			✓	✓	✓
110	*Dischistodus chrysopoecilus* (Schlegel & Müller, 1839)	✓						✓	✓
111	***Dischistodus darwiniensis* (Whitley, 1928)	✓							✓
112	*Dischistodus fasciatus* (Cuvier, 1830)	✓					✓		✓
113	*Dischistodus melanotus* (Bleeker, 1858)	✓		✓			✓	✓	
114	*Dischistodus perspicillatus* (Cuvier, 1830)	✓		✓			✓		✓
115	*Dischistodus pseudochrysopoecilus* (Allen & Robertson, 1974)	✓							
116	*Dischistodus prosopotaenia* (Bleeker, 1852)	✓					✓		✓
117	*Hemiglyphidodon plagiometopon* (Bleeker, 1852)	✓		✓			✓		✓
118	*Lepidozygus tapeinosoma* (Bleeker, 1856)	✓							
119	*Neoglyphidodon bonang* (Bleeker, 1852)	✓					✓		
120	*Neoglyphidodon carlsoni* (Allen, 1975)	✓							
121	*Neoglyphidodon crossi* Allen, 1991	✓					✓	✓	
122	*Neoglyphidodon melas* (Cuvier, 1830)	✓		✓			✓	✓	✓
123	*Neoglyphidodon mitratus* Allen & Erdmann, 2012	✓							

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124	*Neoglyphidodon nigroris* (Cuvier, 1830)	✓		✓			✓	✓	✓
125	*Neoglyphidodon oxyodon* (Bleeker, 1858)	✓					✓	✓	✓
126	*Neoglyphidodon polyacanthus* (Ogilby, 1889)	✓							
127	*Neoglyphidodon thoracotaeniatus* (Fowler & Bean, 1928)	✓					✓	✓	
128	*Neopomacentrus anabatoides* (Bleeker, 1847)							✓	
129	*Neopomacentrus aquadulcis* Jenkins & Allen, 2002	✓							
130	*Neopomacentrus azysron* (Bleeker, 1877)	✓						✓	
131	*Neopomacentrus bankiere* (Richardson, 1846)	✓							
132	*Neopomacentrus cyanomos* (Bleeker, 1856)	✓						✓	
133	*Neopomacentrus filamentosus* (MacLeay, 1882)	✓					✓	✓	✓
134	*Neopomacentrus nemurus* (Bleeker, 1857)	✓						✓	
135	*Neopomacentrus taeniurus* (Bleeker, 1856)	✓							
136	*Neopomacentrus violascens* (Bleeker, 1848)	✓						✓	
137	*Plectroglyphidodon dickii* (Liénard, 1839)	✓					✓	✓	
138	*Plectroglyphidodon imparipennis* (Vaillant & Sauvage, 1875)	✓							
139	*Plectroglyphidodon johnstonianus* Fowler & Ball, 1924	✓		✓				✓	
140	*Plectroglyphidodon lacrymatus* (Quoy & Gaimard, 1825)	✓		✓			✓	✓	✓
141	*Plectroglyphidodon leucozonus* (Bleeker, 1859)	✓						✓	
142	*Plectroglyphidodon phoenixensis* (Schultz, 1943)	✓						✓	
143	*Pomacentrus adelus* Allen, 1991	✓					✓		✓
144	*Pomacentrus albimaculus* Allen, 1975	✓							
145	*Pomacentrus alexanderae* Evermann & Seale, 1907	✓		✓			✓	✓	✓
146	*Pomacentrus alleni* Burgess, 1981	✓							
147	*Pomacentrus amboinensis* Bleeker, 1868	✓		✓			✓	✓	✓
148	*Pomacentrus armillatus* Allen, 1993	✓					✓	✓	✓
149	*Pomacentrus auriventris* Allen, 1991	✓						✓	✓
150	*****Pomacentrus aurifrons* Allen, 2004	✓							✓
151	*Pomacentrus australis* Allen & Robertson, 1974	✓							
152	*Pomacentrus azuremaculatus* Allen, 1991	✓							

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153	*Pomacentrus bankanensis* Bleeker, 1854	✓					✓	✓	✓
154	*Pomacentrus brachialis* Cuvier, 1830	✓					✓	✓	✓
155	*Pomacentrus burroughi* Fowler, 1918	✓					✓	✓	✓
156	*Pomacentrus caeruleus* Quoy & Gaimard, 1825							✓	
157	*Pomacentrus cheraphilus* Allen, Erdmann & Hilomen, 2011		✓						
158	*Pomacentrus chrysurus* Cuvier, 1830	✓		✓			✓	✓	✓
159	*Pomacentrus coelestis* Jordan & Starks, 1901	✓					✓	✓	✓
160	*Pomacentrus colini* Allen, 1991	✓							
161	*Pomacentrus cuneatus* Allen, 1991	✓							
162	*Pomacentrus geminospilus* Allen, 1993	✓					✓		✓
163	*Pomacentrus grammorhynchus* Fowler, 1918	✓						✓	✓
164	*Pomacentrus imitator* (Whitley, 1964)	✓							
165	*Pomacentrus indicus* Allen, 1991							✓	
166	*Pomacentrus javanicus* Allen, 1991	✓							
167	*Pomacentrus komodoensis* Allen, 1999	✓							
168	*Pomacentrus lepidogenys* Fowler & Bean, 1928	✓					✓	✓	✓
169	*Pomacentrus limosus* Allen, 1992	✓							
170	*Pomacentrus littoralis* Cuvier, 1830	✓					✓		
171	*Pomacentrus melanochir* Bleeker, 1877	✓							
172	*Pomacentrus microspilus* Allen & Randall, 2005	✓							
173	*Pomacentrus milleri* Taylor, 1964	✓							
174	*Pomacentrus moluccensis* Bleeker, 1853	✓		✓			✓	✓	✓
175	*Pomacentrus nagasakiensis* Tanaka, 1917	✓		✓				✓	
176	*Pomacentrus nigromanus* Weber, 1913							✓	
177	*Pomacentrus nigromarginatus* Allen, 1973	✓						✓	
178	*Pomacentrus opisthostigma* Fowler, 1918	✓					✓		
179	*Pomacentrus pavo* (Bloch, 1787)	✓							
180	*Pomacentrus philippinus* Evermann & Seale, 1907	✓					✓	✓	✓
181	*Pomacentrus polyspinus* Allen, 1991	✓							

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182	*Pomacentrus proteus* Allen, 1991	✓					✓		
183	*Pomacentrus reidi* Fowler & Bean, 1928	✓						✓	
184	*Pomacentrus saksonoi* Allen, 1995	✓							
185	*Pomacentrus similis* Allen, 1991	✓						✓	
186	*Pomacentrus simsiang* Bleeker, 1856	✓					✓	✓	
187	*Pomacentrus smithi* Fowler & Bean, 1928	✓					✓	✓	
188	*Pomacentrus* sp.	✓		✓					
189	*Pomacentrus spilotoceps* Randall, 2002	✓							
190	*Pomacentrus stigma* Fowler & Bean, 1928	✓					✓	✓	✓
191	*Pomacentrus taeniometopon* Bleeker, 1852	✓							
192	*Pomacentrus tripunctatus* Cuvier, 1830	✓					✓	✓	✓
193	*Pomacentrus vaiuli* Jordan & Seale, 1906	✓					✓	✓	
194	*Pomacentrus wardi* Whitley, 1927	✓						✓	
195	*Pomacentrus yoshii* Allen & Randall, 2004	✓							
196	*Pomachromis guamensis* Allen & Larson, 1975	✓							
197	*Pomachromis richardsoni* (Snyder, 1909)	✓		✓				✓	
198	*Premnas biaculeatus* (Bloch, 1790)	✓					✓		✓
199	*Stegastes albifasciatus* (Schlegel & Müller, 1839)	✓							
200	*Stegastes altus* (Okada & Ikeda, 1937)	✓						✓	
201	*Stegastes apicalis* (De Vis, 1885)	✓							
202	*Stegastes aureus* (Fowler, 1927)	✓					✓	✓	
203	*Stegastes fasciolatus* (Ogilby, 1889)	✓							
204	*Stegastes gascoynei* (Whitley, 1964)	✓							
205	*Stegastes lividus* (Forster, 1801)			✓			✓	✓	
206	*Stegastes nigricans* (Lacepède, 1802)	✓					✓		
207	*Stegastes obreptus* (Whitley, 1948)	✓							
208	*Stegastes punctatus* (Quoy & Gaimard, 1825)	✓							
	Total	**200**	**1**	**26**	**1**	**1**	**87**	**106**	**60**

DISCUSSION

The observed variation in species richness across study sites could have been influenced by the health of the reef, level of protection, and sampling effort. Coral-obligate damselfishes tend to occupy larger coral colonies rather than a smaller one (Nadler et al. 2014). There is also a direct relation between the density of chaetodontid fishes and the diversity of the coral community (Bouchon-Navaro and Bouchon 1989). While we have no record of coral diversity and sizes of colonies in Puerco Island, it is assumed that effective fishing closure favored uninterrupted growth especially of branching *Acropora*, the usual habitats of damselfishes. No take MPAs are known to promote higher coral cover, greater fish biomass (Strain et al. 2019) and stabilized species diversity (Pettersen et al. 2022). The station in Binduyan has the second highest number of damselfishes, is situated right in front of the WPU-Binduyan Marine Research Station, an open-access area for the locals engaging in artisanal fishing while also serving as aquaculture demonstration site for abalone. This is also the site with the highest dive effort and photo-video-documentation activities. Other sampling sites, although part of MPAs (e.g. Hart Reef, Black Rock and Rasa Island), remained susceptible to fishing due to inadequate patrol mechanisms. The use of explosives and drive nets can either cause a decline or increase in abundance of reef associated (Russ and Alcala 1989), while local fishing pressures and hard coral cover have direct influence on the abundance of different fish trophic levels (Elston et al. 2020). Other factors that affect species distribution and abundance include depths and wave exposure (Depczynski and Bellwood 2005; Medeiros et al. 2010; De Chaves et al. 2021).

The current number of damselfish species in Palawan (140 species) is higher than in other biogeographic regions in the country: Celebes Sea (83), Northern Philippine Sea (85), West Philippine Sea (79), Southern Philippine Sea (64), Sulu Sea (87) and Visayan Region (72) species, respectively (see Nañola et al. 2011). In addition, the current number in Palawan is higher than what has been reported (118 species) for the Philippines a few decades ago (see Sin et al. 1994).

Of the 60 species documented in Palawan, three are potential new records. A*mblypomacentrus clarus* are known to occur in the reefs of Cambodia, Indonesia and the Philippines at 15-25 m deep (Allen and Erdmann 2012), hence, its occurrence in Palawan is highly possible. The species occurred in intertidal sandy-rubble flat with patches of seagrass, suggesting an expanded depth range for the species. The second species, *D. darwininensis* are known to occur in silty inshore and coral reefs in northern Australia (Hoese et al. 2006). In Palawan, the species are found in a shallow sandy-rubble substrate with patches of seagrass. Its possible occurrence in the reefs of Palawan reflects a wide geographic range for the species. The third species, *P. aurifrons,* are common at 2-14 m deep coastal and offshore reefs in Western Central Pacific particularly in New Caledonia, Papua New Guinea, Solomon Islands and Vanuatu (Allen 2004). Considering the limited and blurry photos that we have for each species, there is a need for further documentation and capture of specimens to ascertain the identity of the species.

The continued discovery of new damselfish species (Pyle et al. 2008, Randall and DiBattista 2013, Habib et al. 2020, McFarland et al. 2020, Allen et al. 2022), and reports on expanded distribution range (Bourjon et al. 2019, Bennett et al. 2019, Islam and Habib 2020, Sen et al. 2021) suggest that more species remained to be documented. Continued photo-video documentation could help increase in understanding species diversity and discovery of new species.

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ETHICAL CONSIDERATIONS

No animals were captured in this study.

DECLARATION OF COMPETING INTEREST

The authors declare that there is no competing interests to any authors.

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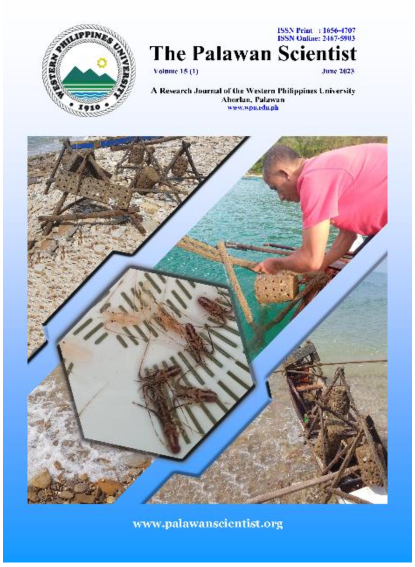
Original Article



Profitability of spiny lobster (*Panulirus* spp.) puerulus collection in Palawan, Philippines

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ABSTRACT

Understanding profitability is crucial when engaging in any fishery business venture. The collection of spiny lobster puerulus has recently caught the interest of many fisherfolks in the province of Palawan, but information regarding its profitability is wanting. This study determined the profitability of puerulus collection based on the information given by two collectors, one from barangay (Bgy.) Rizal, Roxas and and another one from Bgy. San Juan, Aborlan. Information such as the cost of materials, operating expenses, and the monthly catch were gathered with a guide questionnaire. The return on investment (ROI) were based on sales under the prevailing and regulated buying prices. Both collectors invested nearly PHP 100,000 in fabricating 1,000 – 2,000 units of cement discs and for operating the traps for either six or seven months. Under the prevailing and regulated buying prices, only the collector in Roxas had positive total net profits (PHP 13,490 and PHP 182,640) and ROI (15% and 198%), respectively. Local ordinances are needed to assist and protect the puerulus collectors from income loss caused by variable buying prices from traders. The collectors may explore using cheap and equally effective puerulus traps to reduce investment costs.

Keywords: analysis, bioeconomic, regulated buying price, monthly catch, unstable buying prices

INTRODUCTION

In most aquaculture enterprises, seedstocks mostly produced from hatchery propagation are those with shorter larval phases such as shrimps, finfishes, and mollusks (Barnard et al. 2011). In spiny lobster (*Panulirus* spp.), seedstocks solely come from the wild (Priyambodo and Jaya 2009; Jones 2010, 2018; Jones et al. 2019) since hatchery propagation of the species remains a big challenge (Barnard et al. 2011). Although production from laboratory tank experiments is already successful (Jones 2009), the high production cost for commercialization (Jones 2018) and the long larval phase, which involves 20 instars to reach the postlarval puerulus stage are the major constraints (Barnard et al. 2011; Jones 2018).

In Vietnam, where the first aquaculture of spiny lobster was developed in the 1970s, the seedstocks of *Panulirus ornatus* (Fabricius, 1798) and *Panulirus homarus* (Linnaeus, 1758) are still collected mainly within the coastal areas of the country (Jones 2010; Dao and Jones 2015). However, because of overexploitation due to the expanding lobster farms, the occurrence of diseases, the seasonality of wild seedstock settlement (Jones 2010, 2018; Priyambodo et al. 2020), and the shortage of wild seedstock have led to the importation from neighboring countries such as Indonesia and the Philippines (Macusi et al. 2019; Setyanto et al. 2019; NFRDI 2020; Priyambodo et al. 2020; Mecha et al. 2022). With the attractive price for puerulus, the collection of wild seeds has become popular and is considered a lucrative livelihood in various coastal areas in Indonesia and the Philippines (Petersen et al. 2013; Macusi et al. 2019; Mecha et al. 2022). A single puerulus in Indonesia can cost more than USD 1.00 (average USD 1.34; Petersen et al. 2013), while in Davao Oriental, Philippines, the price ranges from USD 1.81 to USD 4.54 (Macusi et al. 2019), and USD 1.44 to USD 3.60 in Palawan (Mecha et al. 2022).

Puerulus collection and grow-out aquaculture are lucrative industries in Indonesia with average returns to a collector of approximately IDR 51 million per year (USD 5,900 per year) and a return on investment of approximately 4.1:1 (Petersen et al. 2013). However, there are no known profitability studies that only focus on puerulus collection since it is mixed with the overall aquaculture fishery (Petersen and Phoung 2010; Petersen et al. 2013; Apriliani et al. 2021). In the Philippines, Macusi et al. (2019) reported that a puerulus collector in Davao Occidental, Philippines, could earn an average monthly income of USD 167-366 during peak season (March-May), but the capital and investment used by the fishers in fabricating and operating gears were not evaluated. Profitability refers to the ability of the business to make a profit which reflects the strength of the business performance (Zhang and Wen 2017). According to Jones (2010), a profitable economic basis is crucial for establishing the spiny lobster industry and for its expansion.

In Palawan, the spiny lobster puerulus collection started in 2019 (Mecha et al. 2022), and become popular in 2020 due to high buying price for white and black (pigmented) puerulus. However, data on its monthly collection in each identified municipality (see Mecha et al. 2022) remained unavailable and no profitability study had validated the certainty of puerulus collection as a lucrative source of livelihood. Hence, this study was conducted to determine the cost, revenue, and profit from puerulus collection, which could be used as a basis for crafting local policies toward a profitable puerulus fishery industry. Specifically, this study aimed to determine the following; cost and return analysis, monthly puerulus catch and prevailing buying prices, and the Return on Investment (ROI) using the prevailing and regulated buying prices.

METHODS

Study Sites

The study was conducted in conjunction with two different projects about livelihood and coastal management conducted in various barangays of two municipalities in northern and southern Palawan. Among the barangays, three localities were purposively selected namely Bgy. Rizal in the municipality of Roxas in northern Palawan and two barangays (Isaub and San Juan) of Aborlan in southern Palawan (Figure 1). These barangays were selected due to the existing puerulus collection in the area (Mecha et al. 2022).

Data Collection and Respondent

Key Informants (KIs) were among the participants who attended the seminars on livelihood opportunities on 10, 26, and 27 September 2021. A total of eight KIs (all were puerulus collectors; one in Bgy. Rizal, Roxas; three in Bgy. Isaub, and four in Bgy. San Juan, Aborlan) were personally interviewed with the aid of a guide questionnaire. Written consent approval was firstly secured before the interview. The eight KIs were purposively selected as they engage in puerulus collection. The questionnaire sought to obtain information about the methods used in puerulus collection and the expenses for the fabrication of traps, installation and operation. Information on monthly catch and buying prices of white and pigmented puerulus were also obtained. Of the eight KIs, only one collector from Bgy. Rizal, and one from Bgy. San Juan had provided complete information, particularly on monthly catch and buying prices. The information from these two KIs was used as the basis for estimating the profitability of the spiny lobster puerulus collection.

Cost and return analysis. All materials, quantities, prices, and operating costs were encoded and computed using MS Excel. The estimated material cost (EMC) was calculated based on the estimated number of materials (M) used by each KI collector multiplied by the selling prices (SP) of puerulus during the collection period in 2021 (Formula 1). The operating cost for trap fabrication, installation, harvesting, maintenance, and repair was also provided based on the number of persons involved, total hours of work rendered, and given wages per hour or day based on the PHP 300 minimum salary in the area. The depreciation cost (DC) was determined using the Straight-Line Method (Formula 2) by subtracting the EMC from the estimated salvage value (SV) of all materials used and dividing by its lifespan for six and seven months per year of collection in Bgy. Rizal and Bgy. San Juan, respectively. The total collection cost (TCC) was obtained by adding the total fixed-material investment cost (TFC) and total operating cost (TOC) (Formula 3).

