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ADDRESS:
Jl. Ir. Sutami 36A Surakarta 57126. Tel. +62-271-7994097, Tel. & Fax.: +62-271-663375, email: editors@smujo.id

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Diversity and abundance of plankton community in Prigi and Tawang Bays, natural settlement habitats of Spiny Lobster larvae in East Java, Indonesia

ENDANG DEWI MASITHAH[1,♥], MUHAMMAD GIANO FADHILAH[2], MUHAMAD AMIN[3], KURNIATI UMRAH NUR[4], LAILA MUSDALIFAH[5], SHIFANIA HANIFA SAMARA[2], YUDI CAHYOKO[3], ALIMUDDIN[6], SAHRUL ALIM[7], BAGUS DWI HARI SETYONO[7]

[1]Department of Marine, Faculty of Fisheries and Marine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Mulyorejo, Surabaya 60115, East Java, Indonesia. ♥email: endang_dm@fpk.unair.ac.id

[2]Aquaculture Study Program, Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Mulyorejo, Surabaya 60115, East Java, Indonesia

[3]Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Mulyorejo, Surabaya 60115, East Java, Indonesia

[4]Aquaculture Study Program, Fisheries Department, Faculty of Marine Science and Fisheries, Universitas Hasanudin. Jl. Perintis Kemerdekaan Km. 10, Tamalanrea, Makassar 90245, South Sulawesi, Indonesia

[5]Research Center for Fishery, National Research and Innovation Agency. Jl. Pasir Putih II No. 3, Ancol, Jakarta Utara 14430, Jakarta, Indonesia

[6]Microbiology and Biotechnology Laboratory, Faculty of Animal Sciences, Universitas Mataram. Jl. Majapahit No. 62, Mataram 83115, West Nusa Tenggara, Indonesia

[7]Program of Aquaculture, Faculty of Agriculture, Universitas Mataram. Jl. Majapahit No. 62, Mataram 83115, West Nusa Tenggara, Indonesia

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Abstract. *Masithah ED, Fadhilah MG, Amin M, Nur KU, Musdalifah L, Samara SH, Cahyoko Y, Alimuddin, Alim S, Setyono BDH. 2023. Diversity and abundance of plankton community in Prigi and Tawang Bays, natural settlement habitats of Spiny Lobster larvae in East Java, Indonesia. Biodiversitas 24: 1642-1649.* Prigi and Tawang Bays have been well-known as settlement areas for spiny lobster larvae, *Panulirus* spp., in East Java, Indonesia. These locations may suggest suitable environments including diet availability for lobster larvae. Therefore, the present study aimed to investigate the type and abundance of plankton in both locations to discover potential live diets for lobster larvae. This study also explored plankton's diversity, uniformity, and dominance indices in both locations. Plankton samples in each location were collected using a plankton net at four depths: 0.3 m, 2.5 m, 5 m, and 20 m with three replicates. The results revealed that 17 plankton species were identified from 0.30 m depth, 13 at 2.5 m, 11 at 5 m, and 13 at 20 m depth at Prigi Bay. In addition, 17 plankton species were discovered at 0.3 m depth, 11 at 2.5 m, 12 at 5 m, and 12 at 20 m at Tawang Bay. Among the most abundant species were *Acartia* sp., *Calanus* sp., *Paracyclopina* sp., and *Oithona* sp. The diversity indices observed in Karanggongso of Prigi Bay and Tawang Bay ranged from 2.02-2.49 and 2.17-2.65, respectively, within the moderate range. Similarly, the uniformity indices observed at both locations were moderate, ranging from 0.38-0.45 at Prigi Bay and 0.41-0.46 at Tawang Bay. There were no dominant species at both locations, as the dominance index values ranged from 0.13-0.30. Among the identified plankton species, *Oithona* sp., *Calanus* sp., *Paracyclopina* sp., and *Acartia* sp. are considered potential live feed for lobster larvae, and thus should be further studied.

Keywords: Diets, diversity, dominance, lobster, plankton, uniformity

INTRODUCTION

Lobster is an important fishery commodity in Indonesia due to its high price, high nutritional contents, and high market demands. According to the Indonesian Central Bureau of Statistics, the total export value of lobsters in 2020 reached USD 8.1 million (BPS 2020). The high export value and continuously increasing marketing demands at national or global markets indicate that lobster is a high-potential fisheries commodity. However, the lobster supply has depended highly on the wild catch because lobster aquaculture has not yet been well developed. One of the main issues faced in lobster aquaculture is larval production, which currently relies on the availability of natural seeds. Many studies have been conducted to study various factors relating to larval production, including spawning-inducing technology and rearing condition, yet the success rates are very low. Several authors have succeeded in breeding and producing larvae. Yet, the larvae can live only 7-14 days after hatching. Therefore, it is hypothesized that the main challenge is diet availability and suitability. According to Amin et al. (2022b), first, one way to start domesticating wild species is by collecting information on their natural habitat as much as possible. Similarly, Kashinskaya et al. (2018) suggest profiling certain animals' natural habitats may reveal their diets.

Environmental conditions, including physical, chemical, and biological factors in natural habitats, highly determine the recruitment rates of lobster larvae (Keulder 2005). Several authors have previously reported the physical and chemical characteristics of the natural

settlement areas of lobster larvae (Amin et al. 2022b; Boudreau et al. 1992; Lillis and Snelgrove 2010). However, studies investigating biological aspects of settlement habitat in the natural environment of lobster larvae are very limited. Meanwhile, many studies conclude that biological factors have important information for the lobster larvae, especially for diets (O'Rorke et al. 2014). Accordingly, biological aspects such as natural dietary aspects and lobster predation processes that occur in nature during larval and post-larval stages could be critical information that must be considered for hatchery production. For example, plankton might be a natural diet source for various aquatic species (Amin et al. 2022d), including lobster seeds, in their natural settlement habitat. Raza'i et al. (2018) added that the availability of plankton as a natural diet source significantly impacts the dependence and growth of marine organisms such as fish, crabs, shrimp, and lobsters.

Profiling plankton diversity and abundance might reveal potential diets for lobster larvae. A similar approach has been done in some studies. For instance, Ihsan et al. (2019) conducted research on plankton as a natural feed for lobster larvae and post-larvae in natural habitats in Teluk Awang, Central Lombok. Trijoko and Pasaribu (2003) conducted another study in Wedi Ombo Bay, Gunungkidul, Yogyakarta. Generally, this study's results suggest that each location has a different structure and abundance, although some species were the same between the area. All these results raised questions about whether lobster larvae are opportunistic or specific feeders. Therefore, to answer these questions, more studies are required by collecting more information in more settlement areas of lobster.

Prigi Bay and Tawang Bay have been well-known as the top two settlement areas for lobster larvae in East Java Indonesia (Amin et al. 2022a); therefore, it is assumed to have important suitable diet availability for lobster larvae. However, studies on the biological aspects of both locations areas are still very limited. Thus, this research aims to investigate the plankton diversity, abundance, uniformity, and dominance indices in the natural settlement habitat of lobster larvae at Prigi Bay and Tawang Bay. The study results are expected to enrich the information on potential diets for lobster larvae for hatchery development.

MATERIALS AND METHODS

Study area

Plankton samples were collected in two common settlement areas of lobster larvae in East Java, Indonesia (Prigi Bay and Tawang Bay), with a protocol as previously described by Amin et al. (2022b). At Karanggongso of Prigi Bay, sampling was performed at three different ordinate points as repetitions: 8°18'13.8"S 111°44'28.4"E (R1), 8°18'16.3"S 111°44'21.6"E (R2), and 8°18'23.0"S 111°44'26.8"E (R3). While at Tawang Bay, the sampling points were 8°15'57.4"S 111°17'46.0" E (R1), 8°15'54.3"S 111°17'48.2"E (R2), and 8°15'51.5"S 111°17'46.2"E (R3) (Figure 1). Plankton sampling in each sampling point was collected at four different depths: 0-0.3 m, 2.5 m, 5 m, and 20 m. First, the water samples collected from three sampling points with the same depth were mixed and filtered using a plankton net and placed in sterile bottles. The filtered sample was then immediately given Lugol which acts as a plankton preservative, up to 1% of the total filtering, and wrapped in Styrofoam. The samples were then examined in the Microbiology Laboratory, Faculty of Fisheries and Marine Science at Airlangga University.

