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Book:

Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds) Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of Rhazya stricta. 50th annual symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.) Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from internet: Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic Escherichia coli predator-prey ecosystem. Mol Syst Biol 4:187. www.molecularsystembiology.com

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Evaluating the stomach content of Wild Scalloped Spiny Lobster (*Panulirus homarus*)

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Abstract. *Amin M, Fitria A, Mukti AT, Manguntungi AB, Amrullah S, Alim S, Martin MB. 2022. Evaluating the stomach content of Wild Scalloped Spiny Lobster (*Panulirus homarus*). Biodiversitas 23: 6397-6403.* The high demand for spiny lobster seeds has placed intense pressure on the lobsters' wild stock in Indonesia. To address this issue, a hatchery was developed to produce scalloped spiny lobster larvae. However, the dietary requirements after the yolk sac has been depleted have proven to be a significant challenge. Thus, the present study aimed to identify the potential live diet of scalloped spiny lobster larvae by identifying the stomach contents of lobster larvae captured in the wild. Fifteen scalloped spiny lobsters at the post-larval stage were collected from three fishing grounds: Gerupuk Bay (Lombok Island), Tawang Bay (East Java), and Prigi Bay (East Java), Indonesia. The stomach of each scalloped spiny lobster was dissected under a dissecting microscope and its contents were observed under a binocular microscope for plankton identification and abundance. The stomach contents of scalloped spiny lobster resulted in five identified plankton species collected from Prigi Bay [*Tintinnopsis* sp. (37.5%), *Grammatophora* sp. (25%), *Synedra* sp. (18.8%), *Phormidium* sp. (4.3%), and *Rhizisolenia* sp. (4.3%)]; six plankton species collected from Tawang Bay [*Ochromonas* sp. (32.3%), *Synedra* sp. (20.6%), *Tintinnopsis* sp. (14.7%), *Uronema* sp. (14.7%), *Coscinodiscuss* sp. (2.9%), and *Planktoniella* sp. (2.9%)]; and six plankton species collected from Gerupuk Bay [*Synedra* sp. (33.3%), *Chlorococcum* sp. (33.0%), *Phormidium* sp. (13.3%), *Gymnidinium* sp. (6.6%), unidentified Cirripedia (3.3%), *Rhizosolenia* sp. (3.0%)]. Of these plankton, *Synedra* sp. and *Rhizolenia* sp. were the most common representatives in the stomach of all lobster samples. Thus, assessing these two plankton genera is highly recommended for future studies.

Keywords: Life below water, live prey, lobster, plankton, stomach content

INTRODUCTION

Lobster is one of the most prominent marine crustacean exports from Indonesia (Jones et al. 2020). According to the Indonesia Central Statistics Agency (2019), lobster is the fourth most valuable crustacean export, worth more than $1 million USD each year (Elvany 2020). Lobsters from Indonesia are exported to several Asian countries including Singapore, Hong Kong, China, Japan, Thailand, Malaysia, Vietnam, and Korea (Muzayyin et al. 2019). The lobster demand has steadily increased throughout time due to its nutritional value and flavour (Apriliani et al. 2021). Economically, the exported lobster has contributed to the actual Indonesian GDP (Firmanda 2021). However, because lobster aquaculture is still in its early stages, increasing market demand has put significant strain on wild populations. According to the exported statistics, the number of spiny lobsters taken in the wild was still much larger than that of cultured lobster. The biggest impediment to lobster aquaculture is a lack of understanding about diets and rearing techniques (Amin et al. 2022d). Furthermore, lobster seed for aquaculture purposes is still heavily reliant on wild stock since artificial generation of lobster larvae has yet to be established. As a result of the increased pressure and exploitation of wild stock lobsters in the larval stage and marketable size, the wild stock of lobsters in Indonesia is under threat. Overfishing of wild spiny lobster supply has reached in some places in Indonesia, including Awang Bay, Lombok Island, and Gunung Kidul, Central Java (Suman et al. 2021). If this trend continues, the natural lobster supply may become extinct in the next few years.

