Kuroshio Science 11-1, 54-62, 2017

Symposium Proceedings

Density distribution of blue crab (*Portunus pelagicus*) larvae with implications to the lying-in concept of stock enhancement

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Abstract

The density distribution of larvae as indicator of spillover effect of lying-in hatchery concept for blue crab (*Portunus pelagicus)* in San Miguel bay was investigated from August to November 2015 to evaluate and verify the usefulness of lying-in concept. Plankton sampling was performed in different stations in Tinambac, Camarines Sur and Mercedes, Camarines Norte. Three concentric centers having one kilometer, two kilometers, and three kilometers diameter from the center (release area) were established in Tinambac, Camarines Sur. Crab larvae were collected from these stations of varying diameters during southwest (August-September) and northeast (November) monsoon. The same set-up was done in Mercedes, Camarines Norte where no lying-in station was established. Selected samples were subjected to DNA barcoding for identification and species composition. Findings revealed increasing pattern of abundance of crab larvae as the distance gets nearer to the release area which can be attributed as an effect of the intervention. Comparatively, such pattern was not observed in Mercedes where there is no lying-in station. This study also confirmed through DNA analysis that the collected crab larvae matched with the DNA of the parent crab thus contributing to stock enhancement. In-depth follow up and rigorous sampling on a monthly basis focusing on density distribution is recommended. Genetic tagging is also suggested for more conclusive information on the survival of the released larvae from lying-in facility.

Key words: Portunus pelagicus, lying-in hatchery, density distribution, DNA barcoding, stock enhancement

INTRODUCTION

Among the invertebrates, crabs are one of the most important invertebrate resources taken, and contribute significantly to global food supply. There are 51 species of swimming crabs reported in the country, but only about 7 are considered marketable. The blue crab (*Portunus pelagicus*) is the main species exploited, comprising over 90% of crab landings (Ingles 2004). It is also one of the most exploited crustacean food sources in the Philippines. In fact, according to FAO statistics (BFAR 2015), Philippines ranked seventh among the top fish producing countries in the world in 2013, with production of 4.87 million tonnes of fish, crustaceans, molluscs and aquatic plants (including seaweed).

Swimming crab fishery is suffering from a boom and bust trend since it started to be exploited for export and commercial purposes in the early 1990's. In Guimaras Strait, the records of buyers from 1992 to 1999 showed steady decline of fisher's average catch by 57.68% while from 1998 to 1999, blue swimming crab catch dwindled by 45.40% indicative of too much fishing pressure (Ingles and Flores 2000). In Panguil Bay, blue swimming crab with the same stock as the Visayan Sea and Guimaras Strait showed a catch dropped off by 76.5% from 50.3 to just 11.8 tonnes (Ingles and Flores 2000). In 2007, Regions V, Vl and Vll represented 51.26% of the total Philippines' blue swimming crabs productions (Romero 2009) but the total Philippines blue swimming crab landings showed two level plateaus, one before the peak in the early 1990's and second is after the peak in the late 1990's (Ingles 2004). It should be noted however that a 50% decrease of the biomass

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in a single fishery indicates overfishing. Recent studies in San Miguel Bay showed an estimated annual production of 524.90 metric tons swimming crabs which resulted to an overexploitation of the stocks (Nieves et al. 2013).

With the above mentioned statistics and realities on the dwindling stocks of swimming crabs, lying-in hatchery concept was identified as one of the doable management options that are fisher's friendly and science-based (Nieves et al. 2013). The concept of lying-in hatchery is holding egg-bearing blue crab in a 40-liter container and monitored until it hatches. After hatching (usually during early morning), breeder is being removed from the container to prevent bacterial infections. The newly hatched zoeae are harvested and restocked in the other containers with aerator. At night, hatched crab larvae will then be packed in fry bags filled with seawater and oxygen and transported by motorized banca and released in a designated area in San Miguel Bay.

With the aforementioned project being implemented, there is no direct evidence yet, or case reported measuring the success of this resource conservation practice in terms of abundance and distribution effect. Having this circumstance, the present study was designed to evaluate and verify the usefulness of lying-in hatchery concept estabished in Tinambac, Camarines Sur. Specifically, the study aimed to (1) identify crab broodstock in lying-in hatchery and the crab larvae collected using DNA barcoding; (2) determine crab larvae abundance and distribution as an effect of lying-in hatchery concept; and (3) identify crab larval stages and its density.

