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Revealing diets of wild-caught ornate spiny lobster, *Panulirus ornatus*, at puerulus, post-puerulus and juvenile stages using environmental DNA (eDNA) metabarcoding

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ABSTRACT

Diets are a critical factor in the artificial production of lobster larvae, yet knowledge of the diet requirements of spiny lobster especially at its early life stages is rarely investigated. Thus, the present study aimed at finding potential diets of spiny lobster larva by analyzing the stomach content of wild-caught ornate spiny lobster, *Panulirus ornatus*, at three different life stages: puerulus, post-puerulus, and juvenile using eDNA metabarcoding. The results showed that 10 plankton species were identified at the puerulus stage, and the top five were *Oithona* sp. (36.30% of the relative quantity of eDNA), *Macropthalmus setous* (19.18%), *Audacallichirus mirim* (13.01%), *Oithona simplex* (5.48%), and *Pseudodiaptomus euryhalinus* (4.11%). Furthermore, 17 species were identified from the post-puerulus stage, and the five most dominant species were *Audacallichirus mirim* (28.60%), *Oithona* sp. (19.36%), *Pichia* sp. (5.96%), *Helice tientsinensis* (5.86%), and *Oithona simplex* (5.36%). At the juvenile stage, 34 diet species were identified, of which the top five most dominant species were *Oithona* sp. (80.88%), followed by *Canthocalanus pauper* (5.66%), *Acartia bispinosa* (4.02%), *Longipedia koreana* (2.30%), and *Oithona davisae* (1.92%). In addition, 56 plankton species were identified from the natural habitat including *Sicyonia laevigata* (33.73%), *Oithona simplex* (23.70%), *Oithona* sp. (17.70%), and *Acartia tonsa* (11.89%). Of the identified species, five were considered highly potential for developing artificially producing lobster seeds which were *Oithona* sp., *Oithona simplex*, *Acartia bispinosa*, *Acartia tonsa* and *Pseudodiaptomus euryhalinus*.

1. Introduction

Ornate spiny lobster, *Panulirus ornatus*, is one of the highest-value seafood traded in the world. The market demand for the lobster species is continuously increasing over time due to its high nutritional content and rich flavour. Thus, ornate spiny lobster has been a major cultured species in several Indo-West Pacific countries including Indonesia (Nguyen et al., 2022). However, the development of ornate spiny lobster aquaculture is hampered by the availability of seeds. Up until now, the ornate spiny lobster seeds are still highly dependent on wild stock because artificial production of the lobster seeds has not been

developed yet. This has put a high pressure and threatened the wild stock of lobster as many studies reported that the recruitment rate of lobster seeds in nature is very low (<0.1%) (Nurfiarini et al., 2016; Sibeni and Calderini, 2013). To respond to the issue, many researchers have directed their studies to the development of lobster hatcheries and the production of lobster seeds.

Some researchers have successfully bred and produced the lobster larvae in a hatchery. However, the produced larvae are only able to survive for a maximum of 2 weeks, entering the early phyllosoma stage (Cox and Johnston, 2003; Francis et al., 2014; Matsuda et al., 2006; Murugan et al., 2004; Payne et al., 2006). At this stage, the egg yolk as

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the main food reserve for the larvae has been exhausted, and the larvae started feeding on external sources (Cox and Johnston, 2003; Francis et al., 2014). Acknowledging these facts, researchers assume that one of the main causes of mass mortality was the availability of suitable types of feed for lobster larvae. Therefore, finding out suitable diets for lobster larvae could be one of the key factors in the development of lobster hatchery. Nonetheless, to the authors' knowledge, there have been no studies that reported the natural diets of ornate spiny lobster, especially at its early life stages. Therefore, a study to identify the natural diet of ornate spiny lobster is urgently required.