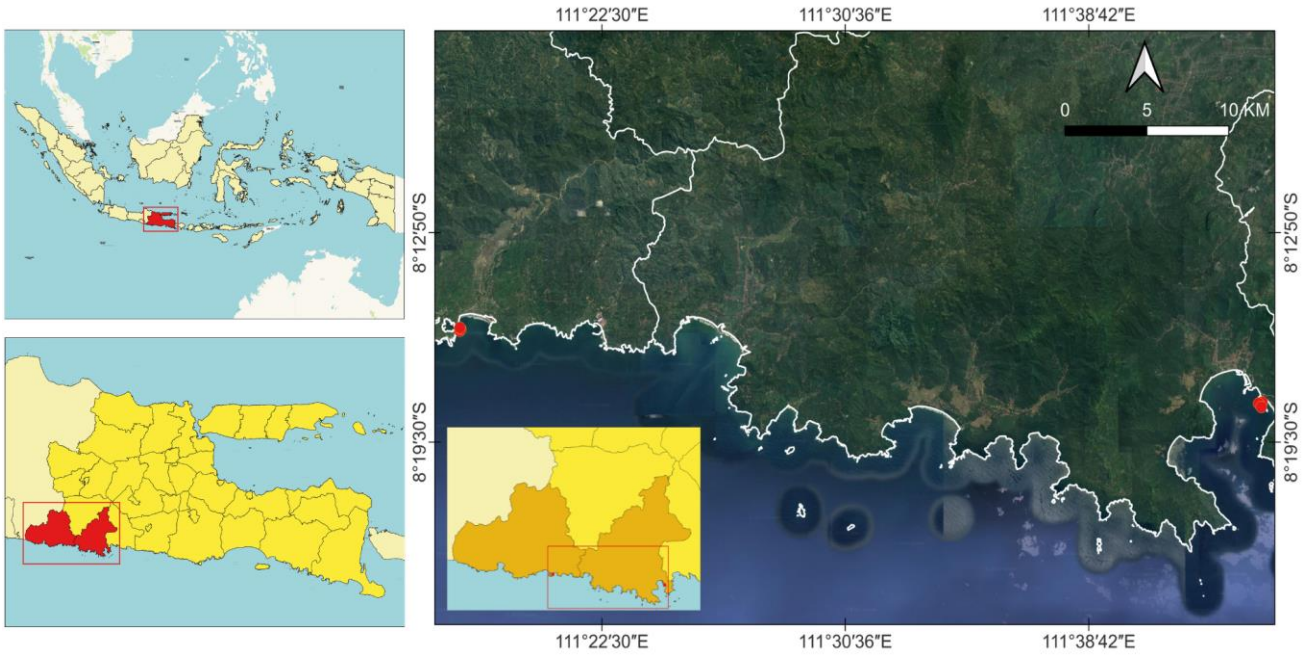

Figure 1. Two sampling locations in Prigi Bay, Trenggalek District, and Tawang Bay, Pacitan District, East Java, Indonesia

Prigi Bay water had temperatures ranging from 27-28ºC, a DO content of 7.48 mg/L, a salinity of 26 ppt, a pH of 7-8, a nitrate (NO_3) content of 0.01 mg/L, and a muddy substrate. On the other hand, Tawang Bay water temperatures were slightly warmer than Prigi Bay, with water temperature ranging from 28.2-28.3ºC, with a lower DO concentration (3.35 mg/L). Moreover, Tawang Bay has a higher salinity (35 ppt), a pH of 8, a NO_3 concentration of 0.01 mg/L, a depth of 20 m, and a sandy substrate.

Abundance and identification of plankton

Firstly, plankton identity and abundance were analyzed using a protocol of LeGresley and McDermott (2010). In brief, plankton samples were placed on a Sedgewick Rafter Counting (SRC) Cell and observed under a binocular microscope with a magnification of 1,000x. Afterward, plankton found in each sample was counted, photographed, and identified according to an identification book guide by Mazzocchi et al. (2012). Then, the abundance index was calculated according to the following formula (Fachrul 2012):

$$N = \frac{a}{b} \times \frac{c}{d} \times \frac{Vb}{Vsrc} \times \frac{1}{Vs}$$

Where: "N" represents the abundance of plankton (plankter/L), "a" represents the number of SRC boxes, "b" is the area of one field of view (mm^2), "c" denotes the number of individuals observed, and "d" indicates the number of boxes observed. "Vb" is the volume of water in the sample bottle (ml), "Vsrc" is the volume of water in the SRC (ml), and "Vs" represents the volume of water filtered in the Field (L).

Diversity, uniformity, and dominant indices

The diversity index value (*H'*) was calculated using the following formula (Fachrul 2012):

$$H' = -\sum PilnPi, where\ Pi = \frac{ni}{N}$$

Where: *H'* is Shannon Wiener Diversity Index, pi is the number of individuals of the i-th species, ni is the number of species, and N is the total individual number. The uniformity index (*E'*) was calculated using the "Evennes Index" formula (Ulfah et al. 2019):

$$E' = \frac{H'}{Lns}$$

Where: E' is the uniformity index, *H'* is the Shannon-Wiener diversity index, *S* is the total number of species The dominance index (d) was calculated using the following equation (Berger and Parker 1970):

$$d = \frac{\mathrm{Nmax}}{\mathrm{N}}$$

Where: "d": Simpson Dominance Index, Nmax: The most abundant number of individual species, dan N = Total individual number.

RESULTS AND DISCUSSION

Plankton abundance in Prigi Bay

Water samples were collected from Prigi Bay and Tawang Bay at four depths (0.3 m, 2.5 m, 5 m, and 20 m). The two bays were located in the Southern part of East Java Province, and both areas face the Indian Ocean, Figure 1. The results showed that 17 plankton species were identified from the surface water (0.0-0.3 m). The top six most abundant species were *Paracyclopina* sp. with 21.21%, followed by *Acartia* sp. (18.18%), *Pteropods* sp. (9.09%,) *Prorocentrum* sp. (6.06%), *Dinophysis* sp. (6.06%), and *Saggita* sp. (2.13%). Other species and their percentage are presented in Table 1. In addition, at a 2.5 m depth, 13 plankton species were identified. Again, the top 6 most abundant species were *Acartia* sp. (26.47%), followed by *Paracyclopina* sp. (23.53%), *Ceratium* sp. (8.82%), *Microstella* sp. (8.82%), *Dinophysis* sp. (5.8%), and *Oncaea* sp. (5.88%). The rest species with their abundance were presented in Table 1.

Furthermore, at 5 m depth, the bay is home to 11 plankton species. The top 4 most abundant species were *Acartia* sp. (30.77%), followed by *Paracyclopina* sp. (23.08%), *Sagitta* sp. (11.54%), and *Oithona* sp. (7.69%). While the other 7 species included *Synedra* sp., *Oikopleura* sp., *Coscinodiscus* sp., *Ceratirum* sp., *Pteropods* sp., *Microstella* sp., and unclassified Lucifer, which were counted for 3.85% each (Table 1). Meanwhile, 13 plankton species were found at a depth of 20 m. Again, the top 6 most abundant species were *Acartia* sp. accounted for 24.14%), followed by *Paracyclopina* sp. with 13.79%, *Pteropods* sp. (10.34%), *Dinophysis* sp. (10.34%), *Ceratium* sp. with 6.90%, and *Sagitta* sp. with 6.90%. The rest species are presented in table 1.

Plankton abundance in Tawang Bay

A total of 17 plankton species were identified from the water sample at a depth of 0.0-0.3m (surface water) of Tawang Bay. The top 9 most abundant species were *Acartia* sp., with an abundance of 12.82%, followed by *Ceratium* sp. (10.26%), *Prorocentrum* sp. (10.26%), *Microstella* sp. (10.26%), *Oncaea* sp. (10.26%) *Pteropods* sp. (7.69%), *Calanus* sp., (7.69%), *Synedra* sp. (5.13%), and *Oithona* sp. (5.13%). At the same time, the rest of the species were counted for 2.56% each and presented in Table 2. In addition, 11 species of plankton were found in a water sample at a depth of 2.5 m in Tawang Bay.