Many researchers have long been working on developing a lobster hatchery to generate lobster larvae for aquaculture purposes (Goldstein et al. 2019; Apriliani et al. 2021; Amin et al. 2022b). Nonetheless, those studies have only succeeded to breed and produce larvae, which lasted for only a few weeks (~1-2 weeks). This is mainly owing to the limited availability of suitable diets after the yolk sac has been depleted. Thus, one of the most challenging concerns faced in the lobster hatchery is the particular lack of understanding on the diet requirements of the larval stage (Nankervis and Jones 2022). Hence, investigations to identify potential diets for lobster larvae should be conducted, as this field of research is currently limited. Several research has reported on the identification of lobster diets, but restricted to specific localities. While investigations have clearly indicated that lobsters are opportunistic omnivores, whose diet reflects their environment (Blamey et al. 2019; Góes and Lins-Oliveira 2009; Suzuki et al. 2006), thorough diet profiling based on specific localities and environments ought to be investigated to better cater to the needs of the lobsters found within specific regions.

To the best of the authors' knowledge, there have been only a few research related to the identification of natural diets for scalloped spiny lobster (*Panulirus homarus* L) in Pacitan and Trenggalek district, East Java, Indonesia. These two locations are considered the most common fishing grounds for spiny lobster in East Java (Amin et al. 2022a; Amin et al. 2022b). Differences in physical, biological, and chemical properties of the fishing grounds may indicate different diet availability. As a result, this research is required to assess the natural feed of *P. homarus* at the post-larval stage in Pacitan and Trenggalek waters in order to obtain a comparison that can be utilised as a reference in sand lobster hatchery cultivation in Indonesia. The current study also included one of the most prolific spiny lobster fishing fields on Lombok Island to provide further information on diet type. Thus, the goal of this study was to access and compare the natural feed of *P. homarus* in different places during the post-larval stage: Tawang Bay, Prigi Bay, and Gerupuk Bay, Indonesia.

MATERIALS AND METHODS

Sampling Sites

The present study was conducted in three locations in which *P. homarus* post-larvae were commonly caught. These locations were Prigi Bay, Trenggalek District, East Java (location 08°17'18" S and 111°43'42" E), Tawang Bay, Pacitan District, East Java (location 8°15'40.71" S and 111°17'3.62" E) and Gerupuk Bay, Central Lombok District, West Nusa Tenggara, Indonesia (location 116°20'3.7" E and 8°56'1.3" S) (Figure 1).

Table 1. The mean measurements for carapace length, total length, and total weight of *Panulirus homarus.*

Location	Carapace length (cm)	Total length (cm)	The total weight (g)
Tawang Bay	0.42±0.25	2.10±0.10	0.16±0.04
Prigi Bay	0.42±0.01	1.82±0.08	0.11±0.02
Gerupuk Bay	0.44±0.11	1.96±0.11	0.14±0.02

Note: Values are average with a standard deviation of five lobster replicates.