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One way to find out the diets of certain aquatic organisms is by investigating their stomach contents (Amundsen Sánchez-Hernández, 2019; Wang et al., 2021). There are two common methods for identifying the stomach contents of aquatic organisms which are microscopic observation (Cob et al., 2014) and environmental DNA (eDNA) metabarcoding. Some researchers have reviewed that molecular approaches such as eDNA metabarcoding are more powerful than microscopic observation, especially in soft body prey (Díaz-Abad et al., 2022). Thus, the present study was focused on identifying the type of stomach content of ornate spiny lobster at three early life stages (puerulus, post-puerulus and juvenile) using eDNA metabarcoding for more accurate results. The results of this research are expected to be potentially useful for developing lobster feed in order to realize the technology for artificially producing lobster seeds in Indonesia and other countries.

2. Material and method

2.1. Study site

The lobsters and water samples were collected at Prigi Bay, Trenggalek Regency, East Java, Indonesia (08°17'18" N and 111°43'42" E), Fig. 1. The location was chosen due to the area being considered one of the most abundant fishing grounds for lobster in Indonesia.

2.2. Sample collection

A total of 15 ornate spiny lobsters, Panulirus ornatus, at three life stages (puerulus, post-puerulus, and juvenile) were collected from Prigi Bay, East Java Indonesia. These lobsters were caught using a traditional trap made of a nylon net by local fishermen in August 2021. The determination of life stages was based on carapace length (CL): 4-6 mm CL for puerulus (Acosta and Butler IV, 1999), 6-16 mm CL for post-puerulus (Herrnkind, 1994), and 16-50 mm CL for juvenile (Childress and Herrnkind, 1997). Each sample was then dissected and the stomach content was taken out aseptically. Five lobster samples at each life stage were pooled and placed into a sterile 5 ml falcon tube previously filled with absolute ethanol to protect DNA. The samples were preserved at -20° C until further analysis.

The potential diet obtained from the natural habitat was investigated by collecting plankton at Prigi Bay, which was performed according to Amin et al. (2022a) with slight modifications. In brief, a water sampler fitted with a 355 µm mesh plankton net was dragged for ~10 min on

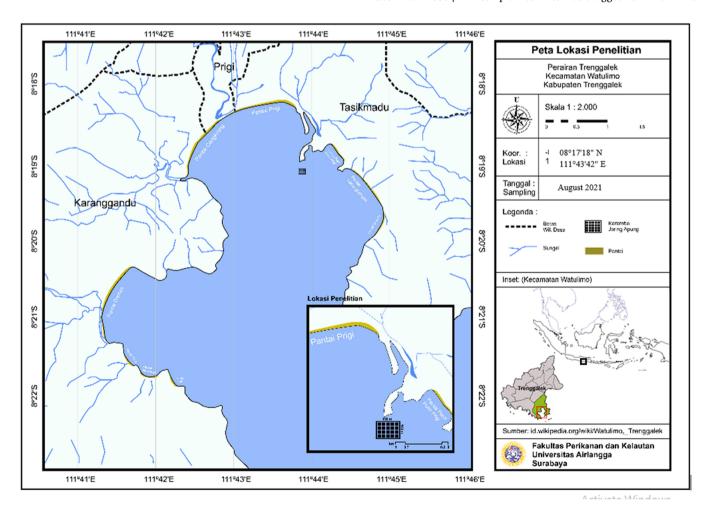


Fig. 1. The geographical location of the sampling site where samples of water and ornate spiny lobster at the three life stages were collected at Prigi Bay, East Java, Indonesia.

surface water (\sim 30 cm depth) at night using a boat in August 2021. The collected planktons in a cod-end of the plankton net were poured into a 1.5 L plastic bottle. The plankton sample was preserved in absolute ethanol and stored at 4 $^{\circ}$ C until further analysis.

2.3. DNA extraction

DNA was extracted using ZymoBIOMICSTM DNA Miniprep Kit (D4300T) as previously described by Amin et al. (2022b) with slight modifications. The pooled stomach content was added into ZRbashingBeadTM Lysis Tubes (0.1 and 0.5 mm) followed by the addition of 750 μ l ZymoBIOMICSTM Lysis solution. The tubes were vortexed at 13, 000 g for 15 min. While the rest of the steps were performed according to the instruction manual of the ZymoBIOMICSTM DNA Miniprep Kit. For the plankton sample, the sample was filtered using Whatman no 5 filter paper. The filter paper was afterwards cut into pieces and added to ZRbashingBeadTM Lysis Tubes (0.1 and 0.5 mm) followed by the addition of 750 μ l ZymoBIOMICSTM Lysis solution. The remaining steps were the same as the sample from the stomach content.