MATERIALS AND METHODS

Data source and collection procedures

San Miguel Bay is located in Bicol region on the Pacific coast of Luzon at around 14°N latitude and 123°E longitude. It is a shallow, estuarine body of water with an area of 1,115 km^2. Depth (average of 7.4 m) and salinity increases northward from the outfall of the Bicol River (near Cabusao) to the mouth of the bay facing the Pacific Ocean (Silvestre, G. T. and Hilomen V.V. 2004). A total of eighteen stations were established in Tinambac and Mercedes (Table 1, Fig. 1). Nine stations in Tinambac and another nine stations in Mercedes. Crab larvae were collected in three varying concentric circles with three stations each. Water quality parameters were characterized prior to sampling to ensure that the experimental sites should have at least the same salinity and temperature.

Sampling of crab larvae was performed during the months of August to September and November 2015 which coincides with the southwest and northeast monsoon seasons, respectively (Nieves et al. 2013). Samples were collected at night and near water surface to ensure more zoeae (Tagatz 1968). Plankton net with a mesh size of 80um and a ring on the mouth part measuring a diameter of one meter was used. The

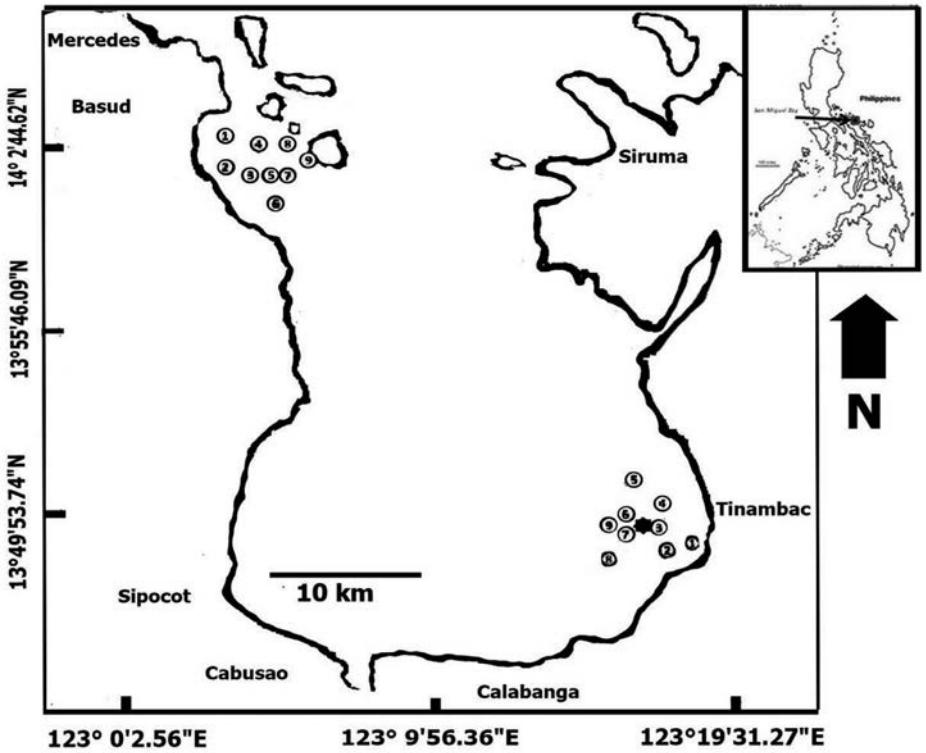

Fig. 1. Map of San Miguel Bay showing the sampling areas in Tinambac and Mercedes. Numbers enclosed within circles express sampling stations and closed star represents lying-in hatchery concept release area.

Table 1. Sampling Stations in Tinambac, Camarines Sur and Mercedes, Camarines Norte.

Stations		Latitude	Longitude
Tinambac (with lying-in station)	1	13°47'52.96"N	123°18'21.31"E
	2	13°47'44.55"N	123°17'34.42"E
	3	13°48'26.83"N	123°17'23.58"E
	4	13°49'25.69"N	123°17'31.84"E
	5	13°50'10.34"N	123°16'33.42"E
	6	13°48'54.61"N	123°16'24.85"E
	7	13°48'16.27"N	123°16'22.93"E
	8	13°47'27.45"N	123°15'38.09"E
	9	13°48'38.67"N	123°15'44.36"E
Mercedes (without lying-in station)	1	14° 3'9.74"N	123° 2'52.95"E
	2	14° 2'0.02"N	123° 2'58.08"E
	3	14° 1'36.32"N	123° 3'47.91"E
	4	14° 2'35.90"N	123° 4'5.21"E
	5	14° 1'40.63"N	123° 4'29.94"E
	6	14° 0'31.86"N	123° 4'41.22"E
	7	14° 1'27.53"N	123° 5'2.11"E
	8	14° 2'43.25"N	123° 4'59.22"E
	9	14° 2'0.88"N	123° 5'45.98"E