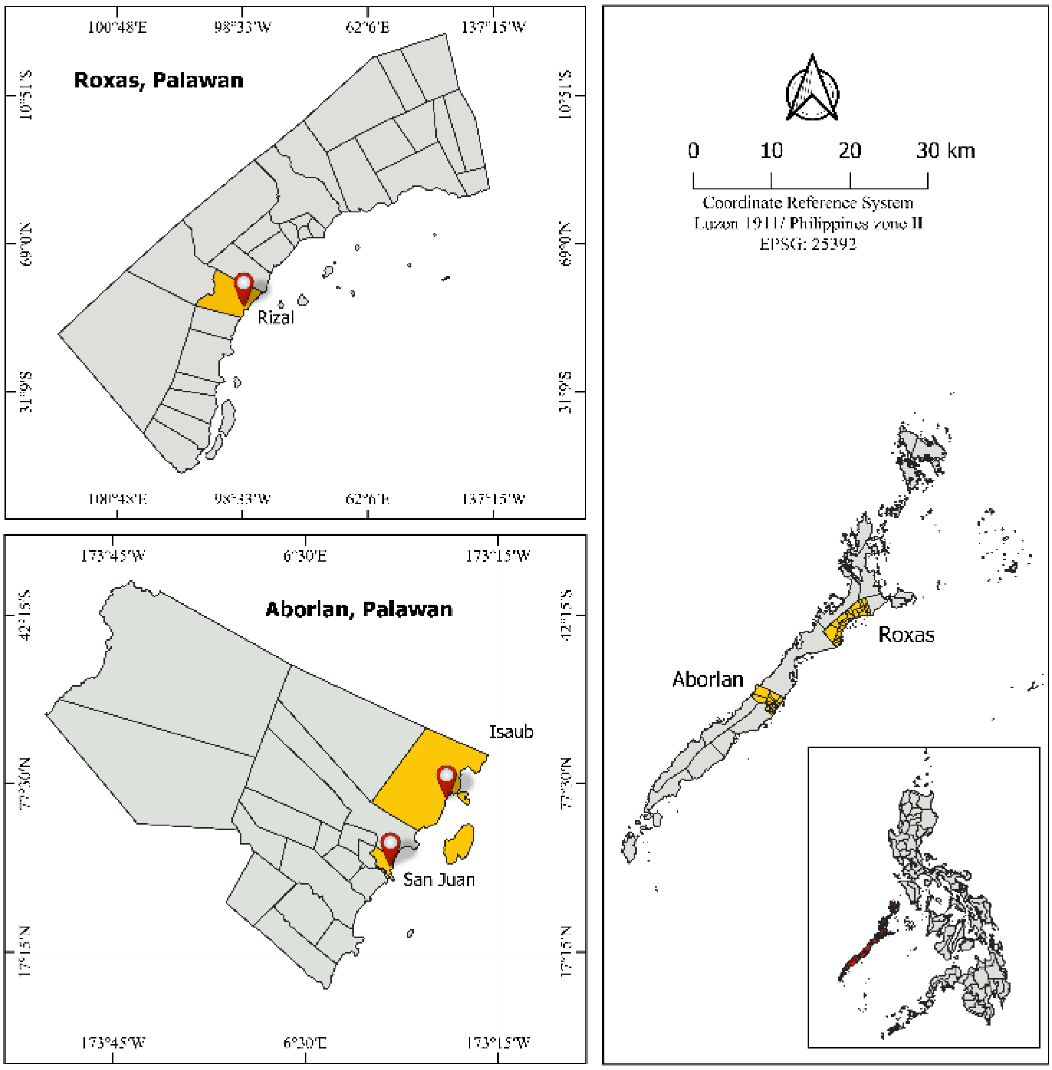

Figure 1. The three coastal barangays (Rizal, Isaub, and San Juan) in the municipalities of Roxas and Aborlan, Province of Palawan.

$$\text{Estimated Material Cost (EMC)} = M \times SP \qquad \text{Formula 1}$$

$$\text{Depreciation Cost (DC)} = \frac{(EMC - SV)}{\text{lifespan of materials}} \qquad \text{Formula 2}$$

$$\text{Total Collection Cost (TCC)} = TFC + TOC \qquad \text{Formula 3}$$

Monthly puerulus catch and buying (prevailing) prices. The number of puerulus caught per month per KI was plotted in a graph along with the number of traps used to determine the relation of monthly catch to the number of traps. Also, it was used to determine the abundance of puerulus settlement per locality. The prevailing buying prices per stage of puerulus were also plotted in a graph per locality.

Profitability. To determine the monthly revenue (MR) of the collectors, the buying prices (BP) (prevailing and regulated) were multiplied by the number of puerulus (NP) caught per month (Formula 4). The prevailing buying price was obtained from the prices of puerulus per category in 2021 as provided by the two KIs. The regulated buying price was based from the Provincial Ordinance No. 2475 series of 2020 and applied to both white and pigmented puerulus. These buying prices were used to determine if the collector per locality has a positive or negative ROI. Meanwhile, the monthly profit (MP) was obtained by subtracting the monthly collection cost (MCC; see Table 1) from the MR (Formula 5). Lastly, the ROI was computed based on the total net profit (TNP) for 6 or 7 months of collection season divided by the TCC × 100 (Formula 6).

$$\text{Monthly Revenue (MR)} = BP \times NP \qquad \text{Formula 4}$$

$$\text{Monthly Profit (MP)} = MR - MCC \qquad \text{Formula 5}$$

$$\text{Return on Investment} = (TNP/TCC) \times 100 \qquad \text{Formula 6}$$

Data Analysis and Interpretation

All data collected from two puerulus collectors were separately encoded and analyzed as they differ in terms of the number of traps, prices of materials, labor cost, monthly puerulus catch, and buying price.

RESULTS

Cost and Return Analysis

Although both KIs used the stake-hanging method, they differed in terms of the number of traps and other materials. The puerulus collector from Bgy. Rizal, Roxas used 14 sacks of cement and 1 m^3 (~40 sacks) of sand for the 1,000 concrete disc type traps (1 cement:3 sand) having 25 cm diameter with 5 cm thick dimension (one-side is perforated), and 100 bamboo poles for installation of traps (Table 1). Overall, the puerulus collector in Bgy. Rizal spent about PHP 92,360 with monthly fixed investment and operating costs of PHP 13,602 and PHP 8,410, respectively (Table 1).

Meanwhile, the puerulus collector from Bgy. San Juan, Aborlan utilized 31 sacks of cement and 217 sacks of sand for the 800-2,200 concrete disc-type traps (1 cement:7 sand) with larger dimension (15 cm diameter and 13 cm thick; both-sides are perforated) than the collector from Bgy. Rizal (Table 2). Overall, the puerulus collector from Bgy. San Juan spent about PHP 93,760 with monthly fixed investment and operating costs of PHP 11,519 and PHP 5,800, respectively (Table 2).

Table 1. Cost and return analysis of puerulus collection in Bgy. Rizal, Roxas, Palawan for the six months of collection season.

Particulars	Quantity	Unit price/ wage (PHP)	Lifespan/ Duration	Total amount (PHP)
Fixed Investment (Trap Fabrication (1,000 concrete disc type; 25 cm diameter with 5 cm thick) and Trap Installation (Stake-hanging method))				
Cement (one sack can produce 71 concrete discs)	14 sacks	300	6 months for 2 years (6 months/ year)	4,200
Sand (1 m^3) ~40 sacks (3 sacks per sack of cement)	1 m^3	1,700		1,700
Bamboo (“Tiring” type)	100 poles	120	6 months	12,000
Rope #6 or 3-mm diameter	40 rolls	350	6 months for 2 years (6 months/ year)	14,000
Trap fabrication (@PHP 350 per day for 5 days per collection season)	2 persons	350	5 days	3,500
Trap installation (@PHP 350 per day for 5 days per collection season)	2 persons	350	5 days	3,500
Miscellaneous (snacks, transport fare, others)	2 persons	300	5 days	3,000
Total investment costs				**41,900**
Monthly Investment Cost (Depreciation of materials + 6 months dividend from the trap fabrication and installation and miscellaneous)				**5,192**
Depreciation cost (Total cost of product-salvage value for 6 months of collection season for 2 years)				**3,525**
Operating Cost				
Harvest (6 hours per day @ PHP 58 per hour for 10 days per month per season)	2 persons	3,480	6 months	41,760
Maintenance and repair (@PHP 350 for 1-day cleaning per month for 6 months)	3 persons	350	6 months	6,300
Miscellaneous (snacks, transport fare, others)	2 persons	200	6 days	2,400
Total operating cost				**50,460**
Monthly operating costs (Total operating cost for 6 months of collection)				**8,410**
Monthly collection costs (Monthly investment cost + Monthly operating cost)				**13,602**
Total collection cost (Investment cost + Operating cost)				**92,360**

Table 2. Cost and return analysis of puerulus collection in Bgy. San Juan, Aborlan, Palawan for the seven months of collection season.

Particulars	Quantity	Unit price/ wage (PHP)	Lifespan/ Duration	Total (PHP)
Investment Cost (Trap Fabrication (800-2200 concrete disc type; 15 cm diameter with 13 cm thick) and Trap Installation (Stake-hanging method))				
Cement (one sack can produce 70 concrete discs)	31 sacks	200	7 months for 2 years (7 months/ year)	6,200
Sand (7 sacks per sack of cement)	217 sacks	20		4,340
Bamboo ("Bayog" type)	160 poles	30		4,800
Rope #12 or 6-mm diameter (for installation of bamboo posts)	7 rolls	240		1,680
Rope #12 or 6-mm diameter (1 roll for 70-disc traps)	31 rolls	240		7,440
Trap fabrication (70-disc per day @PHP 350 days for 31 days)	2 persons	350	31 days	21,700
Trap installation (@PHP 350 days for 5 days)	4 persons	350	5 days	7,000
Total investment cost				**53,160**
Monthly Investment Cost (Depreciation of materials + 7 months dividend from the trap fabrication and installation and miscellaneous)				**5,719**
Depreciation cost (Total cost of product-salvage value for 6 months of collection season for 2 years)				**1,619**
Operating Cost				
Harvest (3 hours per day @PHP 50 per hour for 10 days per month)	2 persons	1,500	7 months	21,000
Maintenance and repair (@PHP 350 per day twice a month for 7 months)	4 persons	350	14 days	19,600
Total operating cost				**40,600**
Monthly operating cost (Total operating cost for 7 months of collection season per year)				**5,800**
Monthly collection costs (Monthly investment cost + Monthly operating cost)				**11,519**
Total collection cost (Investment cost + Operating cost)				**93,760**

Monthly Puerulus Catch and Buying Prices

Data showed that puerulus collection in two localities has different starting periods of settlement . Collection in Bgy. Rizal started in March while in Bgy. San Juan was in February with peak of the collection in May and March, respectively (Figure 2). The puerulus collector from Bgy. Rizal, Roxashad a higher monthly catch ranging from 200-600 individuals (ind.) using the 1,000 concrete disc traps (Figure 2) compared to the puerulus collector from Bgy. San Juan, Aborlan, that used a total of 800-2,200 concrete disc traps which only caught 30-200 ind. puerulus per month (Figure 2).

During the beginning of collection season in each locality, the white and pigmented puerulus had a higher prevailing buying price ranging from PHP 110 (USD 1.98) and PHP 70 (USD 1.26) in Bgy. Rizal to PHP 130 (USD 2.33) and PHP 80 (USD 1.44) in San Juan (Figure 3). During the lean season, the prevailing buying prices for white and pigmented puerulus dropped to PHP 35 (USD 0.63) and PHP 15 (USD 0.27) in both areas, respectively (Figure 3).

Profitability

The buying system of puerulus in two localities involved the separation of white from pigmented puerulus since these two have different buying prices. Forty percent (40%) of catch were considered white, while 60% were pigmented. In addition, the estimated total revenue and net profit of puerulus collectors from Bgy. Rizal, Roxas, for six months of collection season were around PHP 105,850 and PHP 275,000, and PHP 13,490 and PHP 182,640 using the prevailing and regulated buying prices, respectively (Table 3). The puerulus collector in Bgy. Rizal earned 15% ROI using the prevailing buying prices, while 198% for the regulated buying price (Table 3).

Meanwhile, the puerulus collector from Bgy. San Juan, Aborlan had estimated a total revenue of PHP 18,920 and PHP 59,000 for prevailing and regulated buying prices, respectively (Table 4). However, the collector had a negative net profit and ROI (Table 4) during the seven months of collection season.

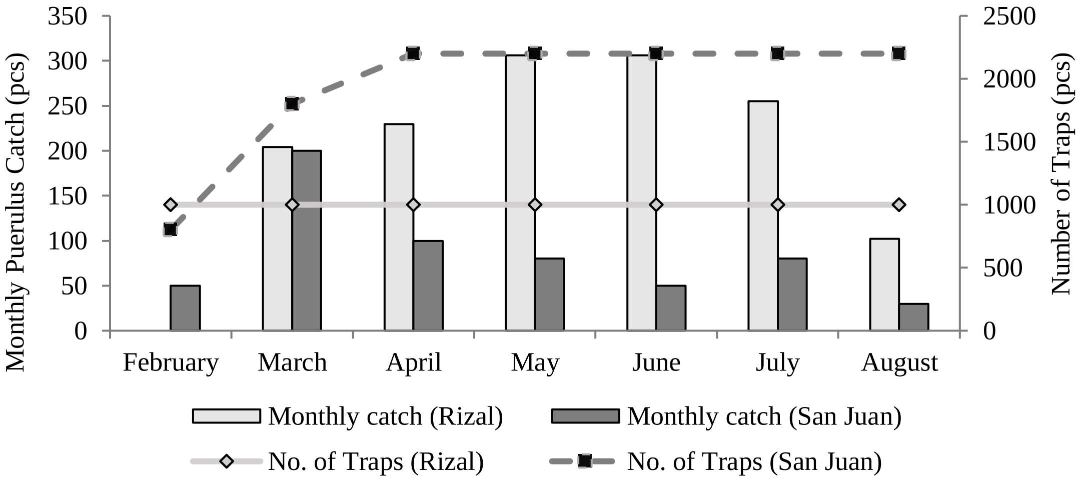

Figure 2. Monthly puerulus catch and the number of traps used by the collector from Bgy. Rizal, Roxas, and Bgy. San Juan, Aborlan, Palawan.

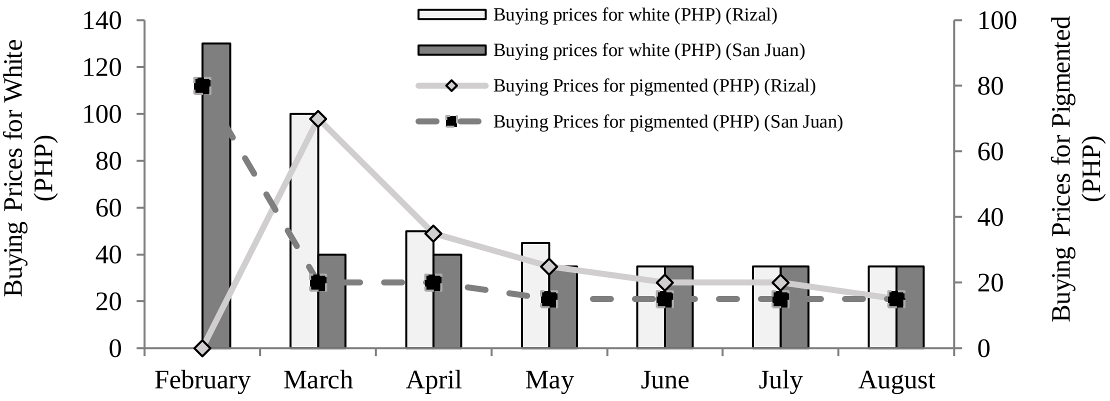

Figure 3. Monthly prevailing buying prices of puerulus (white and pigmented) in Bgy. Rizal, Roxas, and Bgy. San Juan, Aborlan, Palawan.

Table 3. Total revenue and profit of puerulus collector from Bgy. Rizal Roxas, Palawan using the prevailing and regulated buying prices. USD 1 = PHP 55.39 based on the real-time dollar-to-peso exchange of Google Finance in August 2022.

Monthly catch of puerulus per category	Number of puerulus	Prevailing buying prices (PHP)			Regulated buying price (PHP)		
		Price of puerulus	Monthly total revenue	Monthly net profit	Price of puerulus	Monthly total revenue	Monthly net profit
March							
White	160	110	**34,400**	**20,798**	100	**40,000**	**26,398**
Pigmented	240	70					
April							
White	180	50	**18,450**	**4,848**	100	**45,000**	**31,398**
Pigmented	270	35					
May							
White	240	45	**19,800**	**6,198**	100	**60,000**	**46,398**
Pigmented	360	25					
June							
White	240	35	**15,600**	**1,998**	100	**60,000**	**46,398**
Pigmented	360	20					

Monthly catch of puerulus per category	Number of puerulus	Prevailing buying prices (PHP)			Regulated buying price (PHP)		
		Price of puerulus	Monthly total revenue	Monthly net profit	Price of puerulus	Monthly total revenue	Monthly net profit
July							
White	200	35	**13,000**	**-602**	100	**50,000**	**36,398**
Pigmented	300	20					
August							
White	80	35	**4,600**	**-9,002**	100	**20,000**	**6,398**
Pigmented	120	15					
Total			**105,850**	**13,490**		**275,000**	**182,640**
ROI (%)				**15**			**198**

Table 4. Total revenue and profit of puerulus collector from Bgy. San Juan, Aborlan, Palawan using the prevailing and regulated buying prices. USD 1 = PHP 55.39 based on the real-time dollar-to-peso exchange of Google Finance in August 2022.

Monthly catch of puerulus per category	Number of puerulus	Prevailing buying prices (PHP)			Regulated buying price (PHP)		
		Price of puerulus	Monthly total revenue	Monthly net profit	Price of puerulus	Monthly total revenue	Monthly net profit
February							
White	20	130	**5,000**	**-6,519**	100	**5,000**	**-6,519**
Pigmented	30	80					
March							
White	80	40	**5,600**	**-5,919**	100	**20,000**	**8481**
Pigmented	120	20					
April							
White	40	40	**2,800**	**-8,719**	100	**10,000**	**-1,519**
Pigmented	60	20					
May							
White	32	35	**1,840**	**-9,679**	100	**8,000**	**-3,519**
Pigmented	48	15					
June							
White	20	35	**1,150**	**-10,369**	100	**5,000**	**-6,519**
Pigmented	30	15					
July							
White	32	35	**1,840**	**-9,679**	100	**8,000**	**-3,519**
Pigmented	48	15					
August							
White	12	35	**690**	**-10,829**	100	**3,000**	**-8,519**
Pigmented	18	15					
Total			**18,920**	**-74,840**		**59,000**	**-34,760**
ROI (%)				**-80**			**-37**

DISCUSSION

Cost and Return Analysis

This study revealed that a spiny lobster puerulus collector using a stake-hanging method with 1,000-2,200 concrete disc traps spent PHP 92,360 to PHP 93,760 for six and seven months of collection season, respectively. Due to insufficient literature about expenses and operating costs of some fishery activities such as gleaning (collection of marine species with commercial value either by hand or with the aid of various tools in coastal areas during low tide) and fishing, the cost and return analysis of this study was compared to the seaweed farming which is one of the livelihood sources in the coastal area. According to Alin et al. (2015), the operation of a 1-hectare seaweed farm with four rounds of harvest has expenditures of at least PHP 34,800, indicating that the six- or seven-month operation in puerulus collection has much higher total expenses. Despite the high expenses required for spiny lobster puerulus collection, a lot of people are lured to engage in this livelihood possibly because of its high price and abundance of puerulus (Mecha et al. 2022), which is also reflected in the short harvest time (3-6 hours per day) and the number of monthly puerulus catch of two collectors from Bgy. Rizal and Bgy. San Juan (Figure 2). Fishers are marginally poor, and their daily survival depends on the availability of cash resources in the coastal area, which could provide them with a good and immediate income for their day-to-day needs (Macusi et al. 2019). In addition, puerulus collection only needs a few materials for the fabrication of traps, installation, maintenance, and repair, while seaweed farming needs more time, hard labor, and some facility, such as a drying area and a suitable location to establish the farm (Alin et al. 2015). Furthermore, the decline of productivity in some fishery resources, which are mostly done by fishers like gleaning requires more labor, while fishing needs higher capital and effort due to the declining wild stocks (Macusi et al. 2017). These reasons have caused many fishers to look for an alternative livelihood (Muallil et al. 2011, 2014) such as the puerulus collection. According to Macusi et al. (2019), fishers engaged in other income-generating activities to meet their family needs.

In addition, most fishers engaged in livelihood business have no records of their expenses and income, and mostly rely on their memory due to the lack of proper training in entrepreneurial skills. According to Aladejebi and Oladimeji (2019), record keeping of all expenses is crucial for the business to track and adjust the expenditures. This explains the high total collection cost of two puerulus collectors since they were not able to record and track the expenses in the whole collection season as mentioned during the interview. The use of traps made of cheaper and locally available materials is highly recommended to reduce the cost of fabrication thereby increasing profitability. Thus, determining the cost using other traps and methods in spiny lobster puerulus in Palawan would be beneficial as it would provide more information, which is crucial for the selection of a particular collection operation with minimal expenses involved.