2.4. Sequencing and bioinformatics

All DNA samples were sent to a service provider for amplification and next-generation sequencing. Briefly, amplification by polymerase chain reaction (PCR) was carried out using barcoded primers that included the sequences, 5'-CCC TGC CHT TTG TAC ACA C-3' and 5'-CCT TCY GCA GGT TCA CCT AC-3' for amplifying the V9 region of the 18 S rRNA gene (Amaral-Zettler et al., 2009). The barcoded PCR products with proper size were selected by 2% agarose gel electrophoresis. The same amount of PCR products from each sample was pooled, endrepaired, A-tailed and further ligated with Illumina adapters. The amplicons were sequenced on a paired-end Illumina platform to generate 250 bp paired-end raw reads.

Amplicon sequence variant (ASV) generation via the DADA2 workflow has been described by Callahan et al. (2016) with slight modifications. Quality assessment of raw reads was done using fastqc (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), after which the sequences of primers and adapters were removed using Cutadapt 3.5 (Martin, 2011). Paired-end reads were processed and merged using DADA2 V1.18 (Callahan et al., 2016). Chimera screening

and taxonomy assignment were done using the SILVA nr database V138.1. In addition, similarity in composition and abundance of diets among the sample groups were compared using Paleontological Statistics (PASt) software to form a hierarchical cluster based on the Bray-Curtis similarity index.

3. Results

3.1. Metabarcoding of eDNA

A total of 430,805 raw reads were obtained from all the samples consisting of 130, 679 reads from the natural habitat (water), 56,949 reads from the stomach at the puerulus stage, 120,781 reads at the post-puerulus stage and 122 reads at the juvenile stage. After bioinformatics filtering, we obtained a total of 136,508 reads from the ornate lobster (34,172 reads for the puerulus stage, 34,172 reads for the post puerulus stage, and 34,175 reads for the juvenile stage). While 34,172 reads were obtained from the environmental water collected at Prigi Bay, Fig. 2.

3.2. Taxonomic composition of stomach content

The puerulus stage was dominated by the order Crustacea (42.11%), Craniata (0.02%), other orders (10.57%), and unknown (47.30%). For the post-puerulus stage, the stomach content was dominated by Crustacea (62.24%), Craniata (0.90%), other orders (10.53%), and Unknown (26.34%). For the juvenile stage, the stomach was dominated by crustacea (84.97%), Craniata (0.02%), other orders (0.225%), and unknown (14.75%). While the habitat was dominated by crustacea (45.31%), Craniata (5.65%), other oders (0.33%), and unknown (48.71%), Fig. 3.

At the genus level, the puerulus stage was dominated by the genus Oithona (41.78%), Macropthakmus (19.18%), Audacallichirus (13.01%), Acartia (6.16%), Parribacus (5.48%), Pseudodiaptomus (4.11%), Xiphias (2.05%), and unknown (10.27%). At the post-puerulus stage, 16 genera were identified, and the top 10 genera were Audacallichirus (28.60%), Oithona (25.91%), Pichia (5.96%), Helice (5.86%), Halicarcinus (4.47%), Macropthalmus (3.28%), Pseudodiaptomus (2.78%), Xiphias (2.18%), Calanus (2.18%), and Astacus (2.09%). There rest genera including Parribacus, Acartia, Paracalnus, Phallusia, Plagiostomum, and unkown were presented in Fig. 4. At the juvenile stage, a total of 47 genera were identified the stomach, and the 10 genera Oithona (67.14%),