gear was approximately located 2.5 meters away from the hull of the boat to minimize being disturbed by the vessel during the course of sampling. Each run took ten minutes horizontal tow beneath the surface (0-1 meter deep) at a speed approximately one to two knots. During each run, speed of the boat was considered in such a way that the whole net was completely submerged in water. The start and end of each tow was marked using a Global Positioning System (GPS-Etrex Garmin) to determine the total distance covered.

Preservation of samples

The samples collected in the net were washed with water in such a way that all of the organisms caught were gathered in the cod end. Collected samples were fixed with 95% ethanol for analysis in the laboratory. Samples were then brought to laboratory for sorting and identification.

Sorting and identification

The samples were sorted with the aid of a stereomicroscope. The number of crab larvae and stages were counted and sorted for identification of their larval stages. All counts were based on the total samples and expressed in densities (number per m^3). Selected samples were also subjected to DNA barcoding (Alcantara et al. 2014) for further identification and species composition.

Data analysis

The abundance of crab larvae was computed as:

$$\text{Density} = (\text{no.of individual / haul}) / A * d,$$

where ka “no. of individual / haul” refers to the number of individual larvae collected per haul, A refers to the area of the plankton net, and d refers to the distance covered in sampling.

Descriptive statistics was used in computing distribution of the larval abundance. T-test (paired two sample for means) and analysis of variance (single factor) were also used to test the significant difference of crab larvae in terms of abundance among stations.

RESULTS AND DISCUSSION

DNA barcoding

The present study molecularly identified swimming crabs collected from Tinambac, Camarines Sur in San Miguel Bay, Philippines. One crab broodstock from lying-in set-up and fourteen crab larvae collected from San Miguel Bay were molecularly identified as *Portunus pelagicus* based on the generated cytochrome oxidase subunit 1 gene (CO1). Species-level designation was achieved after obtaining 99% to 100% sequence similarity search result when compared with the available reference sequences in the National Center for Biotechnology Information through Basic Local Search Tool (BLAST) and Barcode of Life Database System (BOLD) through BOLD-Identification Engine. BLAST is a reliably established database searching methodology for sequence comparison which optimizes a measure of local similarity through Maximal Segment Pair score (Altschul et al. 1990). On the other hand, the Barcode of Life Data System is a DNA barcoding workbench which holds and stores assembled barcode data and offers specialized services that cannot be given by global sequence databases. Just like the BLAST searching, query sequences are pasted to the Identification Systems (IDS) of the BOLD to facilitate molecular identification. BOLD IDS collects nearest neighbors through linear searching of a globally-aligned reference sequences (Ratnasingham and Hebert 2007).

The obtained DNA barcodes of the crab larvae specimens ranged from 639 to 687 base pairslong with an average length of 659 base pairs. The result of sequence analysis using Kimura-two Parameter model revealed that the mean conspecific genetic distance of the COI sequence barcodes