Monthly Puerulus Catch and Buying Prices

The abundance of settling puerulus in coastal areas is highly dependent on the physical characteristics of the coast and water movement (Dao et al. 2015; Priyambodo et al. 2020). The embayment characteristics of Roxas, Palawan could trap the pueruli carried by the ocean current from open water into Green Island Bay. This explains the high monthly puerulus catch in Bgy. Rizal ranging from 200 to 600 ind. month^{-1} (Figure 2; Table 3). Meanwhile, the short continuous coastline of Aborlan in which the water current continuously moves to the southeast or northeast of Palawan explains the low monthly catches that range from 50 to 200 ind. (Figure 2; Table 4). In Lombok, Indonesia, the volume of puerulus settlement on the west coast and southeast also differed due to the abovementioned conditions of the coastal area (Priyambodo et al. 2020). The collection season in Bgy. San Juan started a month earlier than in Bgy. Rizal. This variation which was also reported in Vietnam and Indonesia is attributed to the differences in water current (Bahrawi et al. 2015; Jones et al. 2019). Despite the seasonality, it is still important to have continuous monthly/yearly puerulus catch monitoring per municipality to provide more robust information on the catch trend of spiny lobster puerulus in Palawan, which is crucial in managing the wild stock population.

The high buying price of the puerulus in Palawan only happened at the start of the collection season, then it dramatically dropped every month until the end of the season which contradicts the law of supply and demand wherein price increases with less supply (Fernando 2021). The unstable price has also been reported in Balete Bay, Davao Oriental, however, in the opposite way, where the buying price starts at PHP 100 (USD 1.81) at the beginning of collection (March) and continuously increases to PHP 250 (USD 4.54) at the succeeding peak seasons (April to May; Macusi et al. 2019). According to Macusi et al. (2019), some suppliers and operators of spiny lobster aquaculture from the CARAGA region ventured into the Davao Oriental for the puerulus supply. This competition makes the puerulus in Balete Bay fetch a high price. In Palawan, there is only one foreign middleman involved in buying puerulus (see Mecha et al. 2022) since there are no local fishers that venture into the spiny lobster aquaculture using the puerulus stage. This may explain the low buying prices despite the existing Provincial Ordinance No. 2475 series of 2020 that regulates the buying price. However, this still needs further investigation, as the system of buying and trading of spiny lobster puerulus in Palawan involves several foreign middlemen and buyers in each municipality.

Profitability

Unlike the puerulus collector from Bgy. Rizal, Roxas that only had two monthly losses based on net profit using the prevailing buying prices (Table 3), the collector from Bgy. San Juan, Aborlan had negative net profit and ROI on both buying prices (prevailing and regulated; Table 4) for the whole collection seasons. Both puerulus collectors failed to include the operating cost in their profitability computation, hence they assumed that the collection is still profitable because it has high prevailing buying prices from the start of collection season. Assuming that the operating costs are excluded in the computation as practiced by some novices in business, the collector from Bgy. Rizal would obtain a much higher net profit (PHP 73,950) and ROI (232%) using the prevailing buying prices (Table 5). Meanwhile, a collector from Bgy. San Juan still has a negative net profit and ROI following such scheme (Table 5). This shows that removing the operating cost does not guarantee a positive profit and ROI which is a big loss for the collector. Since the collector was unable to

Table 5. Comparison of total net profit and Return on Investment with and without operating costs of two puerulus collectors using the prevailing buying prices.

Collectors	**Net Profit (PHP)**		**Return on Investment (%)**	
	With operating cost	Without operating cost	With operating cost	Without operating cost
Rizal, Roxas, Palawan	13,490	73,950	15	232
San Juan, Aborlan, Palawan	-74,840	-5,540	-80	-23

track the expenses and earnings from the collection activities for the whole collection season, it become unnoticeable. According to Nelson et al. (1976), to have a positive and high profit, expenses must be reduced.

In addition, the positive and negative net profit and ROI of two puerulus collectors using the prevailing prices show that the abundance of wild seedstocks in a certain site is one of the factors to have a positive net profit and ROI. This is because the prevailing price and capital of the two collectors are almost the same. Similar to the profitability of spiny lobster grow-out aquaculture in Indonesia, where a person with higher seedstocks can yield a higher profit and ROI compared to those with lower seedstocks (Petersen et al. 2013). Hence, collectors in Bgy. San Juan and other localities in Palawan with existing collection activities should find a suitable site with a high abundance of puerulus settlement and use cheaper biodegradable trap materials to have a good net profit and ROI. This would not only lessen the capital needed for the collection but also help lessen the pollution in the marine environment. On the other hand, a follow-up profitability study involving the use of other collection methods as indicated in the previous study (see Mecha et al. 2022) is suggested with more sources of data from different localities in Palawan.

In conclusion, only the regulated buying price (PHP100) for white and pigmented puerulus showed a positive net profit and ROI for localities with abundant wild seedstocks. While the prevailing buying prices resulted to a very little profit for the collector from Bgy. Rizal, Roxas. Establishing a cooperative or fisherfolk association for puerulus collectors may help address the existing problems. Establishment of an organization is crucial for the proper distribution of benefits like the case of lobster production in Belize (Huitric 2005), assist to market their collected puerulus (Macusi et al. 2019) at regulated prices, and implement the maximum harvest per collector per month to minimize the exploitation of wild seedstocks. Moreover, a follow-up study dealing with more KIs from different localities including their socio-demographic profile is also suggested to provide robust data on the financial capacity, expenses, revenue, and net profit of the puerulus collectors in Palawan. In addition, venturing into grow-out aquaculture utilizing the wild seedstocks of Palawan could help reduce the fishing pressure for the wild population of spiny lobsters. This would also help increase the buying prices since there might be a possible increase in the demand and competition among the spiny lobster farmers for the wild seedstocks like the case in Davao Oriental (Macusi et al. 2019). However, proper management of the puerulus fishery must be considered at the soonest to avoid overharvesting and ensure the long-term benefits of collectors and those involves in its fishery.

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ETHICAL CONSIDERATIONS

A written consent form was secured before the conduct of interview to eight Key Informants.

No animals were captured in this study.

DECLARATION OF COMPETING INTEREST

The authors declare that there is no competing interests among them.

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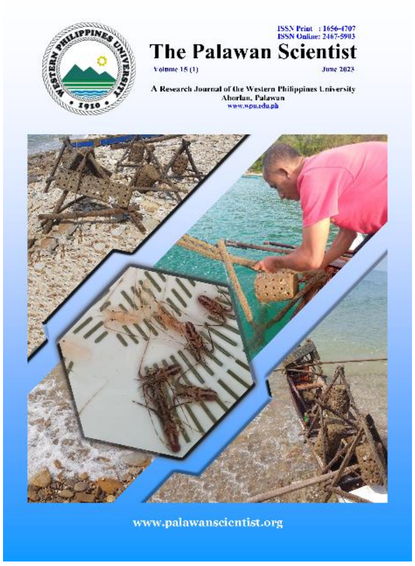

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Histopathology of the gills of Lake Van Fish *Alburnus tarichi* (Güldenstädt, 1814) infected with *Dactylogyrus* spp. parasites.

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ABSTRACT

The Lake Van fish is an anadromous carp endemic to the Lake Van basin. In this study, the histopathological effects of *Dactylogyrus* spp. parasites, which naturally infected fish, on the gills were determined during reproductive migration. Fish gills were stained with hematoxylin and eosin after routine histological procedures and apoptotic cells in the gills were determined immunohistochemically. *Dactylogyrus* spp. parasites were found in 8 of the 60 fish caught in the study. No parasites were found in the fish sampled from the lake. It was observed that the parasites caused hypertrophy, hyperplasia, edema, epithelial desquamation, hemorrhage, fusion of secondary lamellae, and necrosis in the gills. Immunohistochemically, no increase in the number of apoptotic cells was observed in the gills of the parasite-infected fish when compared with the non-infected fish gills. As a result, it can be inferred that the lake water has a restrictive effect in fish against parasites. Histopathologic lesions caused by the observed parasites in fish caught in fresh water could significantly affect gill functions.

Keywords: apoptosis, gill, histopathology, Lake Van

INTRODUCTION

Fish is one of the most important components in the human diet due to its high nutritional quality and content. Although inland waters constitute 0.01% of the world's waters, fishing in these parts provides approximately 40% support to world fish production (Lynch et al. 2016). The Lake Van fish (*Alburnus tarichi* Güldenstädt, 1814) provides approximately 1/3 of Türkiye's domestic fish production. Approximately 10,000 t fish is caught per year, constitutes a great source of protein for the local people (Oğuz and Ünal 2011). The Lake Van fish is a carp species endemic to Türkiye's Lake Van, one of the largest soda lakes in the world. It is the only vertebrate species that has adapted to the extreme conditions of Lake Van such as high pH (9.8), alkalinity (155 mEq/L), and salinity (22‰) (Danulat and Kempe 1992). They migrate in fish schools to the streams pouring into the lake to spawn between April and July every year.

Despite its great importance for the people of the region, the Lake Van fish was included in the category of declining species in 2014 (Freyhof 2014). The decrease in its numbers was due to illegal hunting during spawning, habitat destruction and loss, river sand mining, and waste water pollution have also been shown to decrease egg success in fish as the causes of population decline. The effects of parasites on the decrease in numbers were ignored.

Fish parasites are an integral part of aquatic ecosystems and are common in natural fish populations. Parasites can directly cause the death of fish, as well as having many negative effects such as preventing growth, causing behavioral disorders, reducing resistance to stress factors, and causing histological disorders (Kumaraguru et al. 1995; Feist and Longshaw 2008). Parasitic diseases are among the factors limiting the development of aquaculture (Scholz 1999).

Although many biochemical, physiological, and histological studies have been conducted on Lake Van fish, studies on parasites and their effects on fish are very limited (Oğuz 2015; Oğuz and Kaval Oğuz 2020). The pathogenic effects of parasitic organisms on fish, especially those that cause lesions in tissues, have been studied primarily in fish of economic importance. Monitoring of parasites in cultured fish is of great importance to prevent the spread of pathogens. Histopathological examination of fish tissues is an important method used to determine the health status of fish individually and as a population (Takashima and Hibiya 1995; Genten et al. 2009). The most common gill lesions in fish infected with parasites are hypertrophy, edema, necrosis, epithelial desquamation, hyperplasia, fusion of secondary lamellae, and telangiectasia. However, it is not possible to see the same symptoms in all fish species (Abdelmonem et al. 2010). It has been stated that as a result of gill infection by parasites, fish may die due to a decrease in body weight and condition factor, respiratory disorders, and serious changes in osmoregulation (Raissy and Ansari 2011).

Dactylogyrus is a parasite genus that is common in all fish species, especially cyprinid fish. It is known that especially *Dactylogyrus vastator*, *Dactylogyrus anchoratus*, and *Dactylogyrus extensus* from the family Dactylogyridae infect carp (Trujillo-González et al. 2018). They reproduce with eggs, attach to the host with two hooks at their posterior end (Kennedy 2007), and settle in the fish gill filaments. In large numbers, they spread throughout the fish body. Different levels of tissue damage and necrotic and degenerative pathological changes were observed in infected fish (Abdelmonem et al. 2010).

The present study describesthe effects of the parasite on the gills of *A. tarichi*.

METHODS

Sampling

Lake Van fish were caught from Lake Van and streams pouring into the lake between April and July of 2022, when breeding migration takes place (Figure 1A). Thirty fish each were sampled from the lake and in the River Karasu which flows into the lake. (Figure 1B). After the fish were caught, they were transported to the laboratory in oxygen-connected transport containers.

The total weight and fork length of the caught fish were determined. Age was determined from the operculum. The gills of the fish were removed under anesthesia (phenoxyethanol 320 µl/l), placed in fixatives, and kept in Bouin fixative and 4% paraformaldehyde solutions at +4°C for 24 h (Bancroft and Gamble 2002).

Fish aged between 3 and 5 years, weighing 94-118 g, and of fork length of 17.5-20 cm were used in the present study. The gills of 8 out of the 60 fish caught in the lake and fresh water during the breeding migration of Lake Van fish were infected with *Dactylogyrus* species. Parasitic infection was observed only in the fish adapted to fresh water, but not in those caught in the lake.

All of the animal experimental procedures were performed in accordance with the animal study protocols approved by the Animal Research Local Ethics Committee of Van Yüzüncü Yıl University (Protocol no: 2020/20).

Water Parameter

At the time of fish sampling, water parameters from the lake and freshwater were monitored. The pH, water temperature, dissolved oxygen, and salinity were measured with a multiparameter device (Milwaukee MW805, São Paulo, Brazil). The physicochemical parameters were measured using a HACH spectrophotometer (HACH DR/2010, HACH Co., Loveland, CO, USA). Water samples were analyzed for nitrite (HACH method 8507), nitrate (HACH method 8171), and ammonia (HACH method 8155) according to the manufacturer’s instructions. The water parameters in Lake Van and the River Karasu where the fish were sampled, are shown in Table 1. When the measured values were compared, it was observed that there was a significant difference in the parameters between the two sampling areas. The difference between lake and stream water parameter was analyzed using the t-test (IBM SPSS Statistics 22, USA). The difference between the groups was considered significant at $p < 0.05$.

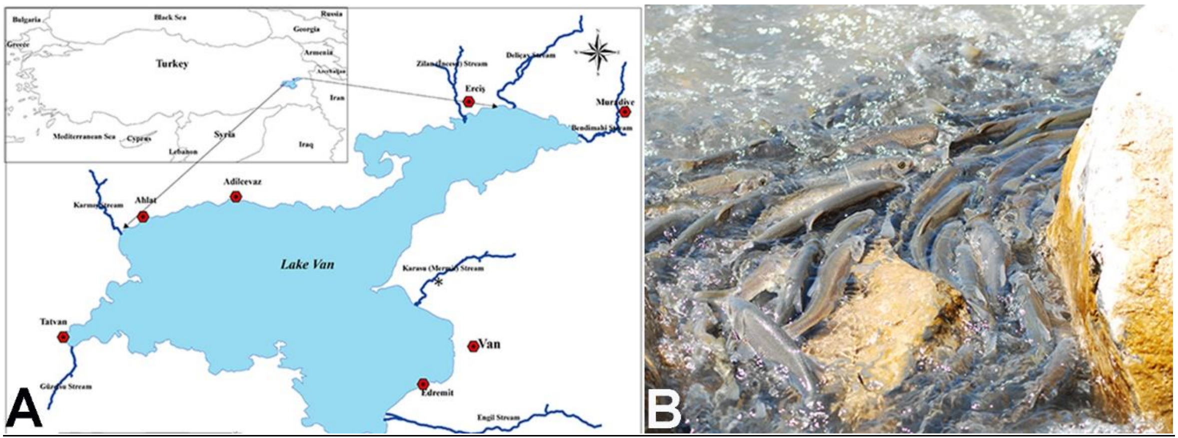

Figure 1. Location of the sampling area of Lake Van fish in Lake Van (A) and the fish school during reproductive migration of Lake Van fish (B).

Table 1. Water quality at the locations where fish were sampled.

Parameter	**Lake Van**	**Karasu River**
Dissolved oxygen (mg/l)	9.100	9.680
pH	9.660	8.960
Temperature (ºC)	12.100	19.90
Salinty (ppt)	16.970	0.200
Conductivity (mS/m)	24.200	0.498
Nitrite (mg/l)	0.001	0.009
Nitrate (mg/l)	0.270	1.900
Ammonia-N (mg/l)	0.420	0.050

Histology

The gills in Bouin fixative were then stored in 70% alcohol at +4°C until paraffin embedding. Tissues passed through graded alcohol series (70%, 80%, 90%, and 100%) and xylol were embedded in paraffin blocks. After 5-µm sections were taken from the paraffin blocks with the help of a microtome (HM 325 manual microtome, MICROM International GmbH, Waldorf, Germany), they were stained with hematoxylin and eosin to determine the general histological structure (Bancroft and Gamble 2002). The stained preparations were covered with Entellan and examined under a light microscope (Leica DMI 6000B microscope, Germany) and photographed using a Leica DFC 490 digital camera (Leica Microsystems, Germany).

TUNEL Assay

A TUNEL Assay Kit HRP-DAB (ab206386, Abcam, UK) was used for detecting cell death in gill sections of the Lake Van fish according to the manufacturer's instructions. Briefly, the tissue sections were deparaffinized and rehydrated at room temperature. Sections on coated glass slides were incubated in Proteinase K solution for 20 min at room temperature and then rinsed with Tris-buffered saline (1X TBS: 20 mM Tris pH 7.6- and 140-mM sodium chloride) for 5 min. To inactivate the endogenous peroxidases, the dried slides were incubated in 100 µl of 3% H_2O_2 at room temperature for 5 min, washed with 1X TBS, and left to dry. Then the specimen were covered with 100 µl of terminal deoxynucleotidyl transferase (TdT) equilibration buffer for 30 min and with 40 µl of TdT labeling mixture solution for 90 min at room temperature. The slides were incubated in a stop buffer at room temperature for 5 min to terminate the labeling reaction, followed by washing with 1X TBS. Then the sections were covered with 100 µl of blocking buffer at room temperature for 5 min, with the conjugate solution for 30 min and with diaminobenzidine (DAB) for 15 min consecutively. Finally, the sections were treated with methyl green counterstain, dehydrated with ethanol, and mounted with DPX. The slides were examined under a light microscope (Leica DMI 6000B microscope, Germany) and photographed using a Leica DFC 490 digital camera (Leica Microsystems, Germany).

RESULTS

When the gills of the uninfected fish were examined histologically, it was observed that the gills were composed of primary lamellae and secondary filaments, and pavement cells, mucus cells, and chloride cells were concentrated in these parts (Figure

2A). When the fish gills of infected fish were examined, it was observed that each gill was infected by parasites at different intensities and the damage varied depending on the parasite density. Tissue loss was observed in the parts where the parasites attached to the gills (Figure 2B). Fish gill epithelial cells infected with *Dactylogyrus* spp. showed hyperplasia, resulting in lamellar fusion (Figure 2B, C). Hemorrhage increased with the intensity of infection in fish (Figure 2D, E). Epithelial separation was observed in the gills of both the healthy and the infected fish, but it was more severe in the gills of the infected fish (Figure 2F).

When the healthy group was compared with the infected group, there was no difference in apoptotic cell density (Figure 3).