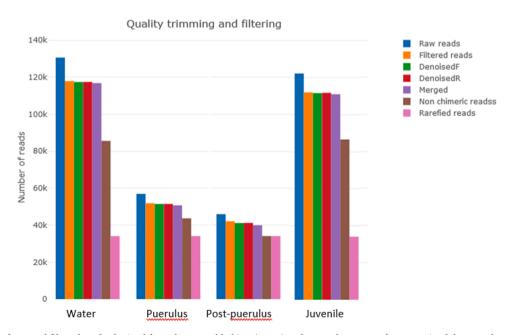


Fig. 2. The numbers of raw and filtered reads obtained from the natural habitat (water) and stomach content of ornate spiny lobster at the puerulus, post-puerulus and juvenile stages collected from Prigi Bay, East Java Indonesia.

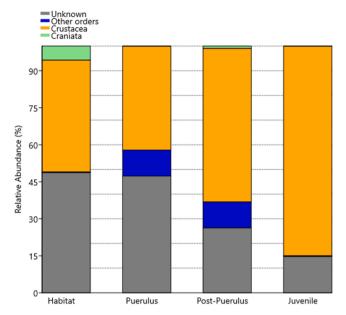


Fig. 3. Taxonomic composition of eukaryotes from the natural habitat (water) and stomach content of ornate spiny lobster at three life stages (puerulus, postpuerulus, and juvenile) collected from Prigi Bay, Trenggalek at the order level.

Pseudodiapttomus (6.27%), Canthocalanus (4.53%), Parribacus (4.19%), Acartia (3.27%), Plagiostomum (2.47%), Longipedia (1.84%), Paracalanus (1.15%), and Canuella (0.82%). While the rest 37 genera were counted as less than 1% each and more details were presented in Fig. 4.

At the species level, a total of 10 species were identified in the stomach contents of ornate spiny lobster at the puerulus stage. The most dominant species was *Oithona* sp. (36.30%), followed by *Macropthalmus* setosus (19.18%), *Audacallichirus mirim* (13.01%), *Oithona simplex* (5.48%), *Pseudodiaptomus euryhalinus* (4.11%), *Acartia bispinosa* (3.42%), *Acartia tonsa* (2.74%), *Xiphias gladius* (2.15%), *Parribacus perlatus* (2.05%), and *Parribacus japonicus* (1.37%). The result also showed that about 10.27% of ASVs were classified as unknown species, Fig. 5.

At the post-puerulus stage, 17 species of potential diets were successfully identified. The top 10 most dominant species were *Auda-callichirus mirim* (28.6%), *Oithona* sp. (36.30%), *Pichia* sp. (5.96%), *Helice tientsinensis* (5.86%), *Oithona simplex* (5.36%), *Halicarcinus ovatus* (4.47%), *Macrophthalmus setosus* (3.28%), *Pseudodiaptosmus euryhalinus* (2.78%), *Calanus finmarchicus* (2.18%), and *Xiphias gladius* (2.18%). The other 7 species were presented in detail in Fig. 6. The present result indicated that about 12.02% ASVs were classified as unknown species.

At the juvenile stage, a total of 34 species of potential diets were identified in the stomach content of ornate spiny lobster. Of the 34, the top 10 most dominant species were *Oithona* sp. (80.88%), followed by *Canthocalanus pauper* (5.66%), *Acartia bispinosa* (4.02%), *Longipedia koreana* (2.30%), *Oithona davisae* (1.92%), *Canuella perplexa* (1.02%), *Oithona aurensis* (1.02%), *Euterpina acutifrons* (0.74%), *Harpacticus* sp. (0.49%), and *Calocalanus improvisus* (0.17%), Fig. 7.

In addition, a total of 56 plankton species were identified from the natural habitat of ornate spiny lobster (the Prigi Bay). Of these numbers, the top 10 most abundant species were *Sicyonia laevigata* (33.73%), followed by *Oithona simplex* (23.70%), *Oithona sp.* (17.70%), *Acartia tonsa* (11.89%), *Xiphias gladius* (6.04%), *Longipedia koreana* (0.95%), *Canthocalanus pauper* (0.58%), *Sphaeramia orbicularis* (0.52%), *Ceratium furca* (0.43%), and *Euterpina acutifrons* (0.35%). The Other species which were counted as less than 0.1% were presented in detail in Fig. 8.