The top 6 most abundant species were *Calanus* sp. (28.00%), followed by *Prorocentrum* sp. (12.00%), *Paracyclopina* sp. (12.00%), *Microstella* sp. (12.00%), *Oncaea* sp. (8.00%), and *Oithona* sp. with an abundance of 8.00%. While the rest plankton species, including *Synedra* sp., *Ceratium* sp., *Pteropods* sp., *Macropthalmus* sp., and *Sagita* sp., were counted at 4.00% each, Table 2.

Table 1. Plankton species identified from Prigi Bay at four depths of water column

Depth	Species	Density (ind./L)
0.3 m (surface)	*Cyclotella* sp.	8
	Penilia sp	8
	Noctiluca sp.	8
	Prorocentrum sp.	16
	Dinophysis sp.	16
	Ceratium sp.	8
	Ceratium sp.	8
	Pteropods sp.	24
	Paracyclopina sp.	56
	Acartia sp.	48
	Microstella sp.	8
	Euphausia sp.	8
	Lucifer sp.	8
	Oipheureidea sp.	8
	Sagitta sp.	16
	Nermatea sp.	8
	Actinulla larvae	8
2.5 m	*Rizosolenia* sp.	8
	Penilia sp.	8
	Ceratium sp.	24
	Dinophysis sp.	16
	Paracyclopina sp.	64
	Acartia sp.	72
	Microstella sp.	24
	Oncaea sp.	16
	Codonelopsis sp.	8
	Oipheureidea sp.	8
	Sagitta sp.	8
	Actinula sp.	8
	Polychaete	8
5.0 m	*Oikopleura* sp.	8
	Synedra sp.	8
	Coscinodiscus sp.	8
	Ceratium sp.	*8*
	Pteropods sp.	8
	Paracyclopina sp.	48
	Acartia sp.	64
	Microstella sp.	8
	Oithona sp.	16
	Lucifer sp.	8
	Sagita sp.	24
20.0 m (Bottom)	Synedra sp.	8
	Penilia sp.	8
	Noctiluca sp.	8
	Dinophysis sp.	24
	Ceratium sp.	16
	Pteropods sp.	24
	Acartia sp.	56
	Paracyclopina sp.	32
	Oithona sp.	8
	Microstella sp.	16
	Euphausia sp.	8
	Protoperidinium sp.	8
	Sagitta sp.	16

Table 2. Plankton species identified from Tawang Bay at four depths of water column

Depth	Species	Density (ind./L)
0.3 m (surface)	*Synedra* sp.	16
	Oscillatoria sp.	8
	Spirulina sp.	8
	Ceratium sp.	32
	Prorocentrum sp.	32
	Pteropods sp.	24
	Acartia sp.	40
	Microstella sp.	32
	Calanus sp.	24
	Oithona sp	16
	Oncaea sp.	32
	Euphausia sp.	8
	Macrophthalamus sp.	8
	Clytemnestra sp.	8
	Cypris sp.	8
	Unclassified Fish larvae	8
	Unclassified flatworms	8
2.5 m	*Synedra* sp.	8
	Prorocentrum sp.	24
	Ceratium sp.	8
	Pteropods sp.	8
	Paracyclopina sp	24
	Calanus sp.	56
	Oithona sp.	16
	Microstella sp.	24
	Oncaea sp.	16
	Macrophthalamus sp.	8
	Sagitta sp.	8
5 m	*Melosira* sp.	8
	Synedra sp.	8
	Bivalve larvae	8
	Prorocentrum sp.	16
	Dinophysis sp.	8
	Microstella sp.	8
	Calanus sp.	48
	Oithona sp.	24
	Naupli Copepoda	16
	Temora sp.	8
	Oncaea sp.	8
	Sagitta sp.	8
20 m (bottom)	*Rhizoselenia* sp.	8
	Pleurosigma sp.	8
	Prorocentrum sp.	24
	Ceratium sp.	8
	Dinophysis sp.	8
	Microsetella sp.	4
	Calanus sp.	6
	Acartia sp.	24
	Oithona sp.	24
	Oncaea sp.	16
	Caridean sp.	8
	Unclassified flatworm	8

Furthermore, 12 plankton species were identified from the water sample at 5 m depth. The top 4 most common species were *Calanus* sp. with an abundance of 28.57%, *Oithona* sp. with an abundance of 14.29%, *Copepoda nauplii* with an abundance of 9.52%, *Prorocentrum* sp. with an abundance of 9.52%. While the rest of the species, including *Melosira* sp., *Synedra* sp., *Dinophysis* sp., *Microstella* sp., *Temora* sp., *Oncaea* sp., and *Sagita* sp., with an abundance of 5.00%, respectively. While in the bottom waters of Tawang Bay (20 m depth), 12 plankton species were identified. The top 9 most abundant species were *Prorocentrum* sp. with an abundance of 16.44%, *Acartia* sp. with an abundance of 16.44%, *Oithona* sp. with an abundance of 16.44%, *Oncaea* sp. with an abundance of 10.96%, *Dinophysis* sp. with an abundance of 5.48%, *Rhizoselenia* sp. with an abundance of 5.48%, *Pleurosigma* sp. with an abundance of the abundance of 5.48%, *Ceratium* sp. with 5.48%, *Caridean* sp. (5.48%). At the same time, the rest species are presented in table 2.

Diversity indices

The diversity index values obtained in the waters of Prigi Bay, Trenggalek District, were 2.49 ± 0.07 at a depth of 0.0-0.3 m depth of water column, 2.18 ± 0.16 at a depth of 2.5 m, 2.02 ± 0.08 at a depth of 5 m, and 2.34 ± 0.10 at the 20 m water column. Those indicate that the Prigi Bay waters have moderate diversity. While the diversity index values obtained in the waters of Tawang Bay, Pacitan District were 2.65 ± 0.03 at 0.0 - 0.3 m depth, 2.17 ± 0.15 at the 2.5 m depth, 2.15 ± 0.22 at a 5 m depth, and 2.32 ± 0.19 at a depth of 20 m which indicates that the waters of Tawang Bay also have moderate diversity, Figure 2.

Uniformity indices

The uniformity index values obtained in the water column of Prigi Bay were 0.45 ± 0.01 at 0.0-0.3 m depth (surface water column), 0.39 ± 0.06 at a depth of 2.5 m, 0.38 ± 0.01at a depth of 5 m and 0.43 ± 0.04 at the 25m depth or bottom of the water column. These index values indicated that the uniformity of plankton in Prigi Bay was moderate. At the same time, the uniformity values obtained in Tawang Bay were 0.46 ± 0.01 at 0.0-0.3 m depth, 0.41 ± 0.05 at a depth of 2.5 m, 0.42 ± 0.07 at a depth of 5 m, and 0.46 ± 0.05 at 25 m depth or bottom of the water column. Similarly, uniformity indices of plankton in Tawang Bay were also considered at a moderate level, Figure 3.

Domination index

Dominance index values obtained from the waters of Prigi Bay were 0.21 ± 0.03 at the 0.0-0.3 m depth, 0.26 ± 0.05 at a depth of 2.5 m, 0.31± 0.02 at a depth of 5 m and 0.24 ± 0.05 at the 20 m water depth. These values mean no plankton species were dominant in the natural habitat of spiny lobster larvae (Prigi Bay). While the dominance index values obtained from Tawang Bay waters were 0.13 ± 0.03 at the 0.0-0.3 m depth, 0.28 ± 0.05 at a depth of 2.5 m, 0.30 ± 0.03 at a depth of 5 m, and 0.16 ± 0.05 at the 20 m depth. Similarly, the values obtained from the surface to the bottom of the waters show that no species dominate in Tawang Bay (Figure 4).