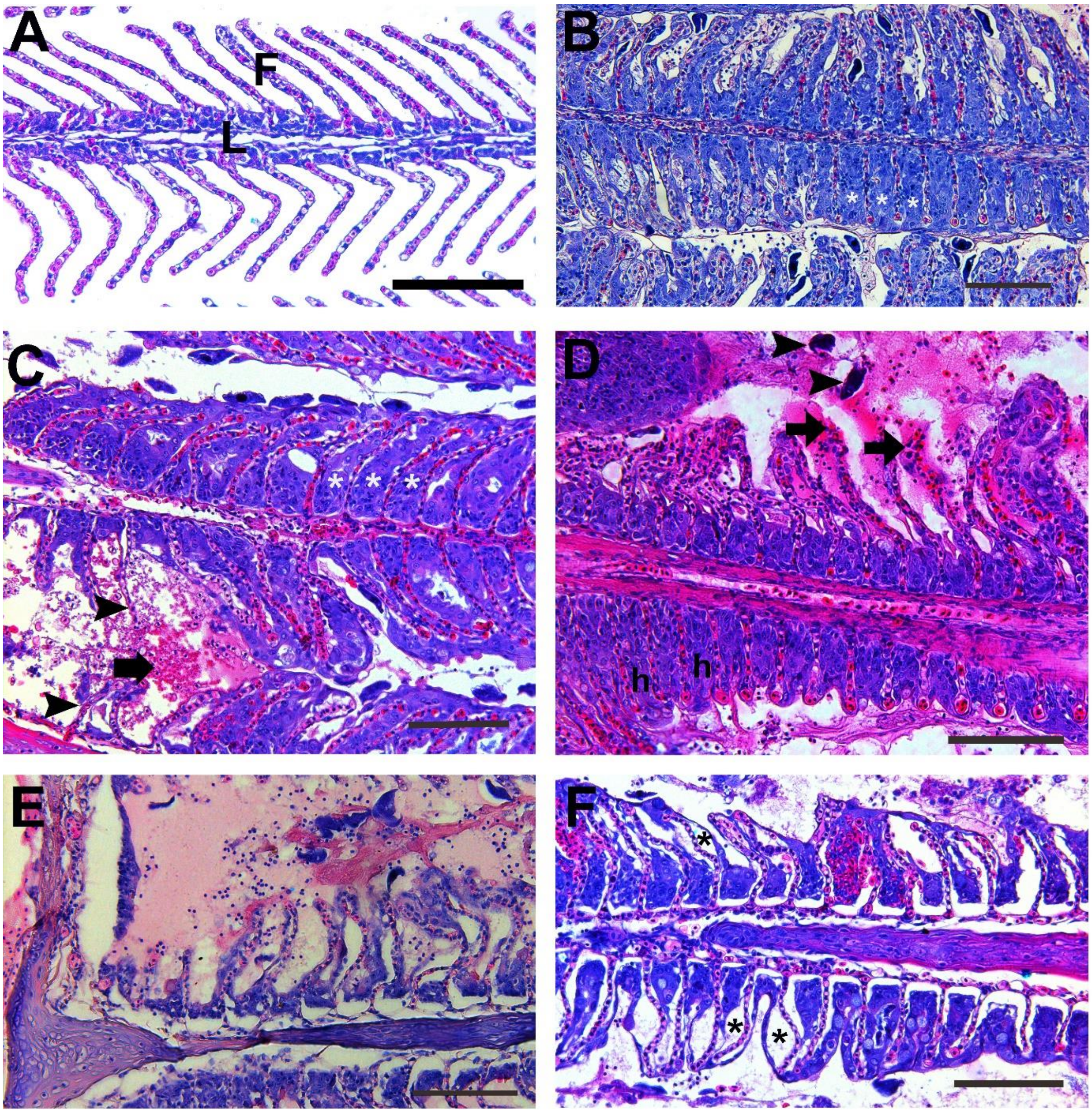

Figure 2. Histopathologic image of the gills of the Lake Van fish infected with *Dactylogyrus* spp. A) General view of normal fish gill histology (L: primary lamellae, F: secondary filament) B) Primary filament hyperplasia with secondary lamellar fusion (asterisk) C) Hemorrhages (arrows), lamellar fusion (asterisk), and necrosis of epithelial cells (arrowhead) D) Hemorrhages (arrows) and hypertrophy of cells (h). Arrowheads show parasites. E) Necrosis and desquamation of epithelial cells F) Epithelial lifting of varying severity (asterisks). Hematoxylin and eosin staining. Scale bars: 100 µm.

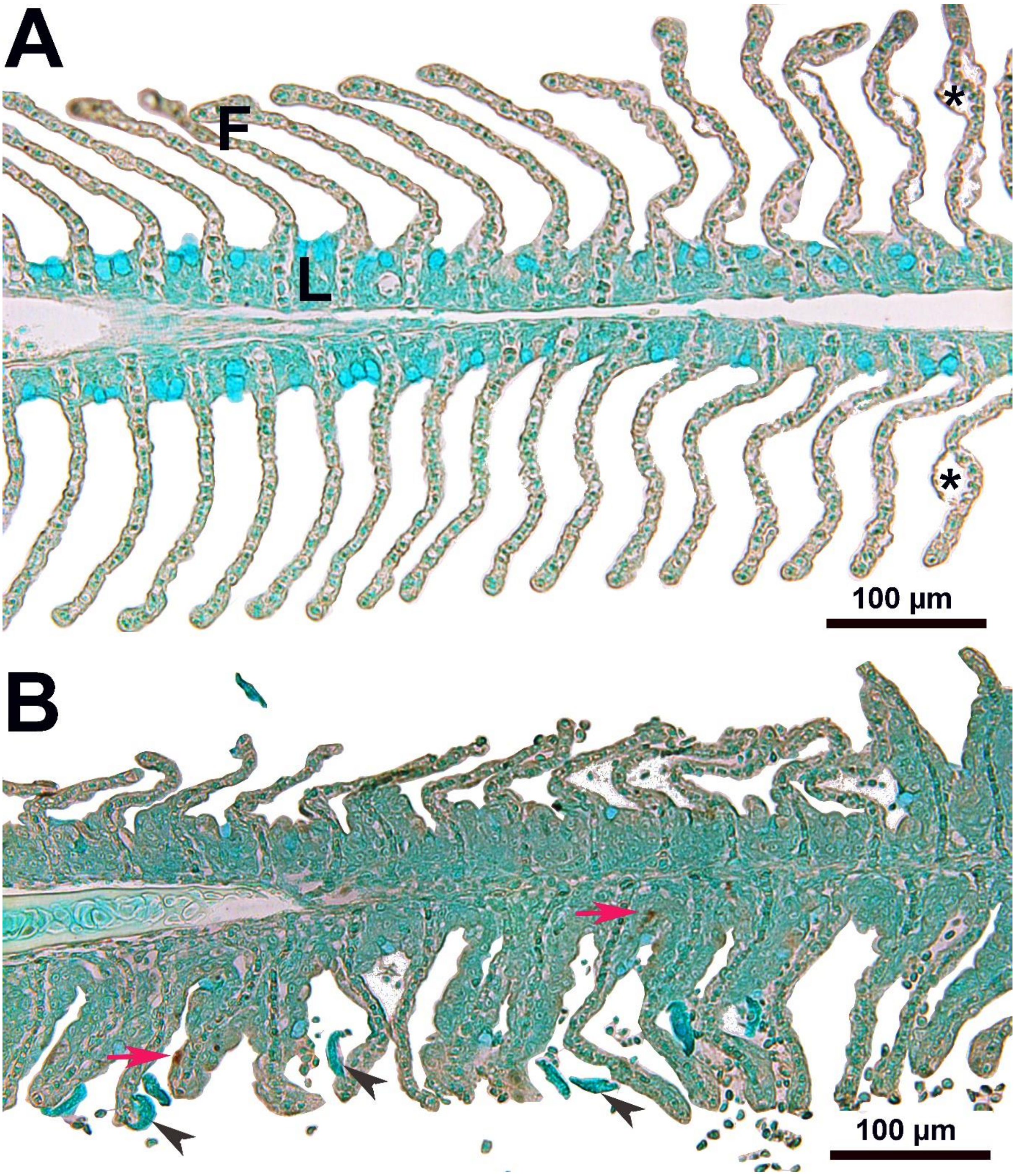

Figure 3. Detection of apoptosis in gill tissues of Lake Van fish. A) Normal fish gill (L: primary lamellae, F: secondary filaments, Asterix: epithelial lifting) and B) *Dactylogyrus* infected fish gill (Red arrow; apoptotic cell, Arrowhead; parasites).

DISCUSSION

This study is the first on *Dactylogyrus* infection and pathology of endemic fish in Lake Van, which is among the largest alkaline lakes in the world. As a result of histopathological examinations, hyperplasia, hypertrophy, lamellar detachment, fusion in the secondary lamellae, hemorrhage, necrosis, and epithelial desquamation were detected in the Lake Van fish infected with *Dactylogyrus*. It has been observed that in intense parasitic infection, necrosis and shedding are more common in the gills, increasing epithelial cell proliferation in the regions where the parasites attach to the gills, causing hyperplasia and, as a result, lamellar fusion. The histopathological findings observed in the present study are similar to those of studies performed in different fish species (Abdelmonem et al. 2010; Santos et al. 2017; Ramudu et al. 2020; Kumari and Nomani 2021). Lamellar separation in the gills was observed in both groups. Although this lesion was reversible, it was quite severe in parasitized gills. Blood congestion and aneurysm may be caused by sudden and intense blood flow to the gills, as stated previously (Rosety-Radriguez et al. 2002). Therefore, it can be inferred that the oxygen deficiency in the tissue caused by parasitic infection in Lake Van fish causes damage to the pillar cells.

It is known that parasitic infection increases apoptosis in animals (Bienvenu et al. 2010; Bosurgi and Rothlin 2021). In a study conducted in zebra mussels, apoptosis was observed only in circulating hemocytes as a result of ectoparasite infection, while no or few apoptotic cells were observed in infected tissues (Minguez et al. 2013). Similarly, in the present study, the number of apoptotic cells in the infected fish gills was very low. In the Lake Van fish, apoptosis may have occurred in immune cells in the blood.

No fish with gills infected with Dactylogyrus were found among the fish caught in the lake. This may have been due to the salty, alkaline, and high pH water of Lake Van (Table 1). In addition, one of the most important factors affecting the presence and density of monogeneans is temperature. Depending on the species, temperature demands also differ (Öztürk and Özer 2014). The lake water temperature, which fell to +4°C in winter, may have caused the elimination of parasites in the gills, and the water temperature, which increased to 19.9°C during the breeding migration, may have caused an increase in the number of parasites.

The present study was carried out in a limited number of fish gills, to further understand the impact of parasites on fish, the examination of other tissues of the fish caught in the lake environment in terms of parasite infection is suggested. *Dactylogyrus* is a genus of helminths represented by more than 900 species worldwide (Kumari and Monari 2021). Despite the diversity of fish in Turkey, the number of *Dactylogyrus* species is quite low when compared to other countries (Soylu 2009). This may be due to insufficient research on fish parasites. Most *Dactylogyrus* parasites infect cyprinids and often have high host specificity. Therefore, this Dactylogyus parasite is thought to be a new species as it is also observed for the first time in Lake Van fish, may be a new species.

Lamellar fusion seen in the gills as a result of parasite infection causes surface reduction and consequently a decrease in oxygen uptake. Advanced histopathological lesions cause the death of adult fish and larvae. In addition, parasite infections negatively affect osmoregulation in the gills (Oğuz and Kaval Oğuz 2020). According to the results obtained, the histopathological changes seen as a result of infection in Lake Van fish increase the effect of stress factors such as hunting pressure, hunger, fish density during migration, and swimming against the flow direction.

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ETHICAL CONSIDERATIONS

All of the animal experimental procedures were performed in accordance with the animal study protocols approved by the Animal Researchers Local Ethic Committee of Van Yüzüncü Yıl University (protocol no: 2020/12) and the Republic of Turkey Ministry of Agriculture and Forestry (08/03/2019-20122).

DECLARATION OF COMPETING INTEREST

No potential conflict of interest was reported by the authors.

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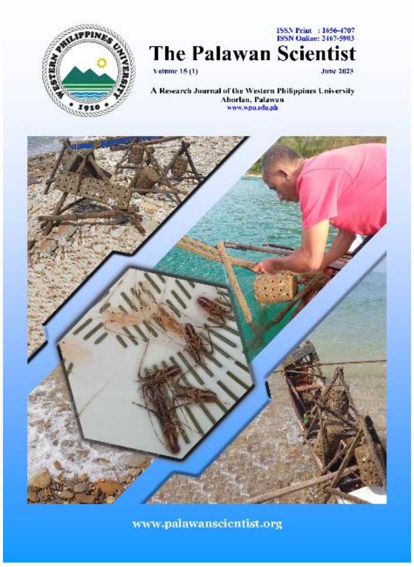
ROLE OF AUTHORS: *ANE, ZABEA and AP - carried out the histology analyzed the data; EKO - wrote and revised the manuscript. ARO - conceived the study, wrote, revised the manuscript, and designed the experiment; All authors read and approved the final manuscript.*

Original Article



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A unified stochastic framework with memory for heat index and sea level dynamics

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ABSTRACT

Monitoring temperature-dependent events is critical for understanding their dynamics since these events have an impact on both animal and human habitation. It is common to see analysis of heat index and sea level that are described separately although these events have a direct connection to temperature. Often these analyses are less effective and less reliable in describing its dynamics vis-à-vis redundancy, flexibility, accounting of uncertainties and optimization. Since both are temperature-dependent events, a unified stochastic model with memory was derived. These events can be effectively described with a collective memory function $(T-t)^{\frac{\mu-1}{2}}e^{-\frac{\beta}{2t}}t^{\frac{\mu+1}{2}}$, modifying the Brownian motion. A good match between the empirical and theoretical MSDs for both heat index and sea level was obtained with memory parameters $\mu_{HI} = 1.0460$ and $\mu_{SL} = 1.0894$, respectively. With $\mu > 1$, heat index and sea level exhibited long-term memory characteristics which have important implications for large timescale prediction. Similarly, analyses using a unified model are simplified and may provide the interrelatedness of these events.

Keywords: collective memory function, forecasting, heat index, non-Markovian, Philippines, sea level

INTRODUCTION

The unprecedented rise in global temperature at a rate of 0.2 degrees/decade over the past thirty years (Hansen et al. 2006) had significantly affected and disrupted both human activities and animal habitation. Among the many issues of elevated temperature, the most pressing is the health-related heat stresses (Dang et al. 2019) including effect on respiratory systems and the weakening of body to maintain temperature balance (Ma et al. 2019; McGregor and Vanos 2018). The elevated island heating, as measured by the heat index (HI), has the greatest impact on human productivity (Wang et al. 2022; Dean and Claassen 2023), while animal habitation decreases as sea level (SL) rises (Paul and Paul 2022; Dixon et al. 2023). In many ways, these observed effects are commonly related to temperature rise. Note that changes in SL is related to rising temperature causing ice melts in both northern and southern hemisphere (Hagen and Azevedo 2022; Orr et al. 2022; Coupe et al. 2023; Park et al. 2023; Purich and England 2023). It is but important to constantly monitor the state of these climactic factors especially in areas that are highly vulnerable to these changes particularly at their extreme state (Balacco et al. 2023; Zagebelnaya 2022).

Since HI and SL are both temperature-dependent events, they may exhibit a similar dynamical model. Because of this, a separate treatment may provide inconsistencies (Meehl et al. 2000; Pielke et al. 2002; Li et al. 2010) and non-conformities of the models resulting to less reliable assessments of current extreme events (Eggleston et al. 2006; Long et al. 2006; Field et al. 2012; Stott et al. 2016). In fact, HI and SL model unification stems from the fact that both phenomena evolve in time can be linked to the same physical processes that may have operated in multiple spatial and temporal scales (Brown et al. 2012). The vantage point is the ability of a unified model for a seamless prediction for these systems (Bhaskaran et al. 2002). For example, the novel Met Office Unified Model for climate change and weather prediction had proven to address model issues on redundancy, flexibility and often outperforms stand-alone models (Brooke et al. 2019; Maher and Earnshaw 2022). On the other hand, it had been shown in Elnar et al. (2021) that interrelated dynamics can have unifying models highlighting similar memory functions with varying degrees. With the interrelatedness between HI and SL, the researchers are driven to demonstrate that these events do, in fact, follow a similar dynamical memory parameter. By employing the analytical stochastic framework with memory (Bernido and Carpio-Bernido 2012, 2014), a direct comparison of the analytical and empirical mean square deviations (MSD) is provided and we obtain the explicit probability distribution function (PDF). In this way, a unified treatment can provide a holistic perspective in the interrelatedness of temperature-dependency of these biophysical events.

METHODS

Stochastic Framework with Memory

The non-Markovian structure of fluctuations of temperature-dependent phenomena such as heat index (HI) and sea level (SL) was modeled using the Hida stochastic functional integral method (Hida 1996). This method enables the researchers us to analyze the PDF and moments analytically. The parametrization of the path of a random variable x was defined as a sum of the initial point and fluctuation (Equation 1; Bernido and Carpio-Bernido 2012, 2014), where $B(t)$ is the ordinary Brownian motion, $f(T-t)h(t) = (T-t)^{\frac{\mu-1}{2}}e^{-\frac{\beta}{2t}}t^{\frac{\mu+1}{2}}$ is a memory function, and $g(T)$ is a modulating factor in $F(T)$. To pin down a particular trajectory of interest, the delta function constraint was applied, $\delta(x(T) - x_T)$ and the probability distribution function (PDF) was obtained for the given endpoint x_T by taking the expectation value of the delta function. The corresponding PDF is given by Equation 2 and $\alpha^2 = (g(T))^2\left(\int_0^T\left[(T-t)^{\frac{\mu-1}{2}}e^{-\frac{\beta}{2t}}t^{\frac{\mu+1}{2}}\right]^2 dt\right)^{-1}$. From the PDF, we can obtain the mean square displacement (MSD) as given by Equation 3. Notice that if $f(T - t) = \sqrt{2D}$, $h(t) = g(T) = 1$, the PDF and MSD correspond to the ordinary Brownian motion. The analyses used in this research were applied to temperature-related occurrences, with a focus on sea level (SL) and heat index (HI). Datasets of these two events are readily available and can be accessed freely through NOAA and NWS websites as described in the next section.

$$x(T) = x_0 + F(T) = x_0 + g(T)\int_0^T (T-t)^{\frac{\mu-1}{2}}e^{-\frac{\beta}{2t}}t^{\frac{\mu+1}{2}}dB(t) \qquad \text{(Eq.1)}$$

$$P(x_T,T;x_0,0) = \left(\frac{2\pi}{\alpha^2}\right)^{\frac{1}{2}} exp\left(-\alpha^2\frac{(x_0 - x_T)^2}{2}\right) \qquad \text{(Eq.2)}$$

$$MSD = g(T)^2\int_0^T [f(T - t)h(t)]^2 dt. \qquad \text{(Eq.3)}$$

Heat Index Dataset

Heat index data were gathered from the National Oceanic and Atmospheric Administration (NOAA) data site in 2018 from 1966 to 2017, totaling 17,588 daily HI data points. In this case, HI datasets obtained were for Mactan Island, Cebu. These HIs reflected the US National Weather Services (NWS) algorithm (Anderson et al. 2013) which provided consistency to environmental results and agreed among the many algorithms to Steadman's apparent temperature (Anderson et al. 2013; Ramirez-Beltran et al. 2017). With a huge amount of data points, Leskovec and Faloutsos (2006) suggested that a subgraph can be surveyed of which 25% of the data points may observe a similar behavior as that of the original graph. Thus, the corresponding subgraphs were plotted and compared to whether they exhibited the same behavior. Only then the representative 25% of the total data points were used in the model. A linear interpolation approach was used in filling the missing data points as it was done in Bucheli et al. (2022). The heat index fluctuation for Mactan Island is presented in Figure 1.

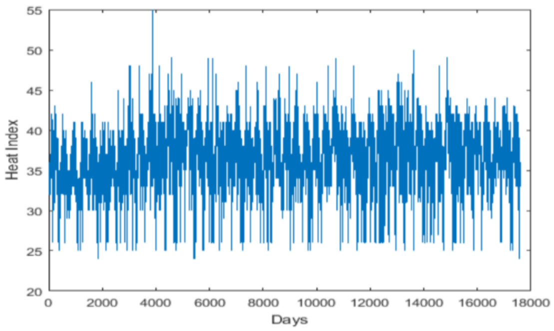

Figure 1. Daily fluctuarions of heat index in Mactan Island from 1966 to 2017.

Sea Level Datasets

The SL data was obtained from the database of the University of Hawaii Sea level center for the Philippines, particularly the Manila Sea level data points. The period covered in the dataset is from 1984 to 2015. For consistency, a 25% representative of the total data points were used in the model. Similarly, missing data points were filled in using linear interpolation (He et al. 2022; Zheng et al. 2022). The fluctuations of SL for Manila are presented in Figure 2, depicting the original data that comprises both deterministic and stochastic components. In order to isolate the purely stochastic part of the data, detrending techniques were applied, and the resulting plot is presented in Figure 3.

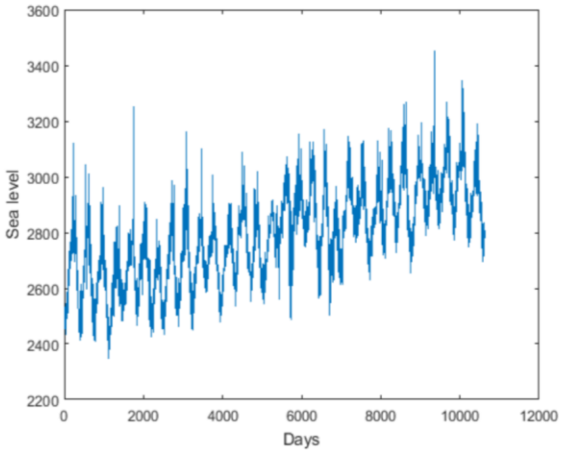

Figure 2. Daily sea level (cm) for Manila, Philippines (1984–2015).