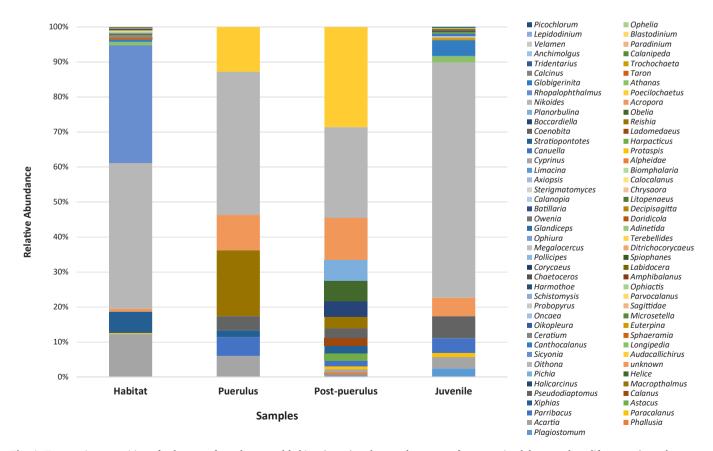


Fig. 4. Taxonomic composition of eukaryotes from the natural habitat (water) and stomach content of ornate spiny lobster at three life stages (puerulus, post-puerulus, and juvenile) collected from Prigi Bay, Trenggalek at the genus level.

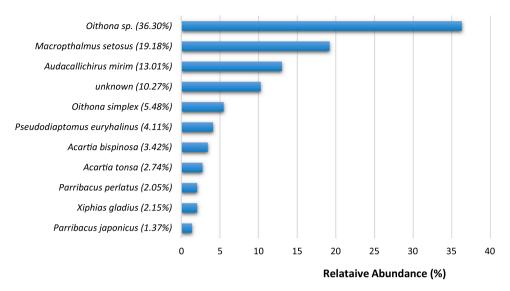


Fig. 5. Taxonomic composition at the species level of potential natural diets identified in the stomach content of ornate spiny lobster at the puerulus stage, collected at Prigi Bay, Trenggalek.

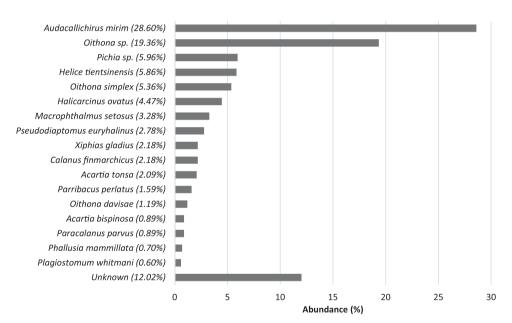


Fig. 6. Taxonomic composition at the species level of potential diets identified in the stomach content of ornate spiny lobster at the post-puerulus stage, collected from Prigi Bay, Trenggalek.

3.3. The similarity index

A hierarchical cluster analysis was performed to compare the similarity of diets between the sample groups based on the Bray-Curtis similarity index. The results showed that a tree diagram where the samples that were viewed as most similar in the composition and abundance of genera were placed on branches that are close together. As presented in Fig. 9, diets identified in natural habitat and diets found in the stomach of Juvenile were placed in cluster 1 with 48.23% similarity. While cluster 2 consisted of Puerulus and post-puerulus had 59.18% similarity. Overall, the similarity of diets among the four samples was ~45%, Fig. 9. Further analysis showed that 6 plankton species were found in all samples which were Acartia bispinosa, Acartia tonsa, Oithona sp., Oithona simplex, Pseudodiaptomus euryhalinus, and Xiphias gladius.