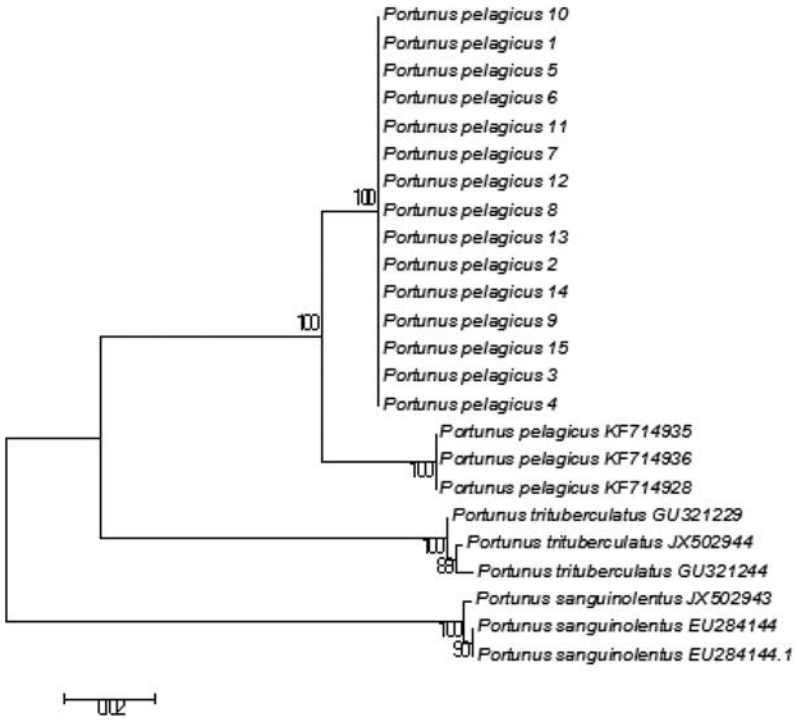

Fig. 2. Monophyletic clade in Neighbour-Joining Tree supporting the belongingness of blue crab (*Potunus pelagicus*) in Tinambac, Camarines Sur.

was 0.80% compared with 14.92% for species within family (*P. sanguinolentus, P. trituberculatus* as reference species). Hence, there was an 18-fold difference in genetic divergence among conspecific individuals compared with confamilial species. To further elucidate the DNA barcoding gap, the Nearest Neighbour Distance (NND) Analysis was provided. The mean distance to the nearest neighbor (*P. sanguinolentus*) was 18.76%, which is almost 23-fold higher than the mean intraspecific distance of 0.8%. Generally, high NND values were consistently obtained in the dataset, supporting clear existence of the DNA barcode gap.

The Neighbour Joining (NJ) tree algorithm in combination with bootstrapping is a heuristic approach to approximate posterior probabilities by progressively selecting taxon pairs from a set of taxa and build a new subtree for pairing (Felsenstein 1985, Saitou and Nei 1987, Munch et al. 2008). Aside from its capacity to handle large data set and faster analysis of sequences to delineate species boundaries (DeSalle et al. 2006, Nei and Kumar 2003), it is also simple which made it as one of the most widely used approach in a tree-based DNA barcoding inference. Moreover, a tree-building method, like NJ tree can assign the taxonomic affiliation of the specimens based on the phylogenetic grouping of the generated query sequence (Hebert et al. 2004a, b). In this study, all specimens formed a cohesive and strong monophyletic clade in the NJ tree supporting the belongingness of the species in the formed tree branch (Fig. 2). The specimens formed a solid clustering against the *P. sanguinolentus* and *P. trituberculatus* respectively. Further, all species clustered together to the reference sequence from the GenBank with mostly perfect bootstrap support values, confirming our claim that all species in the formed clade represent single species. In any DNA barcoding initiatives, a cohesive and distinct clustering in the inferred NJ tree should be prominent in the CO1 sequences to support species delineation (Steinke et al. 2009).

Abundance and distribution

A total of 185 crab larvae and 645 crab larvae were collected during the entire course of the study in Tinambac and Mercedes, respectively. The abundance of crab larvae was significantly higher (T-test, $P < 0.05$) in Mercedes (10.59 crab larvae/100 m^3) than in Tinambac (3.04 crab larvae/100 m^3) with lying-in hatchery concept intervention. This may due to the location of Mercedes which is offshore and near the mouth of the bay where female crabs breed and spawn; hence, the abundance of their larvae (Ong 1964, Hill 1974, Robertson and Kruger 1994 as cited by Quinitio et al. 2001).

The spillover effect of the lying-in hatchery concept in Tinambac was observed (Fig. 3) to show increasing pattern of abundance as the distance gets nearer to the release area which could be an effect of the intervention. On the other hand,

Table 2. Zoeal stages of crab collected during southwest and northeast monsoon in Mercedes and Tinambac stations.

Larval stages	SW Monsoon		NE Monsoon		Total
	Mercedes	Tinambac	Mercedes	Tinambac	
ZOEA 1	476	123	78	19	696
ZOEA 2	38	22	32	0	92
ZOEA 3	5	20	11	0	36
ZOEA 4	1	0	2	0	3
MEGALOPA	2	1	0	0	3
Total	522	166	123	19	830

opposite observation occurred in Mercedes where there is no lying-in hatchery intervention, wherein the pattern decreases as it gets nearer to the center.