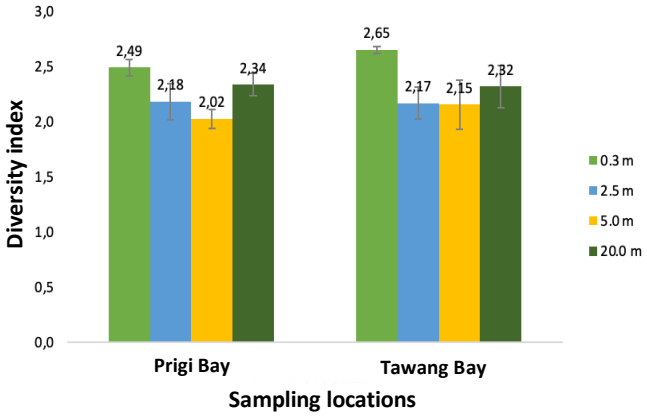

Figure 2. Diversity indices of plankton identified at the water column of Prigi Bay and Tawang Bay, East Java, Indonesia. Bars are the average values with a standard deviation of three replicates

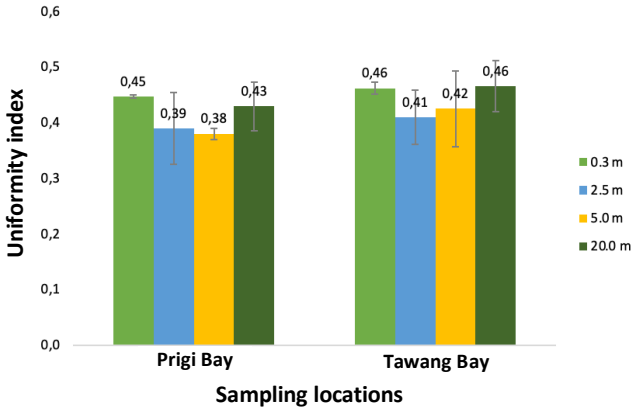

Figure 3. Uniformity indices of plankton identified in Prigi Bay and Tawang Bay, East Java, Indonesia. Bars are the average values with a standard deviation of three replicates

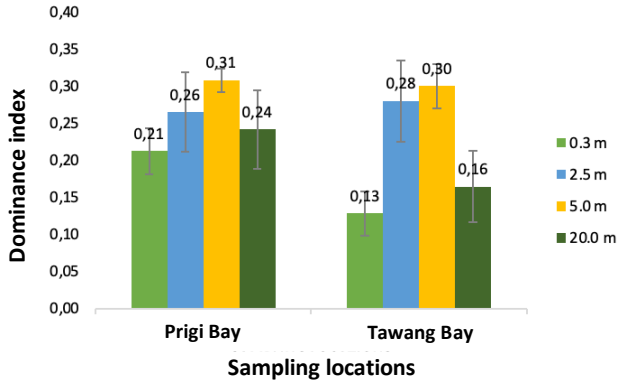

Figure 4. Domination indices of plankton identified at Prigi Bay and Tawang Bay, East Java, Indonesia. Bars are the average values with a standard deviation of three replicates

Discussion

Environmental conditions, including physical, chemical, and biological factors in natural habitats, highly determine the recruitment rates of lobster larvae (Keulder 2005). Several studies have previously reported the physical and chemical characteristics of the settlement area of lobster (Amin et al. 2022b; Boudreau et al. 1992; Lillis and Snelgrove 2010). However, studies viewing biological aspects of settlement habitat in natural environment lobster larvae are still very limited. Meanwhile, many studies conclude that biological factors such as plankton availability would be important information on the natural diets of lobster larvae (O'Rorke et al. 2014). Thus, the present study investigated the diversity, uniformity, and dominance of plankton in two common settlement areas of lobster larvae in East Java (Karangongso of Prigi Bay and Tawang Bay) in Indonesia. The sampling was performed in 4 different depths, which were 0.3 m, 2.5 m, 5 m, and 20 m depth, as lobster larvae vertically migrated from the bottom during the daytime to the surface of the water during the nighttime. It was also discovered that the diversity indices of plankton at both locations were 2.02-2.49 at Karanggongso of Prigi Bay, and 2.15-2.65 at Tawang Bay. The diversity index values found at these two locations were all less than 3, which means at a moderate level**.** The high or low value of plankton diversity can be caused by the evenly distributed abundance of each individual. In another sense, no species have relatively more diversity than other species (Awwaluddin et al. 2017). Therefore, the diversity indices of plankton at Prigi Bay and Tawang Bay, which are at a moderate level may suggest that plankton communities are in relatively equal distribution of different species, with no species being significantly more prevalent than others (Awwaluddin et al. 2017).

Similarly, the uniformity indices of plankton in both settlement areas were classified at a moderate level (0.38-0.45 at Prigi Bay and 0.41-0.46 at Tawang Bay). The uniformity values found at each location, Karanggongso of Prigi Bay water and Tawang Bay water, are categorized as moderate uniformity. The uniformity value is categorized as moderate if the value ranges from 0.4-0.6 (Ulfah et al. 2019). The availability of nutrients, food, and predation processes can affect the high value of uniformity because it affects the type and amount of plankton. Besides that, physical and chemical factors also affect the value of uniformity because it will affect the growth of plankton (Nugroho et al. 2020)**.** In addition, since the distribution of plankton in both water samples is uniform, a high degree of uniformity can be asserted**.** While the dominance indices ranged from 0.21-0.31 at Prigi Bay and 0.13-0.30 in Tawang Bay. The result indicates no dominant species at both locations since all values < 0.05. Dominance index values obtained in both waters indicate the absence of plankton which dominates in Prigi Bay and Tawang Bay. The dominance index value indicates whether organisms are dominant in a water environment. A value between 0.5 to 1 on the dominance index shows the presence of dominant organisms in the water. On the other hand, a value less than 0.5 indicates no dominant organisms are present in the water (Berger and Parker 1970).

Potential diet for lobster seeds

The result also revealed that 17 plankton species were identified from the surface water, 13 species at a depth of 2.5 m, 11 at a depth of 5 m, and 13 at 20 m (bottom) of Prigi Bay. At the same time, 17 plankton species were discovered on the surface of Tawang Bay waters: 11 species at a depth of 2.5 m, 12 species at a depth of 5 m, and 12 species at the seafloor. In general, the number of plankton species identified in the present study is higher than in previous studies reported from other lobster larvae settlement habitats in Awang Bay, West Nusa Tenggara (Amin et al. 2022b) and Wedi Ombo Bay, Yogyakarta (Trijoko and Pasaribu 2004). The most abundant species identified from Prigi Bay are mainly from Phylum Arthropoda, including *Paracyclopina* sp, *Oithona* sp, *Acartia* sp, and *Calanus* sp. Other prominent species included *Prorocentrum* sp., *Dinophysis* sp., and *Ceratium* sp., which belonged to the phylum Dinoflagellata. While phylum Arthropoda, including *Acartia sp., Oithona sp., Oncaea sp., Calanus sp., Paracyclopina,* and *Macropthalmus* sp., also dominated the most abundant species found in Tawang Bay. Plankton species in this area are also dominated by phylum Dinoflagellata such as *Ceratium* sp., *Prorocentrum* sp., and *Dinophysis* sp. Of these identified plankton species, 11 species were found in both locations, including *Acartia* sp., *Ceratium* sp., *Dynophysis* sp., *Euphausia* sp., *Microstella* sp., *Oithona* sp., *Paracyclopina* sp., *Pteropod*s sp., *Rizosolenia* sp., *Sagita* sp., *Synedra* sp. These findings suggest that the planktonic community in both bays is dominated by species belonging to the phylum Arthropoda and Dinoflagellata, which are known to be important components of the marine food web.

Among the identified plankton species, few species have been documented as potential live diets in aquaculture, including *Oithona* sp., for a live diet of European seabass (*Dicentrarchus labrax*) postlarvae (Magouz et al. 2021a) and shrimp larvae (Dinesh Kumar et al. 2017). Therefore, *Acartia* sp. could possess a live diet for seabass larvae, *Lates calcarifer* (Rajkumar 2006), fat snook, *Centropomus parallelus* (Barroso et al. 2013), and many other aquatic larvae (Sarkisian et al. 2019). Some studies also confirmed that these plankton species were identified in the content stomach of lobster larvae. For instance, *Oithona* sp. has been reported from the stomach content of spiny lobsters at the early life stage (Amin et al. 2022c; Khvorov et al. 2012). Furthermore, *Oithona* sp. has been described as a marine calanoid copepod with high protein content, ~59.33% (Santanumurti et al. 2021), therefore frequently used as a live diet for fish or shrimp larvae. Another study has documented that *Oithona* sp. had a high content of fatty acid profiles including polyunsaturated fatty acids (26.47%) and omega-3 fatty acids (36.30), which are higher than a commercial live diet such as *Artemia* sp. (Magouz et al. 2021b). Furthermore, *Acartia* sp. has also been documented to be a good live diet for aquatic larvae such as seabass larvae, *Lates calcarifer* (Rajkumar 2006), and fat snook, *Centropomus parallelus* (Barroso et al. 2013). *Acartia 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). The plankton species have also been identified in spiny lobster larvae's stomach content (Amin et al. 2022b; Amin et al. 2022c). In addition, a member of Acartia (*Acartia tonsa*) had been documented to provide an important nutritional benefit to fat snook larvae undergoing metamorphosis (Vanacor-Barroso et al. 2017).