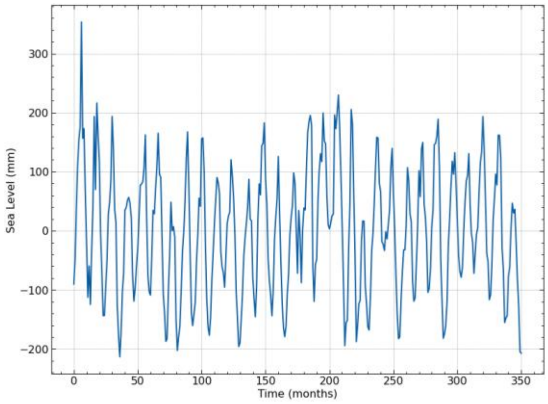

Figure 3. Detrended raw data plot showing long-term fluctuarions in sea level over time.

Mean Square Displacement (MSD) Plots

MATLAB R2018b was used in obtaining the corresponding plots of HI and SL and the plots of their MSD's. The MSD plots were fitted against the theoretical MSD. This theoretical MSD was chosen appropriately to give a good fit to the empirical MSD. Lastly, the corresponding parameters were obtained from this comparison.

RESULTS

Stochastic Framework for HI and SL

The different theoretical MSD's describing stochastic framework with memory was surveyed. The theoretical MSD is exponentially modified for both events and is given by:

$$MSD = g(T)\left(\frac{\Gamma(\mu)\, t^{(\mu - 1)} e^{-\beta/t}}{\beta^{\mu}}\right) \qquad \text{(Eq.4)}$$

where $g(T) = exp\left[\left(b - \frac{t}{\epsilon}\right)sin(ct - k)\right]$ serves as the modulating function where b, ϵ, c and k are just constants. This is an extended form as used in Bernido et al. (2014). This theoretical MSD corresponded to the memory function given by $f(T - t) = (T - t)^{\frac{\mu - 1}{2}}$, $h(t) = \frac{e^{-\beta/2t}}{t^{(\mu + 1)/2}}$. Then, this stochastic model was applied and insights into the dynamics of the heat index and sea level were gained.

Empirical and Theoretical MSDs for HI and SL

Using the theoretical MSD above, the corresponding log-log plots for the empirical data alongside a theoretical fit of the HI datasets are presented in Figure 4. The corresponding coefficient of determination ($R^2 = 0.7309$) between the two is also provided to assess the quality of the fit. This comparison is essential to determine the accuracy of the theoretical model in describing the observed phenomenon. Moreover, as shown, the dynamical parameters derived from the fit corresponds to $\mu = 1.0460$ and $\beta = 0.0792$ with scaling constants of the modulating function obtained as $b = 0.1377$, $c = 0.063$, $\epsilon = 9999$, and $k = 0.9$. Furthermore, the theoretical MSD with values of $t_c = 3.7$ (x-axis) were normalized. The normalization shifts the graph along x-axis.

The corresponding log-log plot of both the empirical and theoretical MSDs for sea level is presented in Figure 5 below. The corresponding coefficient of goodness of fit to be $R^2 = 0.9312$ was determined. From the fit, the parameters associated with the dynamics of the event were $\mu = 1.0894$ and $\beta = 1.4562$. The scaling constants of the modulating function were obtained as $b = 0.9129$, $c = 2.6569$, $\epsilon = 600$, and $k = 0.8$. Normalization of the x-axis had value of $t_c = 10^{0.5}$ which shifts the graph sideways in order for the two plots to match.

Henceforth, using Equation 4, the explicit form of the Probability Distribution Function of Equation 2 is expressed in Equation 5:

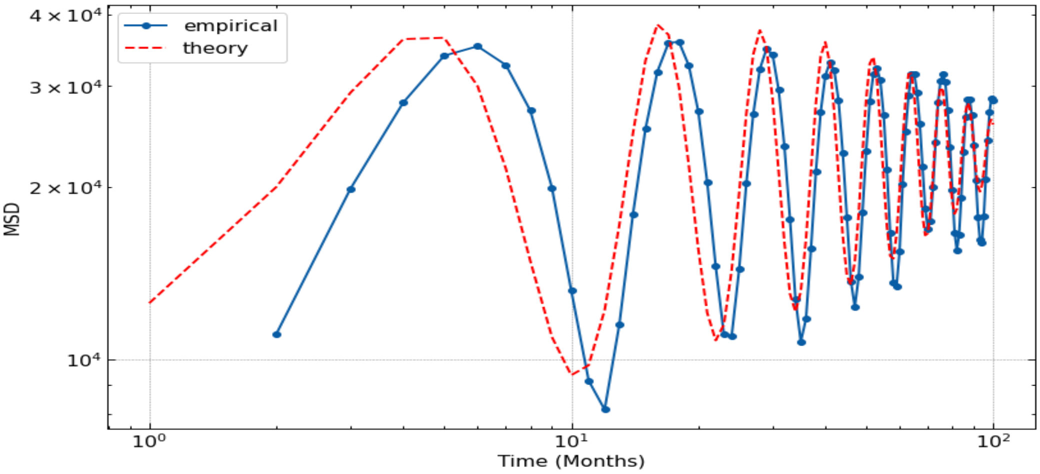

Figure 4. Log-log plots of empirical (blue) and theoretical (red) mean square displacement (MSDs) for heat index.

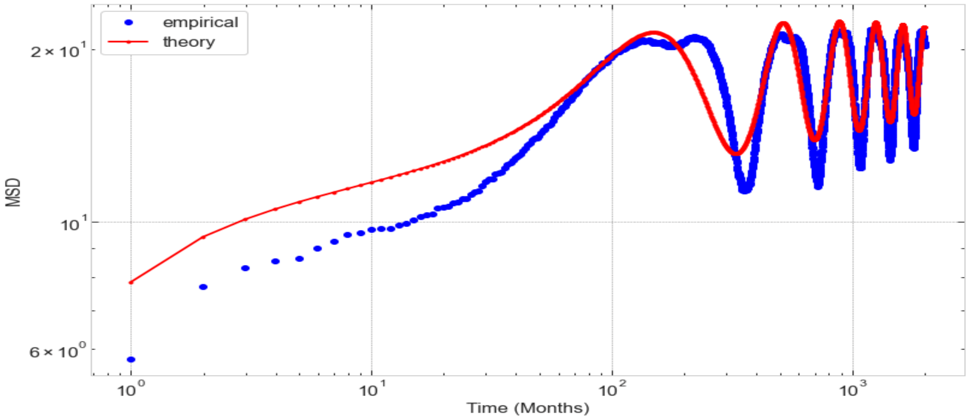

Figure 5. Log-log plots of empirical (blue) and theoretical (red) mean square displacement (MSDs) for sea level (SL).

$$P(x_T,T;x_0,\ ) = \sqrt{\frac{\beta^{\mu}}{2\pi\, exp\left[\left(b - \frac{T}{\epsilon}\right)\right] sin(cT - k)\Gamma(\mu)T^{\mu - 1}e^{-\frac{\beta}{T}}}} \times exp\left[-\frac{(x_T - x_0)^2}{2\, exp\left[\left(b - \frac{T}{\epsilon}\right)\right] sin(cT - k)\Gamma(\mu)T^{\mu - 1}e^{-\beta/T}}\right] \quad \text{(Eq.5)}$$

DISCUSSION

Stochastic Framework for HI and SL

The same stochastic framework for both HI and SL clearly demonstrated that they are, in fact, generally related phenomena. In context, this framework established a dynamical perspective of which both events can be described given that they are two distinct phenomena. Although both are driven by temperature changes, their dynamical behavior is crucial to the impacts because it influences other systems with which they interact. According to World Meteorological Organization (WMO 2021), they had shown the dynamical effects of heat and sea level on extreme weather events including cyclones, drought and wildfire.

It is noted, further, that the HI–SL interactions can be presumed as a driven dynamics resulting to a collective memory between systems as discussed in Elnar et al. (2021), in evo-eco dynamics (Power et al. 2015; Fisher and Pruitt 2020), eco-memory of interacting systems (Baho et al. 2021; Khaligli et al. 2021) or climate – carbon cycle interaction (Page et al. 2021). This collective memory is encoded in the characteristic parameter, μ, of Equation 4. This is anticipated to yield the same characteristic parameter as in the cases of HI($\mu = 1.0460$) and SL ($\mu = 1.0894$).

Empirical and Theoretical MSDs for HI and SL

It is proven that both heat index and sea-level events have similar stochastic memory functions, as indicated in Equation 4. With the memory parameter $\mu = 1.0460$ HI and $\mu = 1.0894$ SL greater than unity suggesting long memory ranges which complemented the views of sea level as random fluctuations with memory (Peng et al. 1994; Li et al. 2011; Dangendorf et al. 2014; Ventosa-Santaularia et al. 2014; Elnar et al. 2021) and other temperature-dependent events, such as surface air temperature (Caballero et al. 2002; Elnar et al. 2021) and ocean circulation (Vyushin and Kushner 2009). The associated parameters in the modulating function *g(T)* can be attributed to factors of the environment referred to as “effective ambient temperature” (Dietrich et al. 2020) both with biotic and abiotic influence. On the other hand, sea level may be modulated by the changes in sea density as caused by temperature (thermostatic) and/or salinity (allosteric) (Antonov et al. 2002; Munk 2003; Ishii et al. 2006). It has poined out; however, the influence of these factors cannot be directly extracted from our model rather we suspect that these environmental parameters have direct implications for the modulation of the HI and SL dynamics as asserted in Barkhordarian et al. (2012).

The long memory characteristics of the model presented herein have an important implication to predicting both HI and SL in larger timescales (Rypdal 2015), thus the decadal prediction of temperature rise (Hansen et al. 2006) as related to the latter can be well described. Since both HI and SL exhibit the same stochastic behavior, analyses can be simplified over these events using Equation 4 above including their interrelatedness. Also, analyzing interrelated events with a unified stochastic model offered more reliable analyses, reducing the degree of errors whereby employing only a few scaling factors. It is presented in this paper that both HI and SL exhibited the same stochastic model with memory, and thus can be analyzed singly using Equation 4. These events' long memory ranges ($\mu > 1$) are often good in predicting their changes over extended durations. Similarly, this unified stochastic model provides more reliable analyses reducing the degree of errors as such only a few scaling factors can be employed.

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ETHICAL CONSIDERATIONS

This research does not have any animal nor human subject. It utilized secondary data under the principles of data science and analyses of big data describing dynamics of heat index and sea level as temperature-dependent climatic systems. The use of accessible data was properly cited in the manuscript.

DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests to any authors. The use of heat -index and sea level data were accompanied with appropriate citations.

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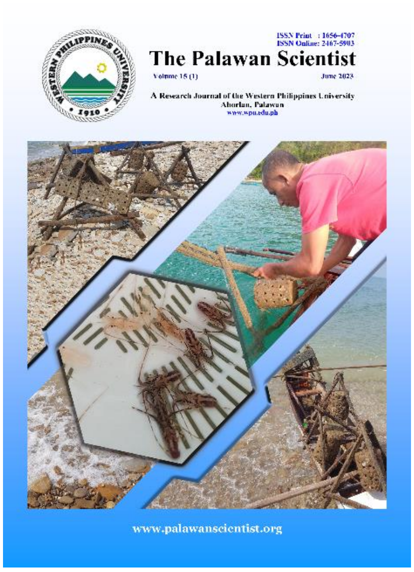
__ROLE OF AUTHORS:__ LRD - Heat index data acquisition and model fitting. Main conception of dynamical model for heat index data; JS – Sea level data acquisition and model fitting. Main conception of dynamical model for heat index data; ARE - Manuscript preparation - rationalization and discusion part; KPC - Manuscript preparation - review of literature and theoretical consideration part; GM - completion of publishable paper; data analysis and unification of dynamical model; corresponding author.

Original Article



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Genetic variation in drought stress tolerant rice variety NSIC Rc9 (Apo) through *In Vitro* mutagenesis

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ABSTRACT

In vitro mutagenesis, a technique combining tissue culture and irradiation, of the drought-tolerant rice variety National Seed Industry Council (NSIC) Rc9 (Apo), resulted in the generation of an induced mutant population. Irradiation of gamma rays at four doses (10 Gy, 30 Gy, 50 Gy, and 70 Gy) was applied to callus pieces derived from tissue-cultured mature seeds. The control (0 Gy) determined the effect of irradiation doses on tissue culture response in callus induction and regeneration. A decreasing trend in callus induction and regeneration efficiency was observed with the increasing dosage of gamma ray. Increasing the gamma ray irradiation doses also increased the incidence of necrosis. The irradiated calli regenerated green plantlets, which produced the IVM$_2$ mutant population. The variability evaluation showed a wide variation in agro-morphological traits, *viz.*, pigmentation in basal leaf sheath, leaf blade, ligule, and collar, angles of leaf blade, culm and flag leaf, panicle exsertion, axis, type and secondary branching, grain size and shape, flowering days, plant height at maturity, panicle length and productive tiller number of the mutant population derived from the combination of tissue culture and gamma irradiation, compared to the variability induced using tissue culture alone identifying 30 Gy and 50 Gy with the most induced variability. The cluster analysis supported the variation of the mutants from the wild type, NSIC Rc9, in terms of phenotypic characteristics. The results showed the efficiency of in vitro mutagenesis in inducing a larger spectrum of mutation compared to using tissue culture and gamma irradiation singly.

Keywords: in vitro mutagenesis, mutation, mutant, tissue culture, variability, wildtype

INTRODUCTION

Extreme effects of the changing global climate remain the most threatening challenge in agriculture, especially in rice production. Development of climate resilient rice genotypes remains the most sustainable technology to mitigate the effects of climate change. Rice breeders utilizes various breeding strategy to deliver rice genotypes that can survive extreme weather conditions, without compromising good traits that are acceptable to farmers and consumers. One of these strategies is induced mutation, to widen genetic pool for rice crop improvement. In the past years, plant breeders used induced mutation for crop improvement, to alter the genetic and phenotypic compositions of the organism. Widening of the crop's genetic pool is commonly done through the exposure of seeds, meristematic cells, tissues, and plant organs to physical mutagens (Sigurbjörnsson and Micke 1974). Physical mutagens are electromagnetic radiations such as gamma rays, X-rays, UV rays, and particle radiation, including fast and thermal neutrons, beta, and alpha particles causing breakage in the DNA double strands (Ulukapi and Nasircilar 2015; Kodym and Afza 2003) due to oxidative reactions resulting from the interaction between the reactive oxygen species produced and the DNA (Morita et al. 2009). A mutation is induced when radiation ionizes nitrogenous bases of the DNA chains, specifically during DNA synthesis. Base change or base deletion creates alterations in critical base sequences of the genetic molecules. The ionization of the bases with free radicals, produced from the radiation particles, alters the structure of the nitrogenous bases, thereby changing the encoded traits before radiation exposure. Ionizing radiation and ion beams are the most commonly used physical mutagens in mutation breeding (Viana et al. 2019). Among the types of ionizing rays, gamma rays are the most adapted for their shorter wavelength and high energy that penetrates deep into the biological matter. Gamma rays cause nucleotide substitutions and deletions of 2 to 16 bp and a frequency estimate of one mutation/6.2Mb. The changes in genetic structure lead to alterations in biotic and abiotic stress responses and plant features of the crops. The widely used gamma ray is Cobalt 60, with a half-life of 5.3 years and a radiation emission of 1.33 MeV (Celik and Cimen 2017).

From 1960 to 2014, numerous mutant crops were released in 60 countries for cultivation and consumption, wherein rice crop has the highest number of mutants of around 700 (IAEA 2016). The registered mutants possess better agro-morphological traits, increased yield and yield components, better quality and nutrition, superior biotic resistance, and abiotic tolerance (IAEA 2016). From 2005 to 2015, the breeding program for rainfed-drought-prone and adverse environments of the Philippine Rice Research Institute utilized various induced mutation techniques to generate and develop improved breeding lines. The lines have durable tolerance to moisture-related stresses, and resistance to diseases, and exhibit good agronomic and grain quality traits. Of these released varieties, two varieties were from seed mutation of another culture-derived Pokkali, approved by the National Seed Industry Council (NSIC) and registered in the IAEA Mutant Variety Database. These are the NSIC Rc272 (Sahod Ulan 2) and NSIC Rc346 (Sahod Ulan 11). *In vitro* mutagenesis (IVM) is a technique of combination of tissue culture and irradiation which enhances genetic variation for morphological traits and resistance to stress (Ahloowalia 2001). IVM technique generates crops that express recessive and dominant mutations (Xu et al. 2012). IVM increases the rate of mutation by ensuring each plant in a treated population will contain at least one mutation, introducing a specific trait improvement, without affecting the existing good traits, such as tolerance to severe drought stress (Yadav et al. 2013). The use of induced mutation techniques in enhancing variation has been a very effective tool in rice breeding. The variation created from this technique resulted in the generation and promotion of numerous crop varieties with exemplary traits for cultivation and consumption (Okasa et al. 2021; Poli et al. 2021; Cabusora et al. 2022).

The rice variety NSIC Rc9, is locally known as "Apo", and is released for cultivation in upland rice ecosystem. This variety is also known to have tolerance to drought conditions, however some characteristics were not acceptable to farmers and consumers, such as its phenotype and grain quality. The study aimed to induce variation in the drought-tolerant rice variety, NSIC Rc9 (Apo) through IVM at different doses of gamma irradiation.

METHODS

NSIC Rc9 (Figure 1a-d), locally known as "Apo" is a rice variety released by the National Seed Industry Council (NSIC) of the Philippines in 1991 for the upland rice ecosystem. It is a variety known for its good performance under an aerobic culture system (Kato and Katsura 2014) and tolerance to drought stress (Venuprasa et al. 2007; Swain et al. 2017;). However, Apo possesses undesirable phenotype, making it less acceptable to Filipino farmers and consumers. Mature seeds of NSIC Rc9 were subjected to in vitro mutagenesis in the 2009 dry season. The mutation technique combines tissue culture and gamma irradiation to enhance genetic variability and for trait improvement.

The study was conducted at the Plant Breeding and Biotechnology Division of the Philippine Rice Research Institute, Central Experiment Station (CES) in the Science City of Muñoz, Nueva Ecija.

Figure 1. Plant type (a), mature (b) and milled (c) grains, and panicle (d) of the wildtype, Apo.

In Vitro Culture (IVC) and Irradiation

Culture media preparation. Callus induction medium (CIM) containing 4.4 gl^{-1} Murashige and Skoog -based macronutrients and micronutrients (Murashige and Skoog 1962), 10 ml^{-1} of 100X ethylenediamine tetra-acetic acid iron (Fluka Chemika, Switzerland), 1 ml of B-vitamins, consisting of 1$mgml^{-1}$ glycine, 1$mgml^{-1}$ nicotinic acid, 1$mgml^{-1}$ pyridoxine-HCL and 1$mgml^{-1}$ Thiamine-HCL (Sigma Aldrich, Singapore), 0.1 gl^{-1} myo-inositol (Sigma Aldrich, Singapore), 30 gl^{-1} sucrose, 6 gl^{-1} casein hydrolysate (Sigma Aldrich, Singapore), 1 ml of 1$mgml^{-1}$ 6-benzyl amino purine (Sigma Aldrich, Singapore), 1 ml of 1$mgml^{-1}$ 2,4-dicholoroacetic acid (Sigma Aldrich, Singapore), and 1 ml of 1$mgml^{-1}$ naphthalene acetic acid (Sigma Aldrich, Singapore) was used. Regeneration media (RM) contains the same components and amount, except for naphthalene acetic acid of 0.5 ml^{-1} only. Both culture media were hardened with 3 gl^{-1} pharmaceutical agar (Pronadisa, Conda Lab, Madrid) and 1 gl^{-1} phytagel (Sigma Aldrich, Singapore). For CIM, 30 ml was dispensed in Gerber bottles covered with Gerber caps and 20 ml of RM was dispensed in a 50 ml Pyrex test tube covered with kaput. Culture media were autoclaved (TOMY SX-7000. Tomy Tech. United States, New York) with 115 psi for 15 min at 115°C.