4. Discussion

Diets are a critical factor for the successful production of artificial lobster seeds, yet knowledge of diets of spiny lobster especially at the early life stages is rarely reported. To the authors' knowledge, the present study is the first study to report natural diets of *Panulirus ornatus* at three life stages (puerulus, post-puerulus and juvenile stages) as well as diets available in their habitat (marine water) using the eDNA metabarcoding technique. The results in general showed that the stomach contents of the ornate spiny lobster at the three life stages were mostly dominated by zooplankton species. This finding was quite similar to several studies where the stomach content of *Panulirus* consisted of bivalves, gastropods, barnacles, fish, algae, crabs, Polychaeta, ascidiacea, and echinoderms (Barclay et al., 2006; Boyd and Mason, 2020; Gnanalingam and Butler IV, 2018; Mashaii et al., 2011). Similarly, Jeffs (2007) also confirmed that several gelatinous zooplankton and small crustaceans in the stomach content of New Zealand lobster, *Jasus edwardsii*.

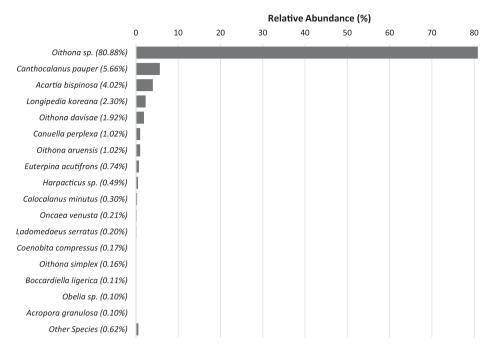


Fig. 7. Taxonomic composition at the species level of potential diets identified in the stomach content of ornate spiny lobster at the juvenile stage, collected from Prigi Bay, Trenggalek.

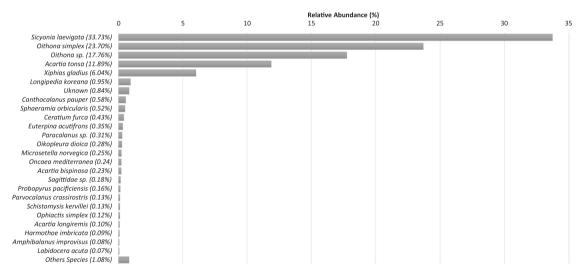


Fig. 8. Taxonomic composition of eukaryotes from the natural habitat (water) of ornate spiny lobster at Prigi Bay, Trenggalek at the species level.

This study found five most abundant species in the stomach content of spiny lobster at the peurulus, post-puerulus and juvenile stages, which are Oithona sp., Oithona simplex, Acartia bispinosa, A. tonsa, and Pseudodiaptomus euryhalinus. Of these species, Oithona sp. is the most common live diet reported for fish and crustaceans in aquaculture (Huanacuni et al., 2021). Similar studies previously reported the presence of Oithona sp. as the dominant live diet species for lobsters at the early life stage (Khvorov et al., 2012) as well as in the natural settlement habitat of spiny lobster (Amin et al., 2022a). Oithona sp. is a marine calanoid copepod which has high protein content, $\sim 59.33\%$ (Santanumurti et al., 2021), therefore frequently used as a live diet for fish or shrimp larvae. Additionally, Magouz et al. (2021) reported that Oithona nana improved growth performance, feed utilization, and survival rate of European seabass (Dicentrarchus labrax) postlarvae. It was further reported that the fatty acid profiles including polyunsaturated fatty acids (26.47%) and omega-3 fatty acids (36.30) of the copepod were higher compared to a commercial live diet such as Artemia. Dinesh

Kumar et al. (2017) reported also that *Oithona rigida* could replace formulated diet to feed post larva of white shrimp, *Litovenaeus vannamei* with the same growth performances. The present study also found *Oithona* spp. in the stomach of ornate spiny lobster at the post-puerulus and juvenile stages as well as from the natural habitat.