Other potential food sources for lobster larvae identified in the present study are zooplankton and phytoplankton. The plankton results found at each station consist of Bachillariophiceae (*e.g., Rizosolenia* sp., *Synedra* sp., *Cyclotella* sp.) and Copepoda (e.g., *Oithona* sp., *Acartia* sp., *Calanus* sp.). These plankton groups were identified at each station, highlighting their potential as a food source for lobster larvae. Diatoms, which belong to the phytoplankton group Bachillariophiceae, contain essential nutrients required for the growth of lobster larvae, such as PUFA (Polyunsaturated Fatty Acid). The PUFA is the major fatty acid in Bachillariopiceae diatoms (Pahl et al. 2010), including EPA (eicosapentaenoic acid, 20:5 n-3) and DHA (docosahexaenoic acid 22:6 n-3). Therefore, PUFA is the major fatty acid in Bachillariopiceae diatoms (Pahl et al. 2010). PUFA content of these diatoms is relatively high, with levels ranging between 23.4 and 60.7% (Valera and Saavedra, 2016). High PUFA content was identified in several plankton species as potential prey for spiny lobster larvae *Jasus edwardsii*, and these long-chain fatty acids are an essential nutrient for spiny lobster (Koshio and Kanazawa 1994; Liddy et al. 2004; Wang 2013).

Copepoda (Hexanauplia) is a rich source of protein, particularly in gastropods; it is also high in calcium content which is important for lobster during molting (Kirno et al. 2012). Several studies, such as those by Alka (2016), Chow (2011), and Connel (2007), have reported the presence of copepods in the digestive tracts of lobster larvae. That suggests copepods are a preferred food for lobster larvae. Protein is the predominant organic nutrient in the spiny lobster larvae and their preferred prey (Wang 2013). This is consistent with prior examinations of digestive enzymes of phyllosoma of *J.edwardsii* and *Panulirus ornatus*, which reveal that they necessitate a high-protein diet and will utilize protein to generate energy during food deprivation (Johnston et al. 2004a, 2004b, 2006). Copepods contain high protein content, ranging from 28.9-84.9 % of dry weight, indicating that lobster larvae consume prey with high protein content (Wang and Jeffs 2014). The protein content of the copepods follows the amount of protein incorporated into artificial feeds for some of the crustacean's larvae, including crab, shrimp, and clawed lobster species, which ranges between 30% to 60% protein (Conklin et al. 1980; Guillaume 1997; Holme et al. 2009). Moreover, copepods are also high in lipids, ranging from 11.3-12.4% (Wang and Jeffs 2014). Rich-lipid diets can be properly digested by the spiny lobster larvae and utilized to supply energy, especially during a food scarcity (Johnston et al. 2004; Liddy et al. 2003; Liddy et al. 2004; Ritar et al. 2003). Furthermore, late-phase phyllosoma of spiny lobster probably targets high lipid prey as they prepare to accumulate an enormous amount of lipid to fuel their non-feeding post-larval stage (Jeffs et al. 2001a, 2001b). The presence of copepods, especially *Oithona* sp., *Acartia* sp., and *Calanus* sp., in a high abundance value at the Karanggongso of Prigi Bay and Tawang Bay could provide a significant source of high lipid natural diets for spiny lobster larvae. These results suggest that these plankton species are a potential diet for spiny lobster larvae. Therefore, in vivo trials using aquatic animals especially for developing ornate lobster hatcheries, should be further studied.

In conclusion, the number of plankton species found in both locations was more abundant in the surface water (0-0.3 m) compared to the deeper water column. A total of 17 plankton species were identified from the surface water, 13 species at a depth of 2.5 m, 11 species at a depth of 5 m, and 13 species at 20 m (bottom) of Prigi Bay. Similarly, 17 plankton species were discovered from the water surface of Tawang Bay: 11 species at a depth of 2.5 m, 12 species at a depth of 5 m, and 12 species at the seafloor. Based on the diversity, uniformity, and dominance indices, both locations had moderate plankton diversity, and no specific species was dominant over the others. Among the identified plankton species, several members of Bachillariophiceae, Copepoda, and Hexanauplia, such as *Oithona* sp., *Calanus* sp., *Paracyclopina* sp., and *Acartia* sp., are considered potential live feed for lobster larvae, and thus should be further studied.

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UNIVERSITAS AIRLANGGA
FAKULTAS PERIKANAN DAN KELAUTAN
Kampus C Mulyorejo Surabaya 60115 Telp. (031) 5911451 Fax (031) 5965741
Laman : https://fpk.unair.ac.id, e-mail : info@fpk.unair.ac.id

SURAT KETERANGAN
Nomor: 1432/UN3.FPK/KP/2023

Yang bertanda tangan di bawah ini:
Nama : **Dr. Eng. Sapto Andriyono, S.Pi., MT.**
NIP : 197909252008121002
Pangkat/Golongan : Penata / III - C
Jabatan : Wakil Dekan III

Dengan ini menerangkan bahwa:
Nama : **Dr. Endang Dewi Masithah, Ir., MP.**
NIP : 196909121997022001
Pangkat/Golongan : Pembina/IV - A
Jabatan : Lektor Kepala

Telah melakukan penelitian yang dipublikasi pada bulan Maret tahun 2023 dengan judul sebagai berikut:
Diversity and abundance of plankton community in Prigi and Tawang Bays, natural settlement habitats of Spiny Lobster larvae in East Java, Indonesia

Adapun penelitian ini sudah mengacu pada prosedur pertimbangan etik dari:
1. *American Fisheries Society* (AFS, 2014) yang berjudul *Guideline for the Use of Fishes in Research* yang menyebutkan bahwa: perizinan baru diperlukan jika penelitian memerlukan pengumpulan spesimen liar dari lapangan berupa ikan (hal 23 ; terlampir), dan
2. *Canadian Council on Animal Care* (CCAC, 2005) yang berjudul *Guideline on the Care and Use of Fish in Research, Teaching and Testing* yang menyebutkan bahwa: pedoman tersebut hanya digunakan untuk hewan uji berupa ikan (Kelas: Chondrichthyes, Agnatha, dan Osteichthyes) dan Avertebrata (Kelas: Cephalopoda) (hal 13,14 ; terlampir).

Sehingga penelitian tersebut tidak perlu dilakukan ***Uji Ethical Clearence*** karena obyek penelitiannya berupa plankton (bukan ikan).

Demikian Surat Keterangan ini kami buat untuk dapat dipergunakan sebagai persyaratan pengusulan Jabatan Fungsional **Guru Besar** atas nama Dr. Endang Dewi Masithah, Ir., MP.