Seed preparation, sterilization, and callus induction. Rough rice of the tested genotype was incubated at 50°C for 24 h and at room temperature for 1 day to break seed dormancy. Seeds were dehusked using a rice grain husker (Satake JLGJ2.5, Hubei-Pinyang Technology Co., Ltd., Hubei, China) to separate the hull from the brown rice. Dehulled seeds were cleaned manually to separate immature seeds and/or mixed grains to maintain the purity of the genotype. Seeds were then washed under running water for 30 min and rinsed with 70% (v/v) ethyl alcohol (Chemline Scientific, Philippines) for 5 s. Seeds were sterilized with 50% (v/v) sodium hypochlorite (Chemline Scientific, Philippines) for 30 min with agitation at 200 rpm, an orbital shaker (MaxQ2000, (Thermo-Scientific, United States) and were then rinsed with sterilized distilled water for three times. This procedure was repeated twice and the seeds were blotted dried in sterile petri plates inside the laminar hood (Hitachi, Japan) for 1 h. The dried seeds were cultured in 30 ml CIM cultures were incubated in the dark at 27 ± 2°C for two weeks until embryogenic callus is formed. Percent callus formation (%CF) was recorded.

Explant excision, irradiation, and regeneration. Explants were excised to separate the scutellar-derived callus from the seed and coleoptile. The calli were inoculated in a Pyrex petri dish, containing 10 ml of MS-based medium. The cultures containing 2-week-old calli were subjected to four different doses (10, 30, 50, and 70) of gamma rays (Chen et al. 2001; Lee et al. 2003a, b) at the Philippine Nuclear Research Institute (PNRI), Quezon City. Non-irradiated calli (0 Gy) were directly sub-cultured in regeneration medium (RM) as control. A total of 60 calli was exposed at each gamma irradiation level with two replications. The irradiated callus tissues sub-cultured in RM. The cultures were incubated at the light condition at 25 ± 1°C on lighted benches equipped with 36-Watt fluorescent lamp (GE, Philippines) at 16/8 h (light/dark) photoperiod until green plantlets were fully regenerated and developed.

Plant hardening, acclimatization, and IVM$_1$ family generation. *In vitro* regenerated plantlets at 4-weeks old, with fully developed shoots and roots, were taken out from the test tube and washed with running tap water to remove adhered medium from the surface of the roots. Plantlets were soaked in a mixture of 1:1 distilled water and MS liquid medium at 1 cm depth. Hardened plantlets were maintained under normal laboratory lighted conditions at 25 ± 1°C for 3 days and gradually decreasing the ratio of MS liquid. Plantlets were transferred into plastic cups with a root conditioning mixture of sterile soil and vermiculite (1:1) and watered with tap water for seven days. The potting medium was autoclaved with 121 psi for 20 min at 115°C prior to hardening. The acclimatized plants were transferred into pots under greenhouse conditions and grown to maturity. Plants regenerated from irradiated callus comprised the IVM$_1$ family. One panicle from each IVM1 family was harvested, which composed the IVM$_2$ generation. Harvested IVM$_2$ seeds were planted panicle-to-a-row under field conditions for agro-morphological traits evaluation.

Evaluation of IVM$_2$ Population for Phenotypic Variability

One panicle from each family was sown in a 1 m × 10 m seedbed with shallow furrows of 2 cm wide × 1 cm depth. Furrows were sprinkled with sawdust and the seedbed was covered with a used sack. A mylar barrier was established to protect the seeds from rodents. Two weeks after sowing, sacks were removed and the seedlings were grown up to 21 days. A water depth of 3 cm was maintained in the canals between seedbeds from three to five days from sowing and was increased to 5 cm before seedling pulling. Seedlings were transplanted panicle-to-a-row with a 20 cm distance between rows and hills. Each IVM2 plant was evaluated for 19 agro-morphological traits at vegetative, reproductive, maturity, and post-harvest stages.

Experimental Design and Data Analysis

Assessment of the tissue culture response of the genotype in the four gamma ray irradiation doses was laid out in a Randomized Complete Block Design, in which variability was analyzed by ANOVA and means were compared by Tukey's using the Statistical Tool for Agricultural Research (STAR), version 2.0.1 (IRRI 2020). Variability in morpho-agronomic traits was evaluated using frequency distribution and histogram, skewness, and kurtosis using the IBM SPSS Statistics Version 20 from the United States of America. Cluster analysis of the derived mutant population in terms of morpho-agronomic traits, by Ward's Method, was carried out using Statistical Tools for Agriculture Version 2.0.1 (IRRI 2020) to generate a dendrogram. The diversity index was measured using the Shannon Weaver Diversity Index (Hutcheson 1970) for qualitative traits (morphological traits) using the formula:

$$D = \sum_{i=1} Pi \log_n(Pi)$$

Where:

D = Diversity Index

P_i = proportion of variant "i" relative to the total population size

n = population size

RESULTS

In Vitro Mutagenesis

Tissue culture of 1,000 mature seeds of NSIC Rc9 produced 600 (60%) calli, which were subjected to four doses of gamma rays from ^{60}Co source. A total of 120 calli was subjected in each irradiation dose. In vitro culture responses include necrosis, tissue proliferation, and shoot and root formation (Figure 2). Among the four gamma irradiation doses, 10 Gy showed the highest regeneration efficiency (%R) of 12.5% which is not significantly different from the control (0 Gy), 30, 50 and 70 Gy which were 8.3%, 5.8%, 1.7%, and 0.8%, respectively. Proliferation and rooting were observed and ranged from 0.8% (30 Gy) to 27.5% (0 Gy), and 10% (70 Gy) to 25.8% (10 Gy), respectively.

Necrotic calli in 0 Gy and 10 Gy were 13.3%, and 15.8%, respectively, and were not significantly different from each other. The highest necrosis was obtained in 70 Gy at 53.3% (Table 1), indicating that at this rate the 50% inhibition dose or the LD$_{50}$ was reached. Results showed that increasing the gamma irradiation doses decreased the regeneration efficiency but increased the percent necrosis. A total of 54 IVM1 plants were produced but only 21 (38.9%) plants survived to maturity.

Trait Correlation of IVC Responses

Pearson's correlation analysis of the tissue culture response with gamma irradiation dose showed a strong correlation (Rho = 0.878) between necrosis and gamma ray irradiation dose (Figure 3a). This may imply that an increasing gamma ray irradiation dose results in a higher frequency of necrotic callus tissues, affecting other tissue culture responses. On the other hand, a strong negative correlation (Rho = 0.924) existed among gamma ray irradiation doses, root formation (Figure 3b), and (Rho = 0.792) regeneration (Figure 3c). This means that as the regeneration of shoots and roots decreases with increasing irradiation levels. A negatively moderate correlation (Rho = -0.249) was observed between gamma ray irradiation dose and proliferation of tissues (Figure 3d).

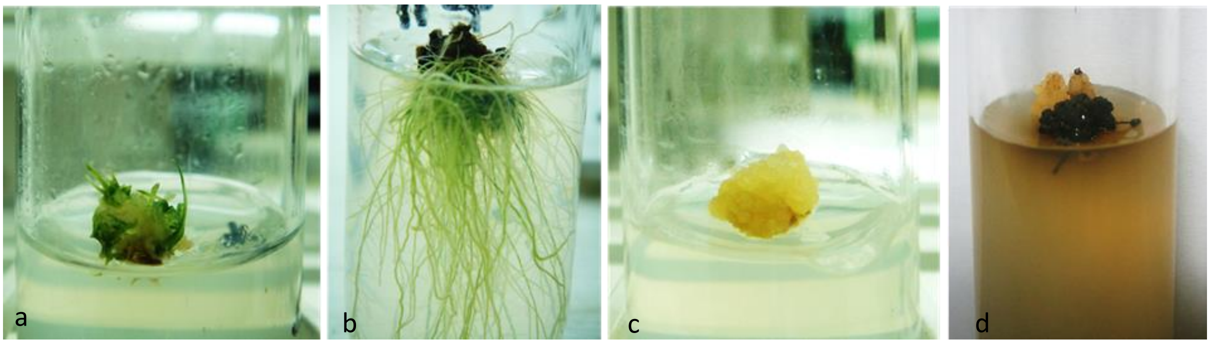


Figure 2. Response of callused tissues in regeneration: shoot formation (a), root formation (b), tissue proliferation (c), and necrosis (d).

Table 1. *In vitro* culture response of irradiated calli with different doses of gamma-ray in regeneration, Philippine Rice Research Institute, Central Experiment Station. Values with the same letter are not significantly different at α = 0.05 by Tukey's comparison of means ***highly significant in comparison to the control (0 Gy) by Dunnett's test SV-survival.

Dose (Gy)	No. of calli irradiated (CI)	Necrosis		Proliferation		Rooting		With regeneration		IVM$_1$ plants		
		no.	%/CI	no.	%/CI	no.	%/CI	no.	%/CI	no.	No. of slants survived	SV (%)
0 Gy	120	16	13.3^{c}	33	27.5^{a}	28	23.3^{a}	10	8.3^{a}	11	8	72.7
10 Gy	120	19	15.8^{bc}	23	19.2^{b}	31	25.8^{a}	15	12.5^{a}	23	9	39.1
30 GY	120	51	42.5^{ab*}	1	0.8^{c*}	24	20.0^{a}	7	5.8^{a}	15	2	13.3
50 Gy	120	65	54.2^{a*}	16	13.3^{b*}	16	13.3^{a}	2	1.7^{a}	9	1	11.1
70 GY	120	64	53.3^{a*}	16	13.3b*	12	10.0^{a}	1	0.8^{a}	1	0	0.0
Total/%	600	215	35.8	89	14.8	111	18.5	35	5.8	54	21	38.9

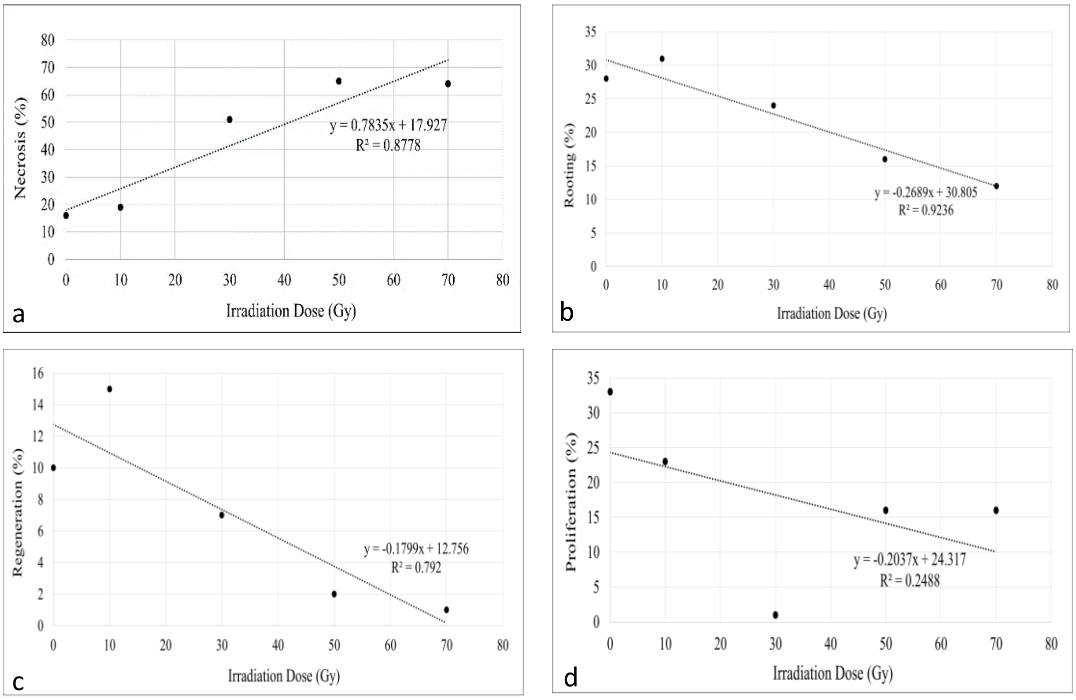


Figure 3. Correlation of *in vitro* culture response to irradiation doses: necrosis (a), proliferation (b), rooting (c), and regeneration (d).

Variability Evaluation of the IVM$_2$ Population

The 39 plants from 0 Gy, 106 plants from 10 Gy, 36 plants from 30 Gy, and 64 plants from 50 Gy, were generated and evaluated for agro-morphological variability for 19 traits.

Variation in morphological traits at the vegetative stage. The IVM$_2$ plants were variable in seven morphological traits across the four gamma ray irradiation doses evaluated at the vegetative stage of the crop (Figure 4). At 0 Gy, the majority of the population from each trait observed was similar to the wild type, NSIC Rc9. This observation indicates that only a minimal variation was induced using tissue culture alone, compared to those explants treated with gamma ray irradiation. In IVM$_2$ plants from 10, 30, and 50 Gy, showed equivalent and/or higher proportion of variable plants compared to the wild type, were observed (Figure 5). Based on the computed Shanon-Weaver Diversity index (SWI), high diversity (1.1 to 1.8) was observed in all of the seven traits across the four gamma ray irradiation doses utilized in the present study (Table 2). The qualitative traits evaluated were dominated by one category in each trait (Table 3). The dominant traits among the mutant plants generated from 0 Gy was green leaf blade with purple margins, intermediate leaf blade pubescence, purple leaf sheath, droopy leaf blade angle and purple color of ligule, collar and auricle. In the population generated from 10 Gy dose, the dominant traits were droopy leaf blades having green color with purple margin and intermediate pubescence. In terms of pigmentation, green leaf sheath was dominant. White ligule, white auricle and pale green collars were also among the dominant traits of the population. At 30 Gy, the majority of the population had leaf blade of green color with purple margin, intermediate pubescence and were droopy. Pale green and green color were dominant for collar and leaf sheath, and white color for the auricle and ligule. The majority of the mutants from 50 Gy, 90% had droopy leaf blades, dark green color and intermediate pubescence, and all of them have green leaf sheath, white ligule, pale green collar and white auricle to 100% of the population belonged to the dominant traits variable from the wild type, indicating a total change in the traits observed from the mutants. Blade pubescence (intermediate) and bland angle (droopy) in the irradiated plants were similar to the wild type. Induced variations were observed in blade color, leaf sheath color, ligule color, collar color and auricle color.

Table 2. Computed diversity index of the morphological traits at the vegetative stage, Philippine Rice Research Institute, Central Experiment Station.

Morphological Trait	Shanon Weaver Diversity Index			
	0 Gy, n = 39	10 Gy, n = 106	30 Gy, n = 36	50 Gy, n = 64
Blade Pubescence	1.3	1.3	1.3	1.5
Blade color	1.2	1.5	1.1	1.6
Leaf sheath color	1.4	1.8	1.3	1.5
Blade angle	1.4	1.3	1.4	1.6
Ligule color	1.3	1.4	1.3	1.5
Collar color	1.3	1.8	1.3	1.5
Auricle color	1.3	1.8	1.3	1.5

Figure 4. Variation in six morphological traits observed at the vegetative stage: basal leaf sheath color (a-purple,b-green), ligule color (c-purple, d-white), collar color (e-purple, f-green), auricle color (g-white, h-purple), blade leaf angle (i-erect, j-droopy) and leaf blade color (k-purple margin, l-dark green), observed in the NSIC Rc9-derived mutant population, induced by different doses of gamma-ray irradiation, PhilRice, Central Experiment Station.

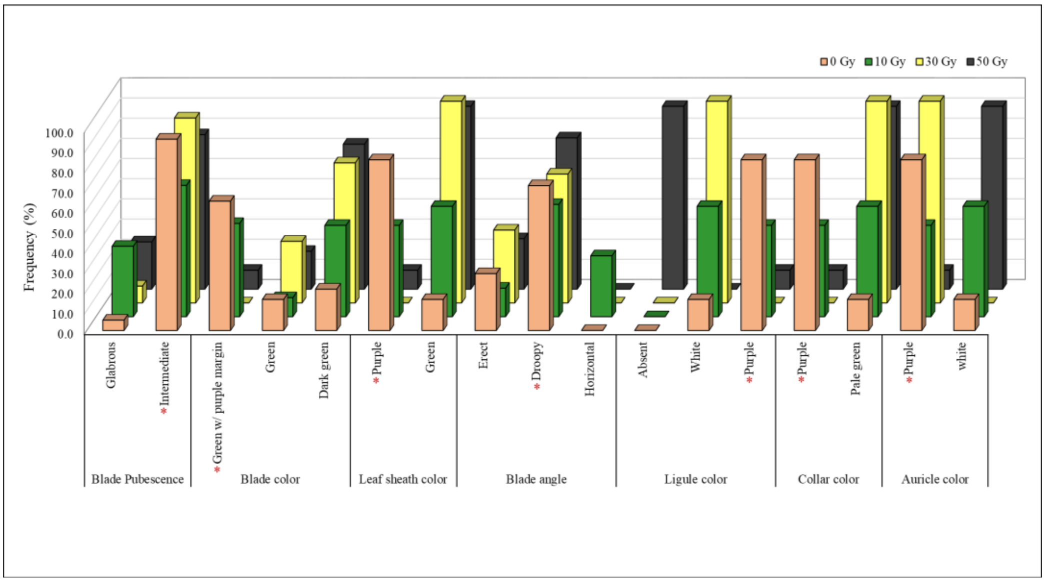


Figure 5. Frequency distribution for the seven morphological traits observed at the vegetative stage in each gamma ray irradiation level, PhilRice, Central Experiment Station (*trait of wildtype).

Table 3. Predominant morphological traits at the vegetative stage observed in each trait and gamma ray irradiation dose, Philippine Rice Research Institute, Central Experiment Station. Note: F = frequency.

No.	Morphological Trait	Predominant Trait per Gamma Irradiation Dose							
		0 Gy		10 Gy		30 Gy		50 Gy	
		Category	F (%)	Category	F (%)	Category	F (%)	Category	F (%)
1	Blade pubescence	Intermediate	94.9	intermediate	65.1	intermediate	91.7	intermediate	76.6
2	Blade color	green with purple margin	64.1	green with purple margin	46.2	dark green	69.4	dark green	71.9
3	Leaf sheath color	purple	84.6	green	54.7	green	100	green	90.6
4	Blade angle	droopy	71.8	droopy	55.7	droopy	63.9	droopy	75.0
5	Ligule color	purple	84.6	white	54.7	white	100	absent	90.6
6	Collar color	purple	84.6	pale green	54.7	pale green	100	Pale green	90.6
7	Auricle color	purple	84.6	white	54.7	purple	100	white	90.6

Variation in morphological traits at the reproductive stage. Variation in six morphological traits at the reproductive stage was observed (Figure 6). At 0 Gy, the majority of the IVM$_2$ plants from each trait was similar to the wildtype, NSIC Rc9. This result indicates that minimal variation was induced compared to those plants subjected to combined tissue culture and gamma ray irradiation tissue culture and gamma radiation. IVM$_2$ populations at 10, 30, and 50 Gy exhibited higher variability, compared to the plants generated from tissue culture alone (0 Gy). The frequency of variants for each of the six traits across the four gamma ray irradiation doses was also observed (Figure 7). Generally, 10 Gy produced the most variable plants that incurred the highest values ranging from 1.9 to 2.1 of SWI (Table 4). The qualitative traits, at the reproductive stage, were dominated by one category (Table 5). At 0 Gy, the dominant traits were intermediate flag leaf angle and culm angle, well exserted and droopy panicles, and semi-compact with dense branching panicle type. At 10 Gy, the majority of the mutants possessed intermediate flag leaf angle and culm angle, well exserted and slightly drooping panicles, and open sparse panicle type. Dominant traits of the mutant population generated from the 30 Gy dose were erect flag leaf and culm angle, well exserted and slightly drooping panicles, and semi-compact and dense branching panicles. At 50 Gy, the majority of the mutants were intermediate in flag leaf angle and culm angle, panicles are well exserted and slightly drooping panicles, and semi-compact sparse panicle type. Induced variations were observed in culm angle, flag leaf angle, panic type and panicle branching. Panicle exsertion (well) and panicle axis (slightly drooping) were similar across the four gamma irradiation doses.

Figure 6. Variation in six morphological traits observed at the reproductive stage: culm angle (a-erect, b-intermediate, c-open, d-spreading), flag leaf angle (e-erect, f-intermediate), panicle exsertion (g-enclosed, h-moderately exserted, i-well exserted), panicle axis (j-droopy, k-upright), panicle type (l-compact, m-semi-compact, n-open) and panicle secondary branching (o-sparse, p-dense), observed in the NSIC Rc9-derived mutant population, induced by different doses of gamma-ray irradiation, PhilRice, Central Experiment Station.