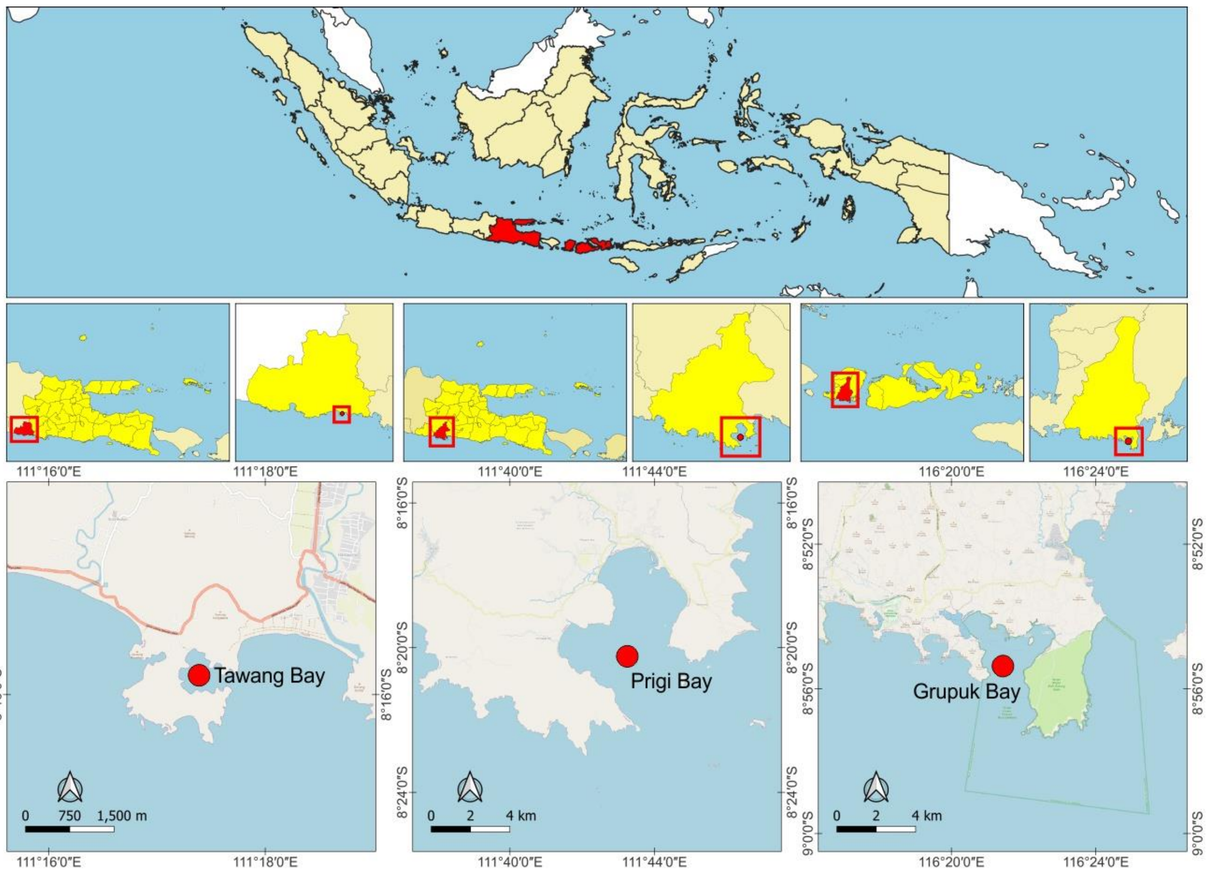

Figure 1. Three sampling locations at which *Panulirus homarus* were collected: Tawang Bay, Prigi Bay (East Java), and Grupuk Bay (West Nusa Tenggara), Indonesia

Figure 2. Post-larval stage of scalloped spiny lobster (*Panulirus homarus*). Five lobster individual were collected from each geographical location.

Samples collection

Post-larval individuals of *P. homarus* were collected from three different geographical locations in August 2021, which was the peak season for catching post-larval lobsters (Amin et al. 2022b). A few '*pocongan'* (traditional lobster traps) were randomly placed at night in strategic areas of the selected sampling sites and were collected in the morning. The lobster samples were then directly preserved in plastic pots filled with 76% ethanol for fixing and preservation. The size of lobster samples collected from three different locations are presented in Table 1 and Figure 2. Thereafter, the stomach of each scalloped spiny lobster was dissected by cutting the cephalothorax lengthwise towards the telson using a sterile sectio-set. Stomach content was then collected and stored in absolute ethanol.

Water sampling

Water sampling procedures was adapted from Amin et al. (2022b). Water samples from three sampling locations were collected using a 2.4L-water sampler at three different depths: 0-0.15 m (surface), 2.5 m, 5 m, and bottom waters at night (20.00-21.00). The water samples were then filtered using a plankton net. The filtered results were stored in a bottle with added Lugol to reach a concentration of 1% to preserve plankton. The samples were kept on ice and transported back to the laboratory for further processing. Samples were observed at the Microbiology Laboratory of the Faculty of Fisheries and Marine Affairs, Airlangga University, Indonesia.