Surabaya, 27 April 2023

Mengetahui,
Dekan FPK Unair

Prof. Moch. Amin Alamsjah, Ir., M.Si., Ph.D.
NIP. 197001161995031002

Wakil Dekan III FPK Unair

Dr. Eng. Sapto Andriyono, S.Pi., MT.
NIP. 197909252008121002

Guidelines for the Use of Fishes in Research

Use of Fishes in Research Committee members:
J. A. Jenkins, Chair, H. L. Bart, Jr., J. D. Bowker, P. R. Bowser, J. R. MacMillan, J. G. Nickum, J. D. Rose, P. W. Sorensen, and G. W. Whitledge on behalf of the American Fisheries Society; J. W. Rachlin and B. E. Warkentine on behalf of the American Institute of Fishery Research Biologists; and H. L. Bart on behalf of the American Society of Ichthyologists and Herpetologists

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American Fisheries Society
5410 Grosvenor Lane, Suite 100
Bethesda, Maryland 20814
USA

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5. Field Activities

5.1 Habitat and Population Considerations

Whether fishes are being collected live for investigations, preserved for study in a museum, or processed to obtain data needed for fisheries management, investigators should observe and pass on to students and employees a strict ethic of habitat conservation, and respectful and humane treatment of the animals in sampling, handling, and euthanasia (ASIH et al. 1987, 1988; AVMA 2013). Collecting should be conducted in a way that minimizes habitat disturbance and "excessive" mortality. The UFR Committee recognizes that currently no field collection techniques exist that will cause zero mortality events in the population being sampled. Research goals will generally dictate appropriate sampling methods. Given a set of alternative sampling methods and collecting gears, investigators can select the ones which cause the minimum levels of habitat disturbance and mortality in target and non-target fish populations. Gathering large series of animals from breeding aggregations should be avoided unless required to meet study objectives. Use of collecting techniques that damage habitat unnecessarily should also be avoided or performed to the minimum extent necessary to achieve study or sampling objectives. For example, trawling or other forms of dragged or towed gears is essential for documenting fish diversity or monitoring the health of fish populations; however, such gears can cause extensive disturbance to substrates, macrophytes, or other important structural elements of fish habitat. Sampling equipment and strategies can be designed to minimize incidental capture of non-target species. Collecting gears, such as gill nets deployed for nonlethal sampling, should be checked frequently to avoid unnecessary mortality. Regardless of the purpose of the experiment—whether to manipulate abundance or to study behavior, reproductive potential, or survivability—mortalities within the population and disturbance to habitat should be kept to the minimum amount that the investigator (along with the IACUC) determines to be acceptable.

The reader should note that some content in section 5 is not restricted to field activities but can extend to laboratory situations as well.

5.2 Field Collections

5.2.1 Permits

Research with fishes frequently requires capturing wild specimens from the field, whether for field-based studies—such as data recording, marking, and relocation—or for laboratory studies of live or preserved specimens. Except when collecting in the open ocean (waters not under the jurisdiction of any particular country), the collection of fishes for all research purposes requires a scientific collector's permit. Permits are issued by natural resource agencies of state, provincial, federal, and tribal entities in the United States and Canada (see section 3.4 Permits and Certificates). Permit applications generally request information about the research to be conducted, sampling methods, the areas to be sampled, and number and disposition of fish specimens to be collected. For a listing of state permitting agencies in the United States, as well

as other useful information about collection of fishes, see Walsh and Meador (1998). Collection of fishes on federal lands often requires a separate special use permit obtainable from the agency responsible for managing the land. The local, state, federal, and tribal authorities that issue collecting permits generally require collectors to notify them of the specific locations, dates, and proposed methods of sampling. Collection of fishes by federal personnel on private lands requires a permit approving access from the landowner.

5.2.2 Natural History Collections

Systematists and taxonomists interested in conducting studies on preserved fishes should be aware of the wealth of specimens archived in natural history collections (see section 5.2.5 Museum Specimens and Other Preserved Specimens) before considering the removal of additional animals from the field. Repeated collections, however, are often warranted to provide information on temporal changes in the study population. The holdings of many ichthyological collections are accessible through database network portals such as Fishnet2 (http://fishnet2.net/) and the Global Biodiversity Information Facility (http://www.gbif.org/). Many state agencies and universities have accessible natural history collections. For listings of fish collections in the United States and Canada, see Leviton et al. (1985), Poss and Collette (1995), and Walsh and Meador (1998). A listing of institutional resource collections available internationally in herpetology and ichthyology, along with symbolic codes and citations, is made possible through ASIH (Sabaj Perez 2013, http://www.asih.org/resources/standard-symbolic-codes-institutional-resource-collections-herpetology-ichthyology).

5.2.3 Representative Samples

Generally, the questions being explored and the study design itself dictate the number of specimens required for an investigation. Acquiring fishes for study generally involves the taking of a very small portion of the population or community present at a location. The general principle applied when sampling fishes is to take the fewest animals necessary to reliably address the hypothesis (see section 2.3 Statistical Design). The minimum number of fishes necessary to provide robust statistical results should inform the sampling protocol. Depending on the gear and methods, and the amount of handling required, high mortality rates may result. This is especially true in investigations involving fish eggs and early life stages. However, high levels of juvenile mortalities and rapid recoveries from population reductions are both characteristic events in the life histories of many fish species.

Sampling by using visual surveys alone is not always sufficient. This is the case in habitats that are structurally and biologically complex, where fish biodiversity data is necessary for their conservation and management. Small, cryptic fishes in coral-reef habitats, for example, are best collected by using small-scale sampling with ichthyocides; increased collection percentages of visually detected fish occurred with ichthyocide application (Smith-Vaniz et al. 2006; Ackerman and Bellwod (2000). The most commonly used ichthyocide is rotenone (see section 8.1

Euthanasia), a naturally occurring ketone from leguminous plants native to Southeast Asia and South America. The use of this chemical option has been diverse (McClay 2000); its use had been indicated with the threat of exotics (Rayner and Creese 2006). Robertson and Smith-Vaniz (2008) reviewed rotenone used by indigenous subsistence fishers and by fishery managers, as well as its toxicity and effects on other organisms. Rotenone anesthetizes and dispatches fishes by blocking the cellular uptake of oxygen (Singer and Ramsay 1994). A manual and SOP (http://fisheries.org/shop/55061p.) detail the proper use of rotenone (Finlayson et al. 2000; Finlayson et al. 2010), and numerous training courses are offered for fishery biologists and public agencies. Finlayson et al. (2010) recommended cautionary use of rotenone as a last resort due to potential harm to unintended targets. Investigation into alternative methods (Marking 2011) is prudent, as is the availability of taxonomic expertise (Walsh and Meador 1998) so to confirm the species present (see section 8.1 Euthanasia).

Sampling fish in contaminants studies is often inherent in biomonitoring of aquatic ecosystems because of capabilities of fish to accumulate environmental contaminants and to respond physiologically. Field procedures for sampling fish for chemical contaminants (Hughes et al. 2006; Schmitt et al. 1999) are useful, with protocols chosen according to study endpoints. A suite of documents and databases are available from USGS Biomonitoring of Environmental Status and Trends (BEST) program (http://pubs.er.usgs.gov/publication/itr19990007) and National Contaminant Biomonitoring Program (http://www.cerc.usgs.gov/data/ncbp/ncbp.html.

5.2.4 Collection of Imperiled Species

The term "imperiled species" applies not only to species officially listed as threatened or endangered by state or federal agencies but also to species that have been identified as candidates for such listings. The number of endangered, threatened, and vulnerable fish species in the southern United States has increased 125% between 1969 and 1989 (Warren et al. 2000). Investigators need to be aware of whether an aquatic habitat to be sampled supports imperiled species, as well as how to identify those species in the field (Warren and Burr 1994). Investigators can also determine if the habitats that support imperiled and nonimperiled species are considered areas of conservation concern and if species could be a focus of conservation concern (Jenkins et al. 2011). State wildlife action plans (Association of Fish and Wildlife Agencies 2007, http://www.teaming.com/state-wildlife-action-plans-swaps) and the network of U.S. Natural Heritage Programs (http://www.natureserve.org/natureserve-network) maintain listings of fishes (and other animal and plant species) of conservation concern. Lists of state-protected species may be obtained from offices that issue collection permits and from the Web sites of NatureServe (originally known as the Association of Biodiversity Information, http://www.natureserve.org/visitLocal/index.jsp) and the USFWS. The USFWS Endangered Species Program (http://www.fws.gov/endangered/) maintains lists of federally protected species. The list of federally threatened and endangered fishes may be also be searched on Web sites of the National Oceanic and Atmospheric Administration (NOAA, http://www.noaa.gov/)

Fisheries Service (http://www.nmfs.noaa.gov/) Office of Protected Resources (http://www.nmfs.noaa.gov/pr/) Web site or NatureServe (http://www.natureserve.org/). A bulletin highlighting protected marine or anadromous fishes (http://www.nmfs.noaa.gov/pr/species/fish/) is also available from NOAA Fisheries Service. Lists of protected fishes in Canada, Mexico, and other foreign countries can be viewed online on the respective national Web sites (such as the Species at Risk Act Public Registry, http://www.sararegistry.gc.ca/default_e.cfm of the Canadian Wildlife Service), as well as via the "Foreign Species" report on the USFWS Endangered Species Program Web site (http://ecos.fws.gov/tess_public/SpeciesReport.do?lead=10&listingType=L). The International Union for Conservation of Nature and Natural Resources (IUCN) maintains The IUCN Red List of Threatened Species™, version 2013.1 (http://www.iucnredlist.org/), providing global coverage of the conservation status of freshwater and marine fishes, as well as other plants and animals.