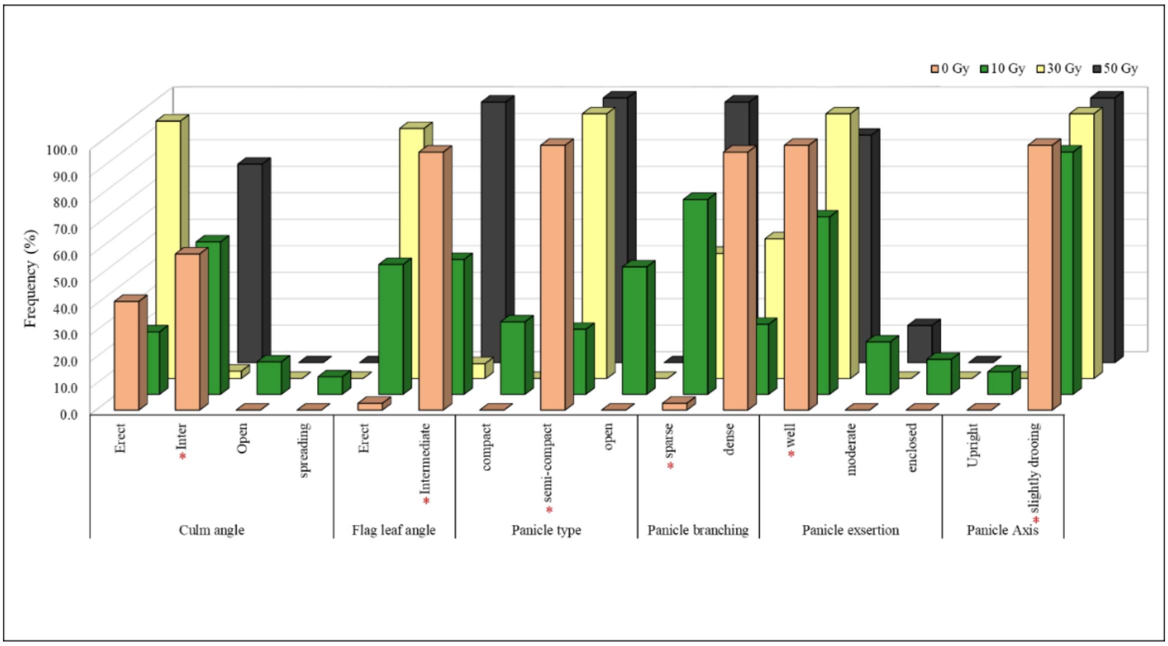
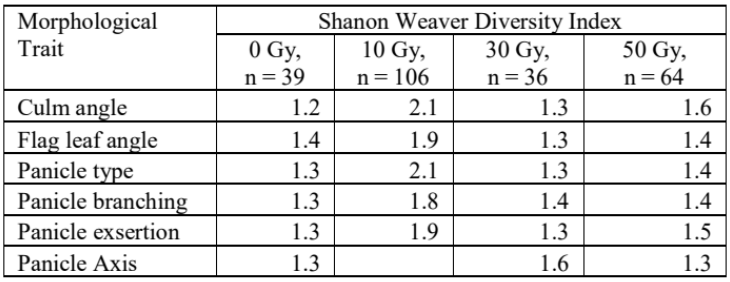


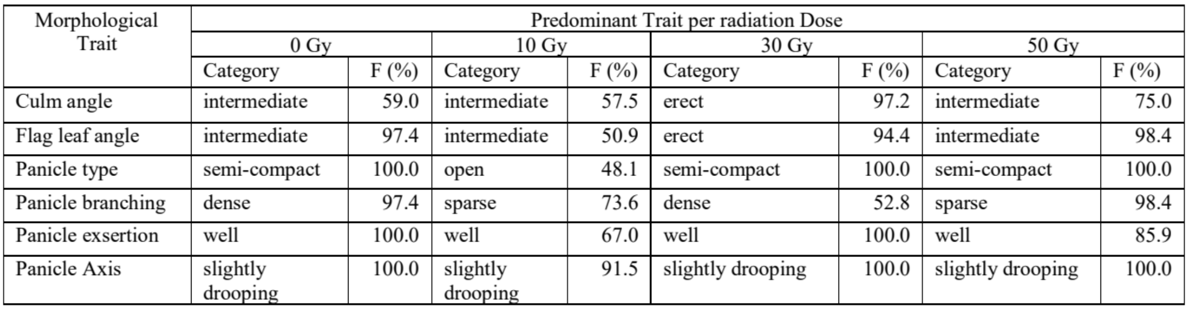
Figure 7. Frequency distribution for the six morphological traits observed at the reproductive stage in each gamma ray irradiation dose, PhilRice, Central Experiment Station. (*trait of wildtype).

Table 4. Computed diversity index of the morphologies at reproductive stage, Philippine Rice Research Institute, Central Experiment Station.



Morphological Trait	Shanon Weaver Diversity Index			
	0 Gy, n = 39	10 Gy, n = 106	30 Gy, n = 36	50 Gy, n = 64
Culm angle	1.2	2.1	1.3	1.6
Flag leaf angle	1.4	1.9	1.3	1.4
Panicle type	1.3	2.1	1.3	1.4
Panicle branching	1.3	1.8	1.4	1.4
Panicle exsertion	1.3	1.9	1.3	1.5
Panicle Axis	1.3		1.6	1.3

Table 5. Predominant morphological traits at the reproductive stage observed in each trait and gamma ray irradiation dose, Philippine Rice Research Institute, Central Experiment Station.

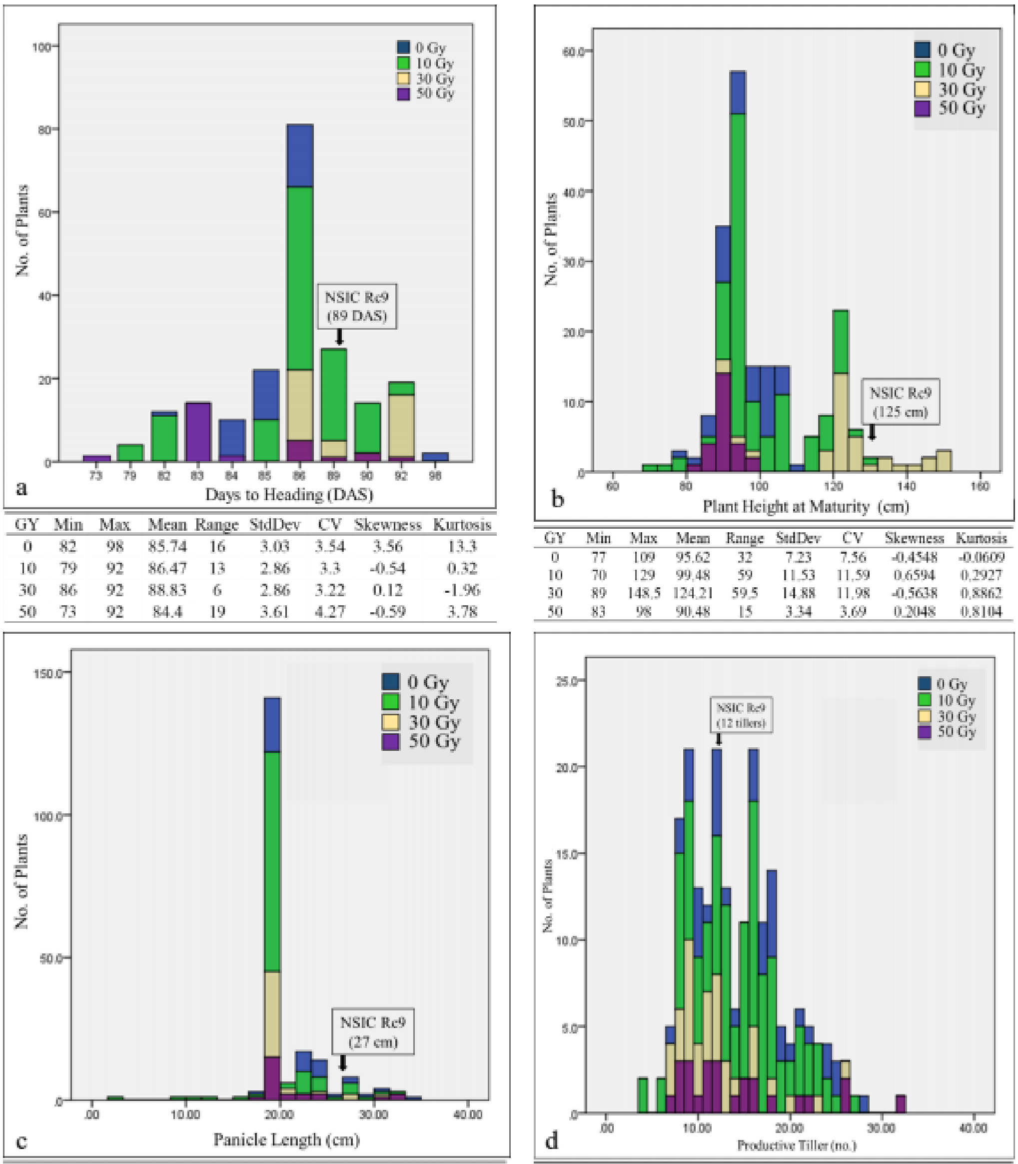


Morphological Trait	Predominant Trait per radiation Dose							
	0 Gy		10 Gy		30 Gy		50 Gy	
	Category	F (%)	Category	F (%)	Category	F (%)	Category	F (%)
Culm angle	intermediate	59.0	intermediate	57.5	erect	97.2	intermediate	75.0
Flag leaf angle	intermediate	97.4	intermediate	50.9	erect	94.4	intermediate	98.4
Panicle type	semi-compact	100.0	open	48.1	semi-compact	100.0	semi-compact	100.0
Panicle branching	dense	97.4	sparse	73.6	dense	52.8	sparse	98.4
Panicle exsertion	well	100.0	well	67.0	well	100.0	well	85.9
Panicle Axis	slightly drooping	100.0	slightly drooping	91.5	slightly drooping	100.0	slightly drooping	100.0

Variation in maturity stage. At maturity stage, variation in grain size and shape was assessed (Figure 8). In 0 Gy, the majority (67%) of the population had medium-intermediate grains that is similar to the widltype, NSIC Rc9, and 33% had long-slender grains. In 10 Gy and 30 Gy, 44% and 61% of the population had long-slender and slender grains, respectively, and the rest had the same grain size and shape with the NSIC Rc9. At 50 Gy, the majority (52%) had long-slender grains and the remaining 48% were medium-intermediate.

Variation in major agronomic traits. Mutant population from each of the irradiation dose were evaluated for variability in four agronomic traits: days to heading, plant height at maturity, panicle length and productive tillers (Figure 9). Heading days was less variable across the four irradiation doses (CV = 3.2% to 4.7%), indicating that this trait was not significantly affected by the gamma ray. Negative skewness was obtained from 10 (Sk = -0.4548) and 50 Gy (Sk = -0.5638), indicating that most of the mutant plants flowered earlier than the population mean, and that majority of the mutants have earlier heading days compared to the wildtype, NSIC Rc 9, which flowered at 89 DAS. Platykurtic kurtosis (-1.96) was observed in 30 Gy, indicating a distributed heading days around the mean (highly variable). Whereas, leptokurtic kurtosis was observed in the 0 (13.3), 10 (0.32) and 50 Gy (3.78) indicating that the heading days are concentrated near the population mean. For plant height, higher variability was observed from 10 Gy (CV = 11.6%) and 30 Gy (12%), compared to 0 Gy (7.6%). Across, irradiation dose most of the mutant plants had reduced plant height compared to the wildtype, NSIC Rc 9. Indicating the efficacy of *in vitro* mutagenesis in inducing variability for this trait. However, variation observed in 0 Gy may be attributed to somaclonal variation induced by tissue culture. Most of the mutants from 0 Gy and 30 Gy had shorter plant height in reference to the population mean. Platykurtic distribution was observed in 0 Gy and leptokurtic in the other irradiation doses. Induced variability for panicle length, across irradiation doses was 11.1% to 17.8%. Positive skewness in 0, 30 and 50 Gy was obtained, indicating that majority of the population have longer culm lengths in reference to the population mean. Positive kurtosis for panicle length was observed across doses. Wide variability was induced in panicle length, with 50 Gy having the highest at CV = 46%. Platykurtic kurtosis was observed in 0 and 10 Gy, whereas leptokurtic kurtosis was obtained in 30 and 50 Gy, indicating an increase or a reduction in the traits observed.

Figure 8. Variation in grain size and shape. Medium-intermediate grains of the wildtype, NSIC Rc9 and mutant exhibiting the same size and shape, and mutant with long-slender grains, PhilRice, Central Experiment Station.



GY	Min	Max	Mean	Range	StdDev	CV	Skewness	Kurtosis
0	82	98	85.74	16	3.03	3.54	3.56	13.3
10	79	92	86.47	13	2.86	3.3	-0.54	0.32
30	86	92	88.83	6	2.86	3.22	0.12	-1.96
50	73	92	84.4	19	3.61	4.27	-0.59	3.78

GY	Min	Max	Mean	Range	StdDev	CV	Skewness	Kurtosis
0	77	109	95.62	32	7.23	7.56	-0.4548	-0.0609
10	70	129	99,48	59	11,53	11,59	0,6594	0,2927
30	89	148,5	124,21	59,5	14,88	11,98	-0,5638	0,8862
50	83	98	90.48	15	3.34	3.69	0.2048	0.8104

GY	Min	Max	Mean	Range	StdDev	CV	Skewness	Kurtosis
0	17	34	22.47	17	3.64	16.21	1.31	1.72
10	3	33	20.52	30	3.54	17.23	-0.8	8.41
30	19	30	20.79	11	2.32	11.14	2.97	8.36
50	17	32	21.84	15	3.89	17.82	1.9	2.79

GY	Min	Max	Mean	Range	StdDev	CV	Skewness	Kurtosis
0	7	28	15.49	21	5.52	35.67	0.39	-0.72
10	4	27	14.47	23	4.88	33.71	0.18	-0.4
30	7	26	11.94	19	4.39	36.74	1.55	2.53
50	7	32	14.32	25	6.57	45.87	1.23	0.94

Figure 9. Variability and frequency distribution of the mutant populations generated from different doses of gamma irradiation, PhilRice, Central Experiment Station.

Cluster analysis. Cluster analysis visualizes further the degree of variation induced in each treatment using morphological traits at vegetative and reproductive stages, and agronomic traits. At 0 Gy, cluster analysis of the 39 mutants generated two major clusters (Figure 10). Cluster 1 consisted of wildtype, NSIC Rc9, and Cluster 2 consisted of the 39 mutant plants that were variable from the wildtype in terms of agro-morphological traits, such as leaf blade attitude, vegetative pigmentations, panicle traits, grain size and shape, and plant height. This clustering indicates that the mutant plants were 17% dissimilar and 83% similar to the wild type terms of phenotype. At 10 Gy, the 106 mutants were grouped into two major clusters (Figure 11). Cluster 1 was composed of 15 (14%) mutant plants and the wild type, indicating their similarity in the presence of purple pigmentation in leaf blades, leaf sheaths, collar, auricle and pistil. Whereas cluster 2 was consisted of 91 (86%) distinct mutant plants with a 16% dissimilarity from the wild type. Mutants in this cluster had no purple pigmentation in their vegetative parts, with long-slender grains. Cluster 2 was further subdivided into two major sub-clusters, wherein the first one consists of mutants with compact panicles with heavy secondary branching, and flowering at more than 90 DAS. The second sub-cluster was composed of mutants with flowering of less than 90 DAS. Cluster analysis of the 36 mutants at 30 Gy dose generated two major clusters (Figure 12) with 20% dissimilarity in reference to the wildtype, NSIC Rc9. The first cluster contains only the wildtype, NSIC Rc9, while the 36 mutant plants clustered together, indicating their variability from the wildtype. This cluster was further divided into two sub-clusters wherein one cluster was composed of mutants with intermediate culm angles and the other was composed of mutants with erect culm angle, and medium-intermediate grains. The second cluster was further sub-clustered into three groups. The first group were mutants with glabrous leaf pubescence, intermediate flag leaf angle and long-slender grains. The second group was composed of mutants with intermediate blade pubescence and erect flag leaf angle, leaf blade attitude, and long-slender grains. The third group were mutants having droopy flag leaf orientation. Cluster analysis of the 64 mutants at 50 Gy, grouped the purple pigmented wildtype and mutants, from the other mutants having no purple pigmentations, (Figure 13), indicating a 20% dissimilarity in agro-morphological traits. The first cluster was further sub-clustered into two groups separating the wildtype from the mutant, because of its droopy flag leaf angle and grain size and shape. Generally, *in vitro* mutagenesis-induced variation resulted in individuals possessing completely distinct characteristics from the wild type, NSIC Rc9 (Figure 14). Cluster analysis by agglomerative clustering showed that the similarity of the mutant plants, clustered independently from the wild type, NSIC Rc9 followed a decreasing pattern.

The variation in reproductive stage, *viz.,* erect flag leaf, erect culm angle, panicle axis and panicle secondary branching, and the variation in grain size and shape improved the farmer's acceptability of the mutants derived from NSIC Rc9.

DISCUSSION

In vitro mutagenesis of the rice cultivar NSIC Rc9, with four different doses of gamma rays, resulted in reduced callus formation and regeneration of callus pieces. Studies by Hossain and Alam (2001) and Islam (2020) showed that both callus growth and plant regeneration were severely reduced when the level of irradiation dose was increased. Similar trends were observed in tobacco (Degani and Pickholz 1973) and Dendrobium (Billore et al. 2019). Radiation could either promote or inhibit cell growth and differentiation of cultured tissues. This inhibition is attributed to the physiological effects of gamma radiation on the cell wall and cell membrane limiting the growth and proliferation of the callus tissues (Hasbullah et al. 2012). The limiting effect of irradiation is also attributed to the effect of radiation on the effectiveness of the exogenous hormones present in the culture media, thereby prohibiting auxin activities (Hughes 1981). Higher doses of gamma rays become toxic to plant tissues, increasing necrosis and reducing green plant regeneration. Induction of variation, by irradiation, in agro-morphological traits was observed at the vegetative and reproductive stages. High variability in agronomic and morphological traits was observed in mutant plants generated from the combination of tissue culture and gamma irradiation, compared to the variability induced by using tissue culture alone. The combined strength of tissue culture and gamma irradiation increased the mutation efficiency by producing more variants. The widened genetic variability provides a bigger venue for selection (Donini 1982; Donini and Sonnino 1998; Ahloowalia 1998). Irradiation of plant tissues results in various effects on the physiology and morphology of plants (Hase et al. 2010), due to the occurrences of mutations in their genetic compositions (Shu et al. 2009). These genetic mutations change the phenotypes of the plants, such as altered pigmentation, floral structures (Cabusora et al. 2020), and reduced plant height and maturity (Choi et al. 2021). In the study of Wu et al. (2005), a generated mutant population from IR64 exhibited huge agro-morphological variations at vegetative, reproductive and maturity stages, including plant architecture, growth habits, pigmentation, and various physiological characteristics.

The hierarchical relationship presented in the dendrogram showed the distinctness of the mutant plants from the wild type, NSIC Rc9, in terms of phenotype. Among the irradiation doses, 30 Gy and 50 Gy induced the highest variation in agro-morphological traits, compared to 10 Gy and, even more, to 0 Gy. The results showed the efficacy of double dose mutation induced by the combination of tissue culture and gamma ray irradiation (Li et al. 2019). Gamma ray irradiation induced a wide range of mutation spectra resulting in large induction of variation in phenotype and genotype of crops (Okamura et al. 2003).