Observation also shows that higher crab larvae were collected during the southwest monsoon than northeast monsoon season (Table 2). A total of 688 with mean density of 11.29 crab larvae/100 m^3 were obtained during the southwest monsoon and 142 with mean density of 2.33 crab larvae/100 m^3 during the northeast monsoon. Spawning of *P. pelagicus* is year round but the peak and lean seasons are influenced by monsoons (Ingles 1996). It breeds throughout year with two main spawning periods, one from February to April and another from July to October (Ingles 1989). Therefore, the

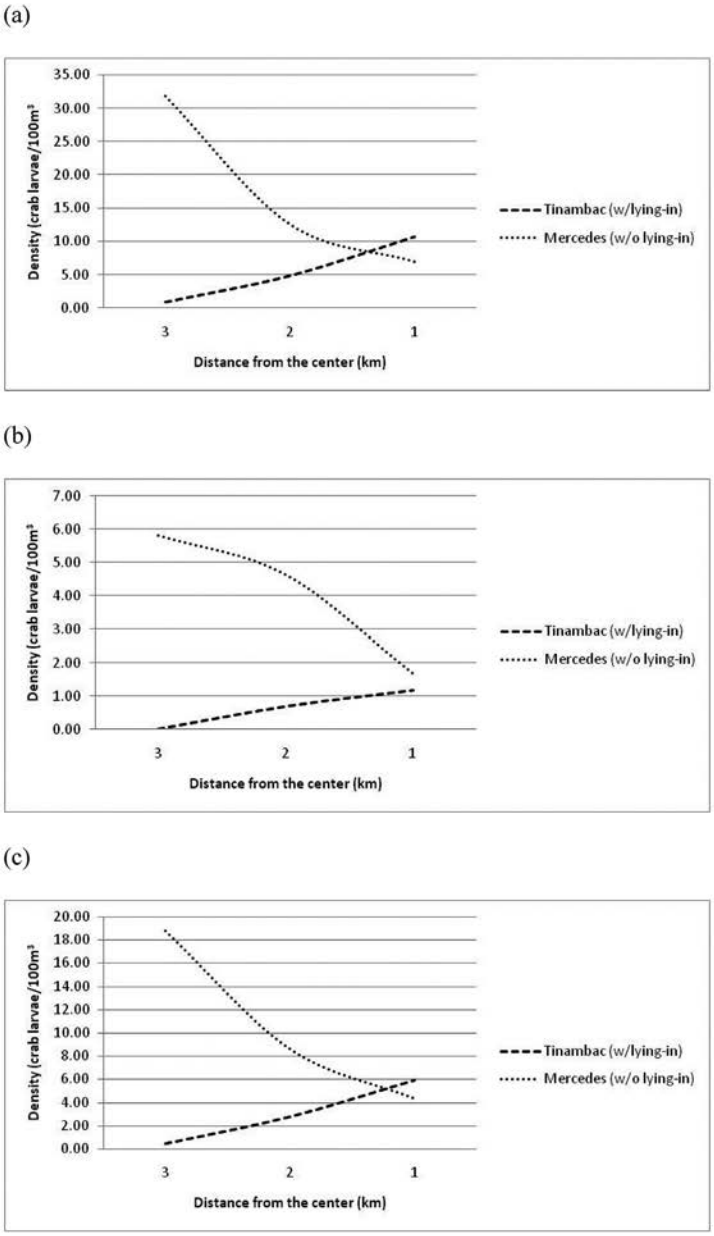

Fig. 3. Pattern of crab larvae density in different distances from the center during Southwest monsoon (a) and during Northeast monsoon (b). (c) Mean pattern of crab larvae density in different distances from the center.

higher abundance of crab larvae recorded during the southwest monsoon (August to September) corresponds with the main spawning periods of *P. pelagicus.* The lower abundance of crab larvae during northeast monsoon also coincides with the rainy season which can be attributed to the low light hence, a limiting factor for phytoplankton growth (Cloern 1987) thus resulted in less food concentration for the survival of crab larvae.

Larval stages

Different crab larval stages were collected and identified in the laboratory. Highest occurrence was observed in zoea 1 with a total of 696 larvae, followed by zoea 2, zoea 3, zoea 4 and megalopa with values 92, 36, and 3 larvae, respectively. Zoeal stages undergo three molting processes to become megalopa, the megalopa stages further molts twice into megalopa stage 1 and stage 2 (Ingles 1996). These stages are very prone to predators such as fishes, jellyfish, shrimp, and other planktivores. As crab larvae grow, the chances for survival decreases wherein one egg per million of crab larvae will survive to become an adult (Whitetaker 2000).