Other common species were a member of the genus *Acartia* including *A. bispinosa* and *A. tonsa*. These species were also found in the stomach contents of ornate spiny lobster at puerulus, post-puerulus and juvenile stages which may suggest that the marine copepod are favourable diet for ornate spiny lobster. Up until now, several members of the genus *Acartia* have been reported as an important live diet for fish/shrimp larvae. For instance, *A. clausi* has been described to have higher contents of proteins (63.12%) and lipids (16.65%), and is also richer in n-3 fatty acids (33.94%) than *Artemia* nauplii and rotifers (Rajkumar, 2006). Therefore, when it was fed to seabass larvae (*Lates calcarifer*), the larvae were found to have better length, weight gain and survival rate compared to those fish larvae fed on rotifer or *Artemia* nauplii

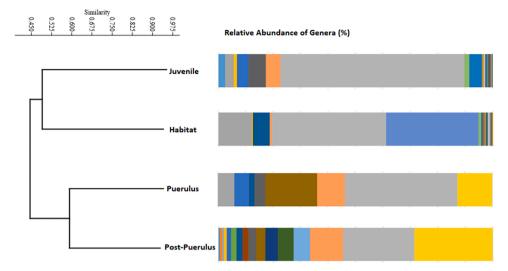


Fig. 9. A hierarchical cluster analysis of all samples based on the Bray-Curtis similarity index. The Vertical axis shows the similarity between each cluster using the unweighted pair group method with arithmetic mean (UPGMA), in which two clusters were identified with a number of 2 samples in each.

(Rajkumar, 2006). Meanwhile, *A. tonsa* found in the present study has also been reported in several studies as an important live diet in marine aquatic hatcheries. Barroso et al. (2013) tried to use *A. tonsa* as a live diet for fat snook, *Centropomus parallelus*, which subsequently showed higher survival and growth rates compared to those fed on *Artemia*. Furthermore, a study by Vanacor-Barroso et al. (2017) concluded that *A. tonsa* provided an important nutritional benefit to fat snook larvae undergoing metamorphosis. Due to its significant nutritional content, *A. tonsa* has been developed for large-scale aquaculture as a live diet for many aquatic larvae (Sarkisian et al., 2019). Meanwhile, studies on the other *Acartia* species (*A. bispinosa*) have not been performed so far, therefore it is highly recommended to characterise its nutritional content followed by in vivo trials using aquatic animals especially for developing ornate spiny lobster hatchery.

The other species found in the stomach content of the ornate spiny lobster was *Pseudodiaptomus euryhalinus*, a marine copepod that belonged to *Subphylum Crustacea*. A similar result has been previously documented in a study by Jernakoff et al. (1993) who identified crustaceans and molluscs as the most common diets of western-rock lobster (*Panulirus cygnus*) at the post puerulus stage in Australia. The marine copepod has been also reported to have high nutritional content as well as high digestibility for fish larvae (McKinnon et al., 2003); therefore is considered an important live diet for a variety of aquatic species including Spotted Rose Snapper *Lutjanus guttatus* larvae (Puello-Cruz et al., 2015). Acknowledging these facts, many studies have been nowadays performed to culture *P. euryhalinus* intensively as the main live diet for fish larvae (Anzueto-Sánchez et al., 2014; Bustillos-Guzman et al., 2002; Chilmawati, 2016; Puello-Cruz et al., 2009).

The present study also found species which is uncommon as a live diet for aquatic species such as *Xiphias gladius* and *Macrophthalmus setosus*. This result might relate to the feeding nature of spiny lobster which is an opportunistic feeder (Blamey et al., 2019a; Jeffs, 2007; Juinio and Cobb, 1992). Góes, Lins-Oliveira (2009), for instance, found different kinds of diets from spiny lobster (*Jasus paulensis*) larvae including algae, hydrozoan, bryozoan, worm, cnidarians, and molluscs. Similarly, Suzuki et al. (2006) found that lobster larvae were opportunistic feeders, preying on various types of live diets including amphipods, copepods, shrimps, actinopterygii, hydrozoa, sagittoidea and gelatinous zooplankton. This large variation in the type of diets consumed by spiny lobsters appears to be highly determined by the availability of the diets in their habitats. A similar conclusion was derived by Blamey et al. (2019a) who found that the diet profiles identified in the stomach content of spiny lobsters were strongly

associated with their natural habitat.