Identification of plankton in stomach content and water samples

The preserved stomach content from each lobster and water sample was assessed under a binocular microscope. The stomach content and water were pipetted and placed on an object glass covered with a cover slip and observed as described by Aqil et al. (2013). Plankton were identified to genus according to the guidelines from Suthers et al. (2019) and Richardson et al. (2019), and abundance (%) follow the work of Sugiharto and Tjahjono (2021):

$$\text{Abundance (\%)} = \left(\frac{\text{Cell numbers/individual}}{\text{Total cell number/total individuals}}\right) \times 100$$

The abundance of natural food in the digestive tract was calculated and sorted based on each taxonomic plankton identified.

RESULTS AND DISCUSSION

Physical and chemical parameters

The chemical and physical conditions of the fishing grounds from Prigi Bay, Tawang Bay, and Gerupuk Bay include measurements of temperature, dissolved oxygen (DO), salinity, pH, and nitrate (Table 2). The temperature was found to be relatively comparable in all three locations, ranging between 27-30°C, with a standard nitrate profile of 0.01 $mg.L^{-1}$. Prigi Bay had a lower salinity (26 ppt) as compared to Tawang Bay (35 ppt) and Gerupuk Bay (31 ppt). Tawang Bay had the lowest dissolved oxygen of 5.35 $mg.L^{-1}$ and Gerupuk Bay has the highest pH of 8. In addition, dissolved oxygen ranged from 5.35 $mg.L^{-1}$, in Pacitan Bay, 7.21 mg/L^{-1} in Gerupuk Bay, and 7.48 $mg.L^{-1}$ in Prigi Bay. There were variances in the type of substrate across the three locations, with Prigi Bay being muddy; and Tawang Bay and Gerupuk Bay being sandy.

Identification of plankton stomach contents

Plankton detected in the stomach contents of *P. homarus* were categorised based on the findings from the three sampling sites (Figure 3). Plankton genera with incomplete forms were categorised as "unidentified" or at least to ahigher taxa with fairly recognisable morphological traits. Figure 4 shows representative images of the detected plankton from the post-larval stage stomach content of *P. homarus*. Five plankton genera were readily identified in the lobsters' stomach contents from Prigi Bay: *Tintinnopsis* sp. (37.5%), *Grammatophora* sp. (25%), *Synedra* sp. (18.8%), *Phormidium* sp. (4.3%), and *Rhizisolenia* sp. (4.3%), while the remaining 10.1% of stomach contents were unidentified (Figure 3A).

Table 2. Water quality parameters measured in the three different sampling locations.

Location sample	Temperature (°C)	DO (mg.L^{-1})	Salinity (ppt)	pH	Nitrate (NO_3: mg.L^{-1})
Prigi Bay, Trenggalek	27-30	7.48	26	7	0.01
Tawang Bay, Pacitan	28.2-28.3	5.35	35	7	0.01
Gerupuk Bay, Central Lombok	27.4-28.1	7.21	31	8	0.01