The collection of imperiled species is allowed only under special circumstances (e.g., conservation status surveys) and requires special permits. Only noninvasive handling techniques (handling that results in no harm whatsoever to the animal) are to be used. Examples can include blood and milt collection, and certain fin clipping and tagging methods. If the goal of the research is to collect an imperiled species for live study, or if incidental capture is anticipated as bycatch, then any collection methods that may be injurious (e.g., gill net catch without close monitoring) or lethal (e.g., ichthyocides) should be avoided.

Conservation efforts for imperiled fish species frequently involve translocations, either among natural localities or from nature to propagation facilities and then back to nature. The environmental laws governing translocations of imperiled fishes are complex and based on such matters as resource use, suitability and security of transplant sites, and the appropriateness of transplanted individuals among sites (i.e., sufficient numbers or freedom from disease; Minckley 1995). All translocation efforts must be conducted by the agency with authority and responsibility for the species and area in question and should not be attempted by unauthorized individuals.

5.2.5 Museum Specimens and Other Preserved Specimens

The collection of fishes from natural populations for museum preservation is critical for (1) understanding basic biology and life history, (2) documenting and recording biodiversity, and (3) establishing reference collections essential for understanding evolutionary relationships and environmental effects (ASIH et al. 1987, 1988). Studies of ecosystem variation or delineation of new species frequently require collection of relatively large series (sufficient for computing statistics on counts and measurements) from multiple populations across geographic ranges (Hughes and McCormick 2006). Sampling natural fish populations for these purposes typically involves broad surveys and collection of specimens in proportion to their occurrence in natural

populations; moreover, such sampling may not be hypothesis-driven. Studies of molecular systematics typically involve very small numbers of specimens, or small amounts of tissue removed from study fishes. However, it is just as important in these studies as in general ecological surveys to deposit voucher specimens in natural history museums, where samples are maintained frozen or preserved in a fixative such as 95% alcohol (isopropanol) or 70% ethanol, for future reference (Wheeler 2003). Museum collections of fishes are also available for use in other types of research. Two important principles that should be followed in collecting fishes for museum preservation are (1) the numbers of specimens collected should be the minimum necessary to accomplish study goals, and (2) animals collected should serve a variety of studies. Precise notations containing specific field data (such as date, exact location, habitat type, etc.) should accompany each collection.

Specimens collected for museum deposition should be preserved in a manner that maximizes their utility for study and minimizes the need for additional collecting. Formalin fixation is the standard practice used to ensure long-term preservation quality of fish specimens. The preferred method for archival storage is direct immersion in a 10% formalin (3.7% formaldehyde) solution, followed by transfer to alcohol (70% ethanol, un-denatured preferred) for long-term preservation and storage, as with voucher specimens. Chemicals are often added to formalin to buffer the solution or to preserve color (e.g., Ionol) (Fink et al. 1979). Although formalin is the fixative of choice for vertebrate tissues, other fixatives are sometimes used for specialized study purposes such as histology (Bouin's or Gilson's fluid) and electron microscopy (glutaraldehyde) (Luna 1992, 1992; Presnell et al. 1997; Clark 1981). Fixation by these methods typically involves small pieces of tissue dissected from specimens that may be sacrificed by means other than immersion in formalin. Carcasses for long-term archiving as voucher specimens should be fixed in formalin and later transferred to alcohol. Euthanizing fish prior to immersion in formalin should be practiced, provided that the sedative does not cause effects detrimental to the objectives of the research. A variety of chemicals, such as tricaine methanesulfonate (MS-222), may be used to anesthetize or euthanize fishes (see section 7.11 Restraint of Fishes: Sedatives and Related Chemicals). When study interests demand that specimens be fixed without prior treatment with sedatives, the specimens can be numbed in ice water, or for small fishes, immersed directly in liquid nitrogen (see section 8.1 Euthanasia).

Portions of animal specimens, including sperm, ova, embryos, tissues, and serum, are sometimes tissue banked. For example, the National Animal Germplasm Program (http://nrrc.ars.usda.gov/A-GRIN/main_webpage/ars?record_source=US) acquires and preserves genetic resources to secure biological diversity for population reconstitution or genomic studies. The San Diego Zoo Institute for Conservation Research (http://www.sandiegozooglobal.org/ICR/purpose) uses stored genetic resources in multiple technologies. Various iterations of specimen banking for retrospective analyses occur globally

for a multitude of investigations, including environmental monitoring, genetics research, and systematics. Fish tissue (liver and muscle) has been collected for the long-term storage of a variety of environmental specimens by the National Institute of Standards and Technology (NIST, http://www.nist.gov/index.html) through the National Biomonitoring Specimen Bank (Wise and Koster 1995; Becker and Wise 2006).

5.3 Live Capture Techniques and Equipment

The choice of a sampling method should be dictated by worker safety, research objectives, seasonal considerations, and the habitat type to be sampled. Capture techniques should prevent or minimize injury and stress (see section 4.2 Stress) (McMichael et al. 1998; Henry and Grizzle 2003; Henry et al. 2003). Live wells or tanks should be provided if fishes are to be kept for more than the time needed to collect essential metrics. Care should be taken to avoid accidental capture of nontarget species and to ensure release of incidentally collected individuals with minimal or no injury (ASIH et al. 1987, 1988). Species that may be dangerous to workers due to size or species-characteristic behavior or capabilities require additional precautions (see sections 5.5 Dangerous Species and Specimens and 7.10 Dangerous Species and Specimens in Captivity).

Several studies have shown electrofishing to be among the most effective techniques for obtaining fish assemblage data in freshwater habitats (Yoder and Smith 1998). Electrofishing can be performed by wading methods or boat-mounted methods. Appropriate electrofishing protocols should consider the sampling purpose and physical constraints of the environment (e.g., conductivity, water depth, and presence of obstructions), as well as use of gear and techniques that minimize potential for electrofishing injury to fishes (Snyder 2003; Dean and Temple 2011). Alternative sampling methods, such as seining, gill or trammel nets, trawls, cast nets, lift or push nets, rigid traps (e.g., minnow traps, slat traps), hoop nets, fyke nets, weirs, or angling, can be just as injurious to fishes if not conducted properly. The sampling methods chosen should allow for efficient capture of the species and sizes of fish needed to address research objectives while minimizing injury and mortality of collected fishes and non-target organisms. Multiple sampling gears may be required for the collection of a broad range of fish sizes or species or if diverse habitats are covered. Passive capture methods, such as set nets and traps, should be checked frequently enough to prevent unnecessary mortality of both target and non-target species. Nets and traps should be carefully positioned, anchored, and flagged and then removed at the cessation of sampling to avoid “ghost fishing” (lost or abandoned fishing gear that continues to kill fish and other sea life). Bonar et al. (2009) and Zale et al. (2013) provide additional information concerning standard sampling methods for fishes in freshwater environments, as well as the efficiency and specificity of various collecting gears.

5.4 Field Restraint of Fishes: Sedatives

Prolonged restraint that causes physiological stress should be avoided. In some cases, use of a sedative or anesthetic agent to minimize stress may be advisable. Although the terms

Canadian Council on Animal Care

guidelines on:

the care and use of fish in research, teaching and testing

This document, the CCAC *guidelines on: the care and use of fish in research, teaching and testing*, has been developed by the *ad hoc* subcommittee on fish of the Canadian Council on Animal Care (CCAC) Guidelines Committee.