Overall, some plankton species identified in the present study were similar to plankton species identified in the stomach content of temperate lobster species. For instance, Oithona sp., Acartia sp., and Calanus sp. found in the present study were also found in the stomach content of American lobster, Homarus americanus at post-puerulus stage (Juinio and Cobb, 1992). These studies might suggest that these plankton species are an important live diet for lobster regardless of the lobster species, tropic spiny lobster or temperate spiny lobsters. However, several studies identifying the stomach content of spiny lobsters reported different species (Gnanalingam and Butler IV, 2018; Jernakoff et al., 1993; Mashaii et al., 2011). These differences might be due to different types of diets available in the environments. Accordingly, Blamey et al. (2019a) concluded that the type and dominant diet found in the stomach of spiny lobsters generally reflected the habitats in which the lobster live. The present study showed also that the similarity of planktons identified in natural habitats and diets of spiny lobster increased as the spiny lobster are growing bigger. Additionally, several studies concluded that spiny lobster was an opportunistic omnivore feeder, preying on diverse diets such as amphipods, copepods and shrimp, radiolaria, thaliacea, actinopterigii, hydrozoa, sagittoidea and Gelatinous zooplankton (Blamey et al., 2019b; Suzuki et al., 2006).

However, the result of the present study was also contradictory to previous studies where spiny lobster at puerulus stage was commonly regarded as a non-feeding stage, reliant on stored energy reserves until it moults to the first instar benthic juvenile (Fitzgibbon et al., 2014; Phillips and Booth, 1994). The present study identified 10 plankton species in the stomach of ornate spiny lobster peurulus. The possible reason might be that these planktons were DNA residuals from feeding during earlier life stages (e.g., phyllosoma). Hosseini et al. (2008) reviewed that DNA of prey may last long enough in the predator's stomach, depending on several factors including temperature, time since feeding, subsequent food intake, sex, weight, and age of predator species. However, the above possibility might be weak due to some previous studies. For instance, Kittaka et al. (1997) reported that pueruli of Jasus verreauxi molted into the first instar juvenile took an average of 25.5 days. While other studies indicated that prey detection in fish starts decreasing after 2 h post-feeding and maximum detection times range between 24 and 48 h (Carreon-Martinez and Heath, 2010; Hunter et al., 2012; Waraniak et al., 2019), which suggests that this possibility of DNA residue is very small. Acknowledging these facts, the other possibility is that puerulus of P. ornatus were actively feeding. Nevertheless, this speculative possibility required further studies in order to get

comprehensive conclusions.

In conclusion, the stomach contents of ornate spiny lobster at the puerulus, post-puerulus and juvenile stages appeared mostly dominated by zooplankton species. Of the identified species, the five most dominant species found in the stomach at all life stages were *Oithona* sp., *Oithona* simplex, *Acartia bispinosa*, *Acartia tonsa*, and *Pseudodiaptomus euryhalinus*. These species have been commonly used as live diets for larvae of several aquatic species including seabass, fat snook, and white shrimps. The results of this study suggest that ornate spiny lobsters at their early life stages fed on the five crustacean members, and therefore are considered potential diets for ornate spiny lobster larvae. However, further study by culturing and feeding these species to ornate spiny lobster larvae is highly recommended.

CRediT authorship contribution statement

Muhamad Amin: Conceptualization, Investigation, Data curation, Data analysis, Writing - original draft, Visualization, Funding. Hussein Taha: Supervision, Data analysis, Writing - original draft, Reviewing, Funding. Syifania Hanifah Samara: Investigation, Data curation, Data analysis, Writing - original draft. Anis Fitria: Investigation, Data curation, Data analysis, Writing - original draft. Nur Aini Muslicha: Investigation, Data curation, Data analysis, Writing - original draft. Laila Musdalifah: Investigation, Data curation, Data analysis, Writing - original draft. Olumide A. Odeyemi: Investigation, Data curation, Data analysis, Writing - original draft. Takaomi Arai: Supervision, Data analysis, Writing - original draft, Reviewing, Funding.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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