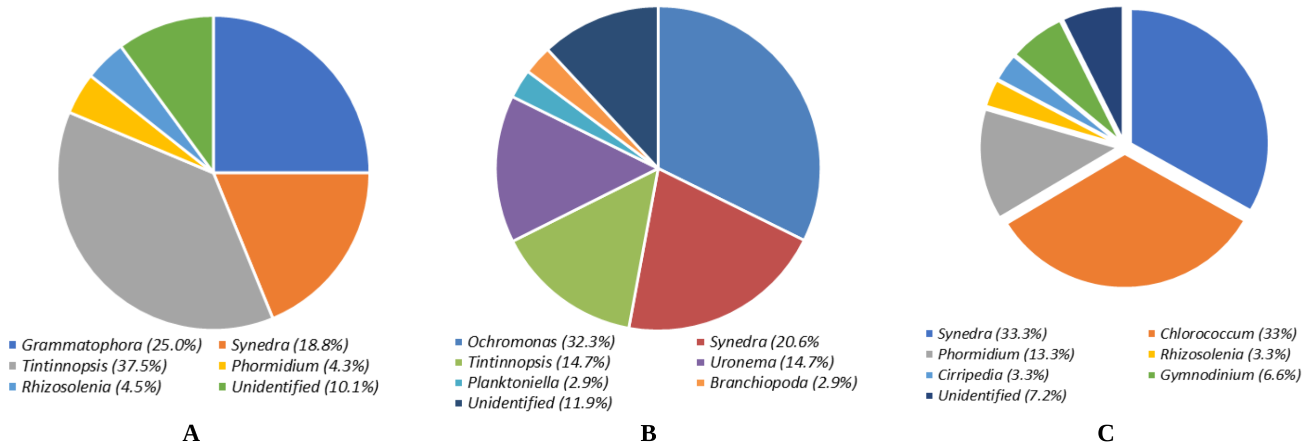

Figure 3. Stomach content analysis of *Panulirus homarus*, collected from the three different natural habitats: (a) Prigi Bay, (b) Tawang Bay (East Java), and (c) Gerupuk Bay, West-Nusa Tenggara Indonesia

As for Tawang Bay, six plankton genera were identified, with *Ochromonas* sp. being the most common (32.3%), followed by *Synedra* sp. (20.6%), *Tintinnopsis* sp. (14.7%), *Uronema* sp. (14.7%), *Coscinodiscuss* sp. (2.9%), *Planktoniella* sp. (2.9%), and an unidentified Branchiopoda (2.9%), while, 11.9% of stomach contents were unidentified (Figure 3B).

At Gerupuk Bay, Lombok Island, *Synedra* sp. (33.3%) was the most dominant species, followed by *Chlorococcum* sp. (33.0%), *Phormidium* sp. (13.3%), *Gymnidinium* sp. (6.6%), an unidentified Cirripedia (3.3%), and *Rhizosolenia* sp. (3.0%), while 7.2% of stomach contents were unidentified (Figure 3C).

All lobster stomach contents resulted in *Synedra* sp. being the most common plankton from the three localities. *Tintinnopsis* sp. were commonly recorded from the stomach contents *P. homarus*, collected from Prigi Bay and Tawang Bay, whereas *Phormidium* sp., and *Rhizosolenia* sp. were common plankton collected from the stomach contents of *P. homarus* from Prigi Bay and Gerupuk Bay.

Plankton in the natural habitat of scalloped spiny lobster

Plankton genera and their abundance from waters of the fishing grounds of *Panulirus homarus* were identified, with *Synedra* sp., *Grammatophora* sp. and *Tintinnopsis* sp. being present in all three localities (Figure 5). When compared to the results of Figures 3 and 5, several plankton species that were present in the marine environment were also present in the digestive tracts of *P. homarus*. From Prigi Bay, these includes: *Synedra* sp. *Tintinnopsis* sp., *Phormidium* sp., *Rhizosolenia* sp., and *Grammatophora* sp.; Tawang Bay: *Synedra* sp. *Tintinnopsis* sp. and *Ochromonas* sp.; Gerupuk Bay: *Synedra* sp.

Discussion

Live diets at the larval phase have become one of the current challenges in producing spiny lobster larvae in hatcheries. Amundsen and Sánchez-Hernández (2019) reviewed that one potential approach to finding potential diets of the organism is by identifying stomach contents. Thus, the present study investigated the stomach contents of spiny lobster at the post-larval stage, a transition from floating to a sedentary phase, as a way to identify potential natural diets. The results showed that the types and abundance of plankton identified in the stomach of scalloped spiny lobster varied, and grouped based on their sampling locations. These results might imply that the diets of spiny lobster larvae are not determined by lobster species, but rather the diets readily available in their respective environments (opportunistic feeders). Conel et al. (2014) suggested a similar hypothesis in which the spiny lobster was an opportunistic predator that fed on numerous species of zooplankton that were accessible in the environment. Butler and Kintzing (2016) described that scalloped spiny lobster tended to be carnivorous and feed on zooplankton, crustacean, molluscs, and gastropods, although they also discovered that lobster fed on several microalgal species. Ihsan et al. (2019) added that various types of phytoplankton, zooplankton, and diatoms in the ocean ecosystem can be a natural feed for lobsters in the larval phase.