Results also revealed a higher concentration of early zoeal stages of crab larvae such as zoea 1 and 2 near the release area (center) which is probably a contribution of lying-in hatchery concept to stock enhancement in Tinambac. Zoea 1, zoea 2 and zoea 3 in 1 km distance have densities of 4.92 crab larvae/100 m^3, 0.69 crab larvae/100 m^3, and 0.29 crab larvae/100 m^3, respectively. However, these values decrease to 0.25 crab larvae/100m^3 for zoea 1 and only 0.10 crab larvae/100 m^3 for zoea 2 and zoea 3 stages in 3 km distance. On the contrary, early zoeal stages such as zoea 1, 2 and 3 are more concentrated in 3 km distance away from the center of the sampling stations in Mercedes (Fig. 4). Early zoeal stages from the natural spawning ground particularly from the mouth of the bay (Ong 1964, Hill 1974, Robertson and Kruger 1994 as cited by Quinitio et al. 2001) are being transported to Mercedes by means of the water circulation (Villanoy et al. 1994) which shows a pattern moving near shore and circulates

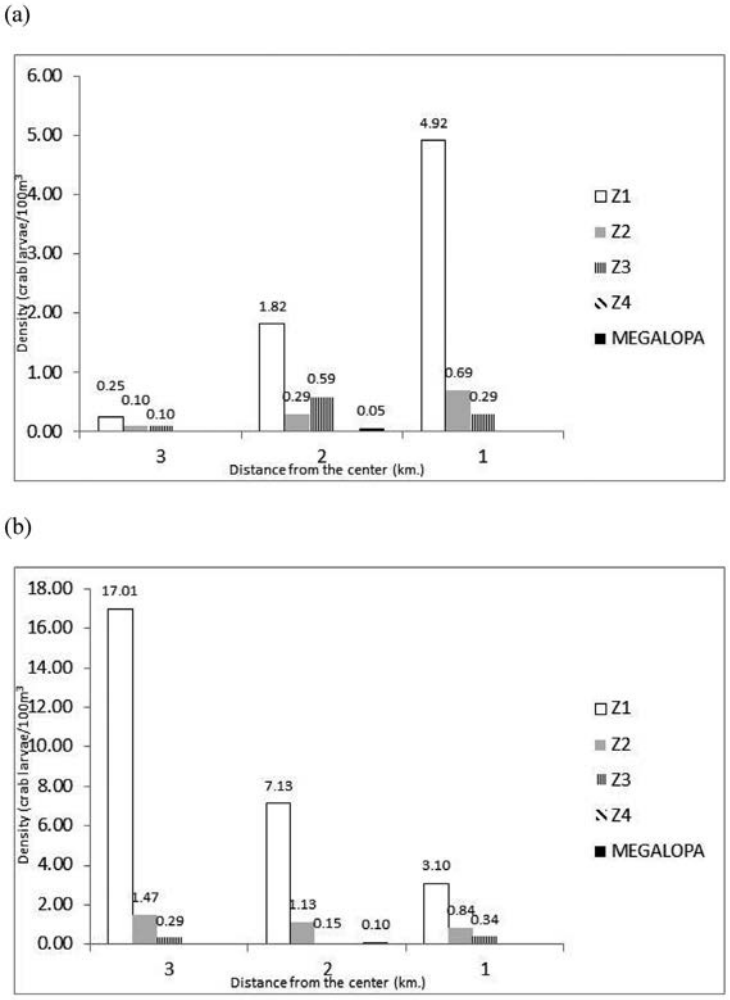

Fig. 4. Crab larval stages density in different distances from the center in Tinambac, Camarines Sur (w/lying-in, a) and in Mercedes, Camarines Norte (w/o lying-in, b).

to the center. Tidal, wind and water surface currents govern the transport of crab within the coastal ocean and estuaries (Tilburg et al. 2005).

CONCLUSION AND RECOMMENDATION

This study demonstrated the spillover potential of the lying-in hatchery concept as an approach for stock enhancement in San Miguel Bay. It revealed increasing pattern of abundance and occurrence of early zoeal stages as the distance gets nearer to the release area which can be attributed as an effect of the intervention. Comparatively, such pattern was not observed in Mercedes where there is no lying-in station. On the other hand, the abundance of crab larvae was higher during the southwest monsoon sampling (August-September) which coincides with the main spawning periods of blue crab (*Portunus pelagicus*) (Ingles 1989). This study also confirmed through DNA analysis that the collected crab larvae matched with the DNA of the parent crab thus contributing to stock enhancement. In-depth follow up and rigorous sampling on a monthly basis is recommended. Genetic tagging is also suggested for more conclusive information on the survival of the released larvae from lying-in facility.

ACKNOWLEDGEMENTS

The authors would like to thank LGU-Tinambac, BFAR-V, and Bicol University Tabaco Campus for the support of this study.

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