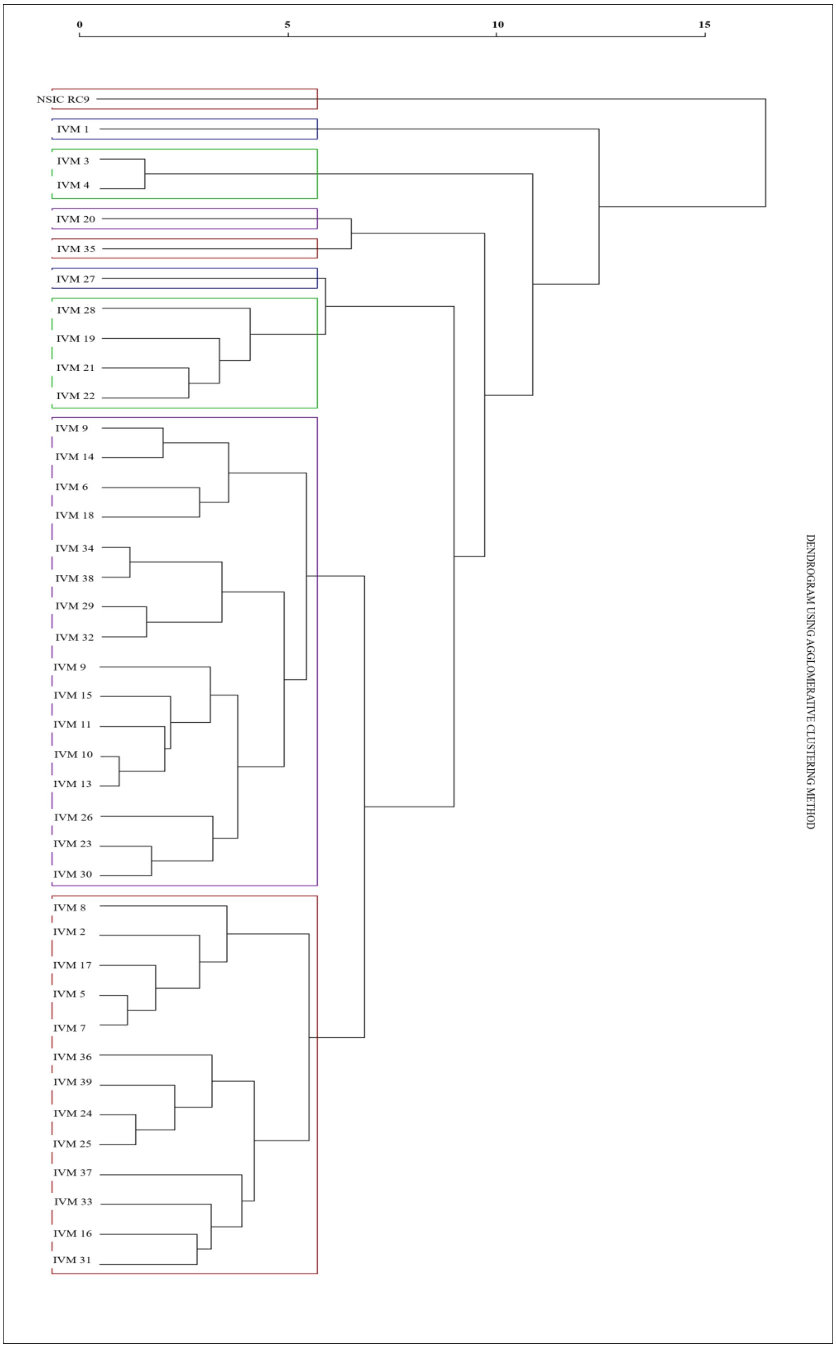


Figure 10. Dendrogram of the mutant population (N = 39) irradiated with 0 Gy gamma ray, PhilRice, Central Experiment Station.

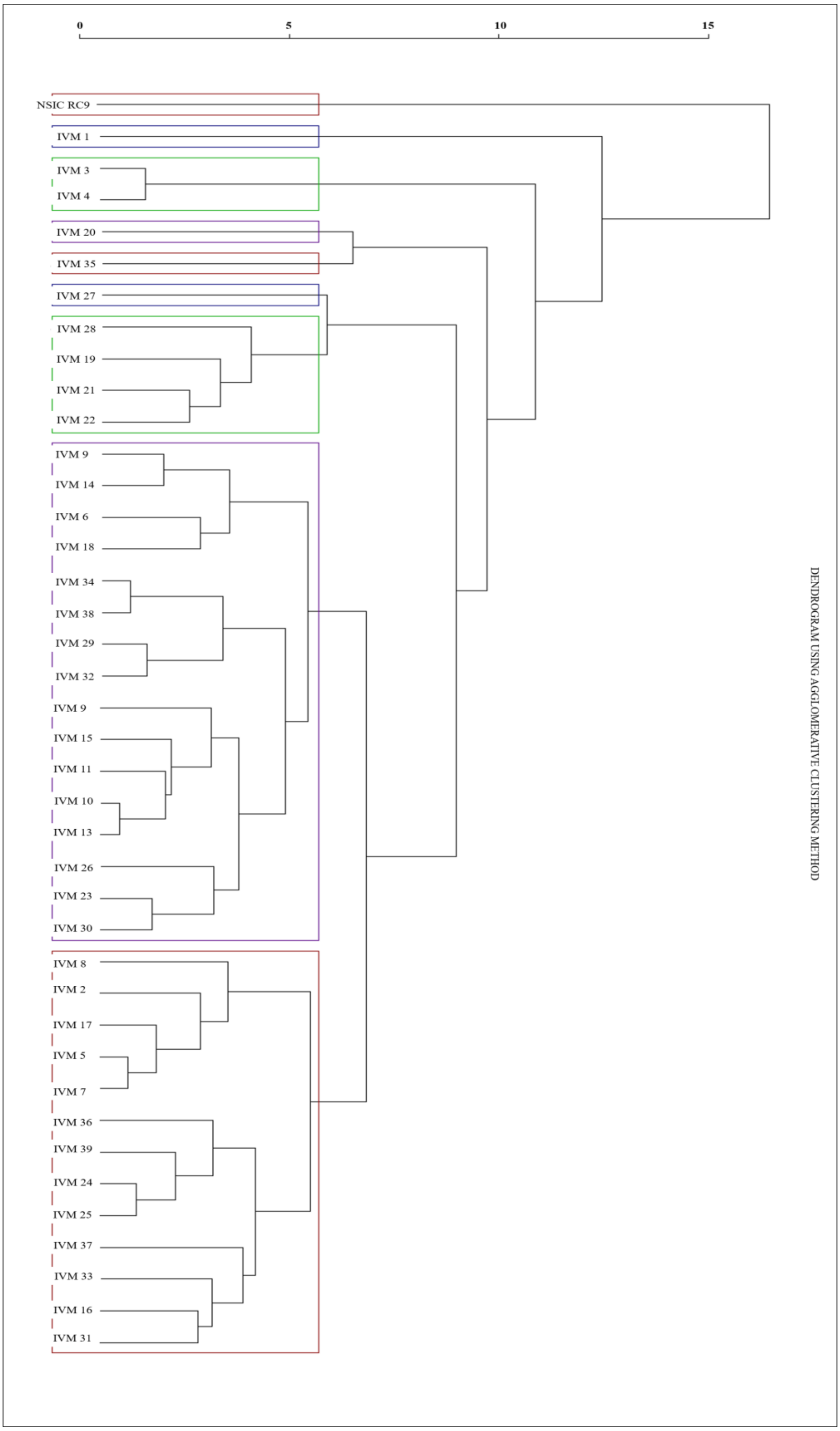


Figure 11. Dendrogram of the mutant population (N = 106) irradiated with 10 Gy gamma ray, PhilRice, Central Experiment Station

Figure 10.

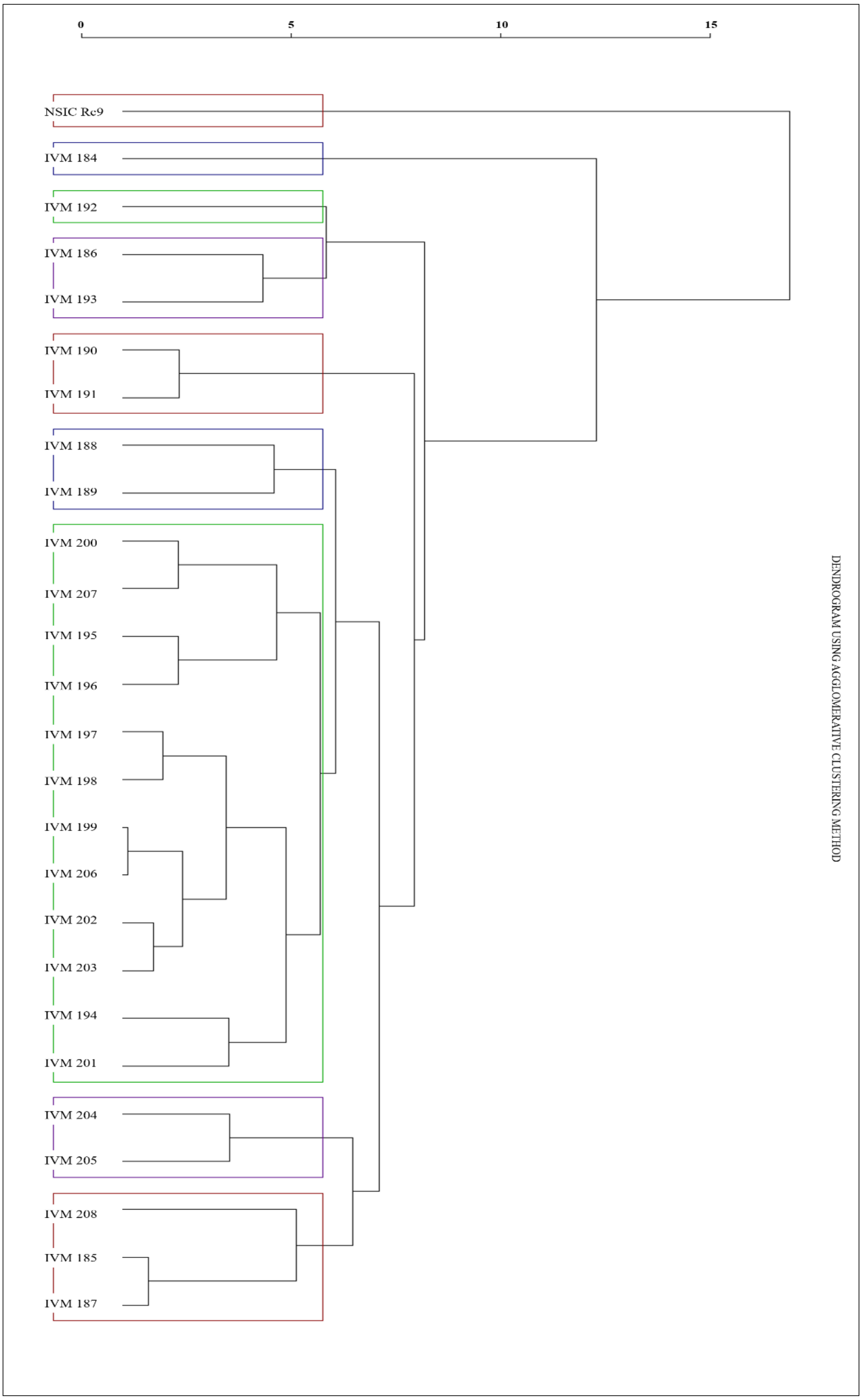


Figure 12. Dendrogram of the mutant population (N = 36) irradiated with 30 Gy gamma ray, PhilRice, Central Experiment Station.

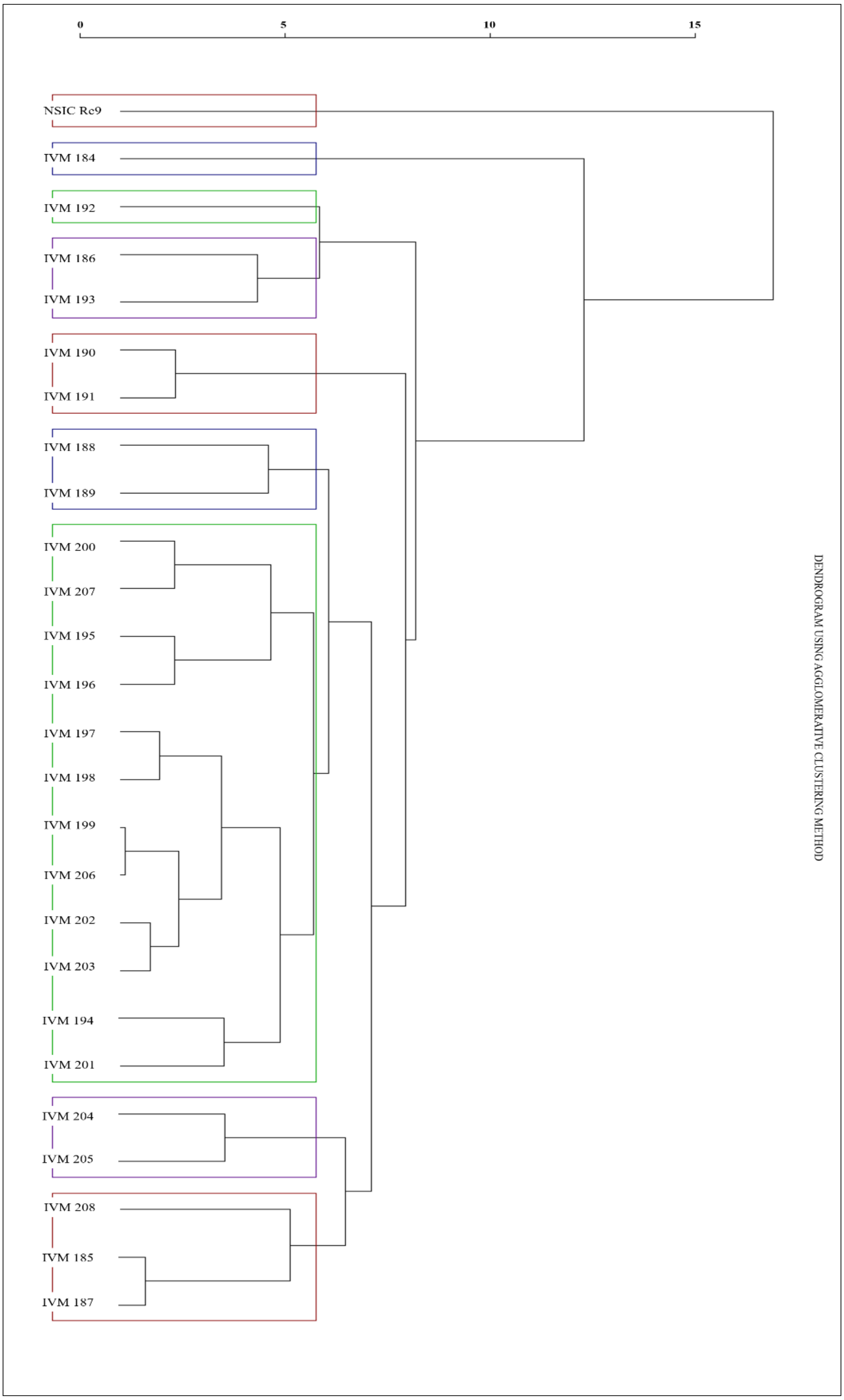


Figure 13. Dendrogram of the mutant population (N = 64) irradiated with 50 Gy gamma ray, PhilRice, Central Experiment Station.

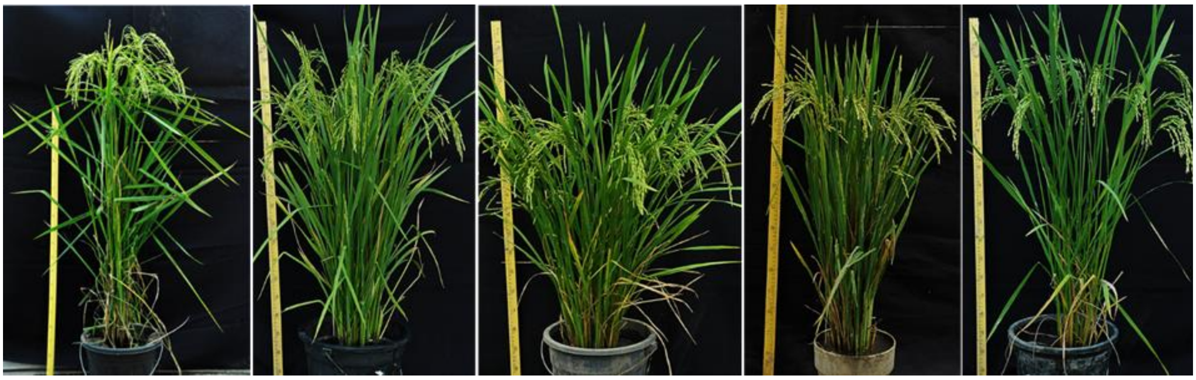


Figure 14. The phenotype of the generated mutant lines showing distinct morpho-agronomic traits compared to the wildtype, NSIC Rc9 (Apo), thereby grouping them in separate clusters, PhilRice, Central Experiment Station.

FUNDING

The research was funded by the Department of Agriculture, Philippine Rice Research Institute, Central Experiment Station (DA-PhilRice).

ETHICAL CONSIDERATIONS

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The authors declare that there are no competing interests to any authors.

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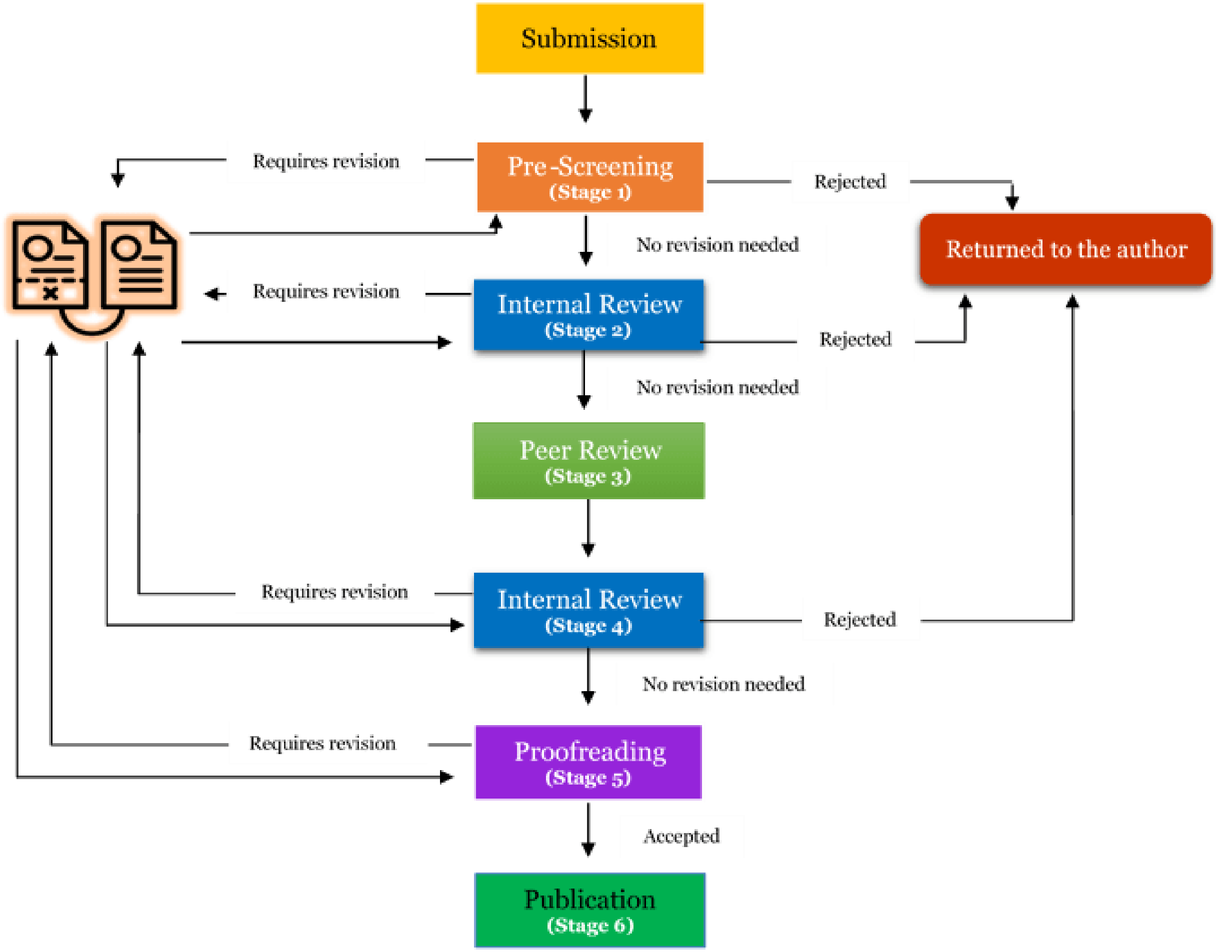

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