The present study identified at least 13 plankton genera from the stomach of scalloped spiny lobsters at post-larval stages. These are *Grammatophora* sp., *Synedra* sp., *Tintinnopsis* sp., *Uronema* sp., *Ochromonas* sp., *Chlorococcum* sp., *Planktoniella* sp., *Phormidium* sp., unidentified *Rhizosolenia*, unidentified Branchiopod, unidentified Oligochaete, unidentified Cirripedia, and unidentified *Gymnodinium*. Of these, the highest prevalence was *Synedra* sp., detected in the stomach of scalloped spiny lobster collected from the three sampling locations: Prigi Bay (18.8%) and Gerupuk Bay (33.3%), and Tawang Bay (20.6%). In addition, *Synedra* sp. was also detected in the natural habitat of lobster in all locations. *Synedra* sp. has been previously reported as a live diet for crayfish (Bahadir Koca and Argun uzunmehmetoğlu 2018), fish (*Puntioplites bulu*) (Intan-Faraha et al. 2020), and found in tilapia and catfish guts (Zaidy 2022). These studies suggest that *Synedra* sp. is a common diet for aquatic species, including lobster larvae. This might be due to the high tolerance of *Synedra* sp. that enables the diatom (Bacillariophyta) to thrive in both freshwater (Harmoko and Krisnawati 2018) and marine water (Van de Vijver and Ector 2020).

Figure 4. Plankton representatives identified from the post- larval stomach contents of *Panulirus homarus*

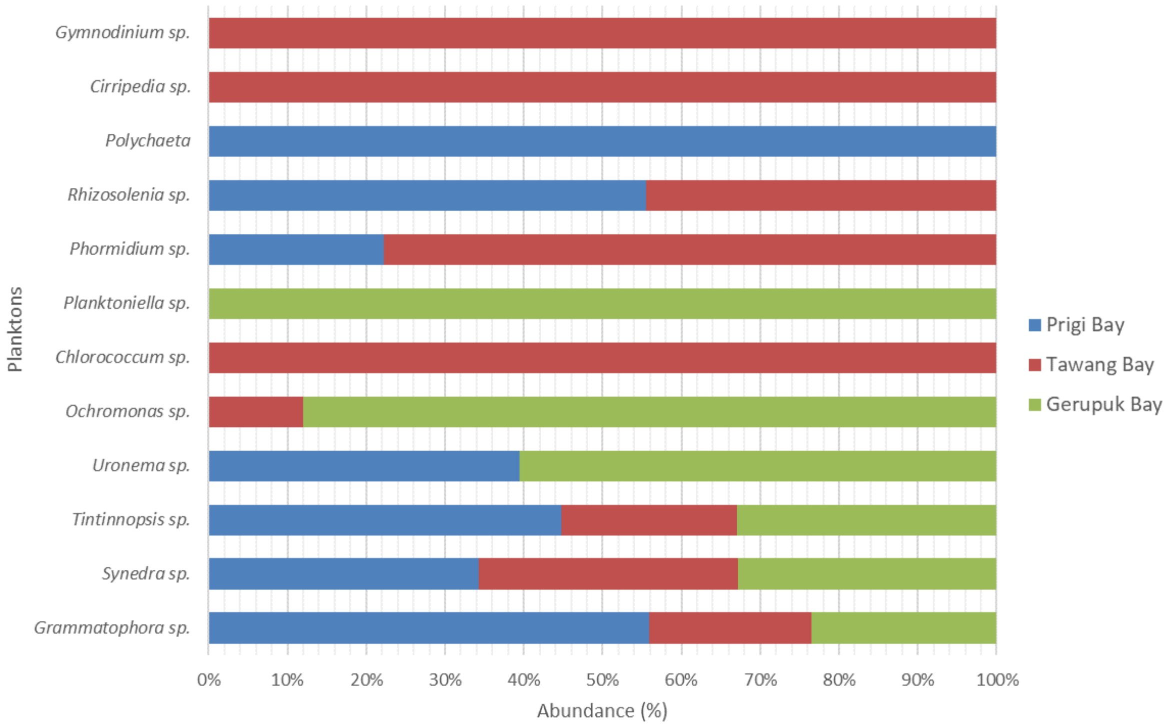

Figure 5. Plankton species and their abundance identified from waters of the three fishing grounds of *Panulirus homarus.*

Rhizosolenia sp. is another diatom that was also found in the stomach content of scalloped spiny lobster and its natural habitat (Tawang Bay and Prigi Bay). The diatom genus was also previously reported as the most dominant live diet of Cladocera (Goldstein et al. 2019). In addition, a study by Van Stappen et al. (2020) reported *Rhizosolenia* sp. as a live natural diet of brine shrimp larvae (*Artemia* sp.) collected from Albastross Bay, Gulf of Carpentaria, Australia. Similarly, *Rhizosolenia* sp. has been reported as one of the most common diets of black oyster (*Hyotissa hyotis*, linné, 1758) collected at Espíritu Santo island, Gulf of California (Villalejo-Fuerte et al. 2005). Another study by Hu et al. (2014) reported that 57% of copepod live diets collected at the South China Sea were *Rhizosolenia* sp. All these studies suggest that *Rhizosolenia* sp. is a very common diet for aquatic organisms, especially small invertebrates. It may potentially be one of the most important diets for scalloped spiny lobster at the post-larval stage.

The new study's findings slightly differ from prior studies on lobster diets in several ways. Góes and Lins-Oliveira (2009), for instance, found different diets from spiny lobster (*Jasus paulensis*) larvae including algae, hydrozoan, bryozoan, worm, cnidarians, and molluscs. Jeffs (2007) documented the spiny lobster larvae/phyllosoma stage (*Jasus edwardsii*) mostly fed on gelatinous zooplankton and small crustacean prey. Another study investigating the natural diets of American lobster (*Homarus americanus*) found bivalves and animal tissues (flesh) as the most dominant diets. Accordingly, Jernakoff et al. (1993) identified caroline algae, molluscs, and crustaceans as the most common diets of western-rock lobster (*Panulirus cygnus*) at post-larval stage in Australia. These variations may be attributed to changes in habitats or environmental circumstances (e.g., coral reef, mangrove) that influence the types of nutrition available. As previously reported by Blamey et al. (2019), the type and dominant diet found in the stomach content of certain lobsters reflected the habitats in which the lobsters live. In addition, various studies concluded that lobster was an opportunistic omnivore feeder (Blamey et al. 2019; Jeffs 2007). Although being considered omnivore feeders where diets are highly determined by the availability of diets in their habitats, lobsters were also observed to eat soft or fleshy prey items, especially in early life stages such as the phyllosoma stage (Jeffs 2007). Similarly, Suzuki et al. (2006) concluded that lobster larvae were opportunistic predators that prey on crustaceans such as amphipods, copepods and shrimp, as well as soft-body organisms including Radiolaria, Thaliacea, Actinopterigii, hydrozoa, sagittoidea and gelatinous zooplankton.

Methods for diet identification may also contribute to the discrepancies in outcomes between the current research and prior investigations. For instance, a study by Hu et al. (2014) employed a molecular identification approach (polymerase chain reaction; PCR) to identify the stomach contents of investigated lobsters, which is more powerful than the current study's conventional method of microscopic examination. Few studies have shown that gut remnants that cannot be recognised via binocular microscopic examination may still be identified using a molecular technique such as PCR (Amin et al. 2022c; Macías-Hernández et al. 2018). The weakness of the conventional approach used in the present study may also become the main reason for less correlation between plankton found in the stomach and the environment. Some plankton found in lobster stomachs that were not found in their environment, and vice versa, appears to contradict the findings of Blamey et al. (2019). Therefore, higher resolution methods such as environmental DNA (eDNA) metabarcoding or Eukaryote-Inclusive PCR are highly recommended for future studies to increase the result accuracy.

In conclusion, post-larval scalloped spiny lobsters seem to be opportunistic omnivore feeders, and prey on various groups of phytoplankton and zooplankton genera including *Grammatophora* sp., *Synedra* sp., *Tintinnopsis* sp., and *Uronema* sp., *Ochromonas* sp., and *Coscinodiscus* sp. *Chlorococcum* sp., *P. chlorinum*, *unidentified* Gymnodiniaceae, unidentified *Cirripedia*, *Rhizolenia* sp. Of these planktonic genera, *Synedra* sp. and *Rhizolenia* sp. were found as the most common plankton genera present in the stomach of lobster samples and their natural habitat.

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