Mr John Batt, Dalhousie University
Dr Kristina Bennett-Steward, Bioniche
Mr Cyr Couturier, Memorial University
Dr Larry Hammell, University of Prince Edward Island
Dr Chris Harvey-Clark, University of British Columbia (Chair)
Mr Henrik Kreiberg, Fisheries and Oceans Canada
Dr George Iwama, Acadia University
Dr Santosh Lall, National Research Council
Dr Matt Litvak, University of New Brunswick at St John
Dr Don Rainnie, University of Prince Edward Island
Dr Don Stevens, University of Guelph
Dr Jim Wright, University of Calgary
Dr Gilly Griffin, Canadian Council on Animal Care

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Canadian Council on Animal Care
1510–130 Albert Street
Ottawa ON CANADA
K1P 5G4

http://www.ccac.ca

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the care and use of fish in research, teaching and testing

A. PREFACE

The Canadian Council on Animal Care (CCAC) is the national peer review agency responsible for setting and maintaining standards for the care and use of animals used in research, teaching and testing throughout Canada. In addition to the *Guide to the Care and Use of Experimental Animals*, vol. 1, 2nd ed., 1993 and vol. 2, 1984, which provide the general principles for the care and use of animals, the CCAC also publishes detailed guidelines on issues of current and emerging concerns. The CCAC *guidelines on: the care and use of fish in research, teaching and testing* is the seventh of this series. This document supersedes Chapter I - Fish, *Guide to the Care and Use of Experimental Animals*, vol. 2 (CCAC, 1984).

These guidelines aim to provide information for investigators, animal care committees, facility managers and animal care staff that will assist in improving both the care given to fishes and the manner in which experimental procedures are carried out.

The present document has drawn substantially from the work of organizations listed in Appendix A. Their contributions to the development of these guidelines are gratefully acknowledged.

The guidelines have been developed by the CCAC subcommittee on fish and were reviewed by a total of 69 experts. A preliminary first draft was agreed on by the subcommittee and circulated to experts in June 2002 (including representatives of the organizations listed in Appendix A), and a second draft was circulated for widespread comment in June 2003. A final review was carried out in August 2004 involving all individuals who had previously provided significant input to the development process. The development of these guidelines also involved consultation with the Canadian Association for Laboratory Animal Science (CALAS) and the Canadian Society of Zoologists (CSZ) through workshops held at annual meetings in Québec City (June 2003), Acadia University (May 2004), and Hamilton (June 2004). Consultations were also held at the Aquaculture Association of Canada and AquaNet annual meetings in Québec City (October 2004), and at the CCAC Workshop on the Fish Guidelines in Vancouver (April 2005).

The guidelines have been organized in a format that should facilitate easy access to relevant sections. Early sections provide an ethical overview relevant to the use of fishes in research, teaching and testing. This is followed

by a brief overview of regulations and responsibilities relevant to the care and use of fishes in science in Canada. The remainder of the document provides guidelines to assist in caring for fishes in laboratory facilities, followed by guidelines to help in the development and review of experimental protocols. An overview of the CCAC *guidelines on: the care and use of fish in research, teaching and testing* is provided through a summary of the guidelines listed in this document prior to the beginning of the main text.

The refinement of animal care and use guidelines is a continuous process. These guidelines are intended to provide assistance in the implementation of best practices, and should not be viewed as regulations. Where regulatory requirements are involved or where it is absolutely imperative to adhere to a particular guideline, the term *must* has been used.

B. INTRODUCTION

The greatest challenge in providing *guidelines on: the care and use of fish* is the wide variety of fishes used in Canada and the diversity of their habits, behavior, life history, and environmental and husbandry requirements. In addition, the scientific information required to define the preferred conditions for fish well-being is limited. While considerable research has been conducted on culture strategies and environmental and water quality requirements, such studies have generally been aimed at determining conditions that optimize production in aquaculture systems, rather than improving the welfare of fishes, and have not usually addressed the difference between *tolerance* and *preference* (Fisher, 2000).

An important consideration in these guidelines is the naturally high mortality rates of juveniles in species whose ecological strategies include the generation of large numbers of progeny to ensure adequate survival in the wild. In addition, many experimental populations of species with usually high survival contain individuals that will not thrive to adulthood even under the best environmental conditions. In some situations, a population-based (or a group of study fish) approach to well-being may be appropriate, but individuals that are not likely to thrive should be euthanized as soon as they are identified.

Another consideration for these guidelines is the general acceptance by the public of the current killing methods used in harvesting wild fishes or in recreational angling. In general, the public appears to be willing to accept these killing methods for food production but not when fishes are used for research. These guidelines accept that for research, teaching, and testing use of any animal, including fishes, more emphasis will be placed on individual well-being than is generally accepted for the commercial harvesting or production of animals for food. It is recognized, however, that in some instances investigators may obtain fishes from people involved in commercial or recreational harvesting and have little influence over the capture methods.

These guidelines apply to fishes held in facilities for research, teaching and testing, as well as to fishes that are studied in their natural habitats.

1. Definition of Fish

For the purpose of these guidelines, fishes are defined as all bony and cartilaginous fish genera (classes Chondrichthyes [cartilaginous fishes], Agnatha, and Osteichthyes [bony fishes]). Fish eggs, embryos or larvae that have not developed beyond exclusive reliance on their own yolk nutrients are not covered by these guidelines. Similarly, invertebrates (except cephalopods) are not covered under the CCAC system of surveillance, but institutions are encouraged to foster respect for these animals by ensuring that holding facilities and levels of husbandry meet standards equivalent to those used for fishes.

2. Rationale for Guidelines on the Care and Use of Fish

The use of fishes as experimental subjects has increased substantially over the past two decades. This increase in use is a result of the rapid development of the aquaculture industry, requirements for testing involving fishes as indicators of environmental change, and the use of fishes as a replacement for mammals in biomedical, pharmacological and genetic research (DeTolla *et al.*, 1995; Fabacher & Little, 2000). The trend toward the use of fishes as a replacement for studies that would previously have used mammals as experimental subjects is not discouraged. However, it must also be recognized that fishes have the capacity to perceive noxious stimuli. Noxious stimuli are those stimuli that are damaging or potentially damaging to normal tissue (e.g., mechanical pressure, extremes of temperature and corrosive chemicals). Whether or not fishes have the capacity to experience any of the adverse states usually associated with pain in mammals is subject to a great deal of debate in the scientific literature (FAWC, 1996; FSBI, 2002; Rose, 2002; Braithwaite & Huntingford, 2004). Nonetheless, fishes are capable of behavioral,

physiological and hormonal responses to stressors (including noxious stimuli) which can be detrimental to their well-being. These CCAC guidelines both support the leadership role that Canadians play in fish research, and ensure that the welfare of fishes is carefully considered during the use of fishes for research, teaching and testing, recognizing that better welfare will result in better science.

3. Ethical Overview

Guideline 1:

Fishes used in research, teaching and testing must be treated with the respect accorded to other vertebrate species.

The CCAC's surveillance system for animals used in research, teaching and testing is based on the principles of humane science, i.e. the Three Rs of Russell and Burch (Russell & Burch, 1959) - Reduction, Replacement and Refinement. For the CCAC, these principles are laid out in its *policy statement on: ethics of animal investigation* (CCAC, 1989). The *ethics of animal investigation* applies to all species covered by the CCAC system, i.e. all vertebrates and cephalopods.

In addition, the CCAC system takes a "moral stewardship" approach to the use of animals in science as explained in the CCAC Experimental Animal User Training Core Topics - Module 2, Ethics in Animal Experimentation (http://www.ccac.ca/en/CCAC_Programs/ETCC/Module02/toc.html).
The first guideline statement in the CCAC *guidelines on: institutional animal user training* (CCAC, 1999a) states, "Institutions must strive through their training programs to sustain an institutional culture of respect for animal life".

3.1 Principles of the Three Rs

According to the CCAC *policy statement on: ethics of animal investigation* (CCAC, 1989), it is the responsibility of the local animal care committee (ACC) to ensure that fishes are used only if the investigator's best efforts to find a non-animal model have failed.

As for any other species covered by the CCAC system, investigators using fishes are required to use the most humane methods on the smallest number of animals necessary to obtain valid information. This requires the use of a sound research strategy, including: identification of key experiments that determine whether a particular line of enquiry is worth pursuing; use of pilot studies; staging of *in vitro* to *in vivo* experiments where possible; and implementation of staged increase in test stimuli where possible (Balls *et al*., 1995). The numbers and species of animals required depend on the questions to be explored. Field studies, aquaculture studies and laboratory studies require different statistical designs; field studies and aquaculture production typically require the use of larger numbers of animals. The life stage of the fishes used in each study will also affect the numbers of animals needed. Studies of early life stages typically require large numbers of individuals. In all cases, studies should be designed to use the fewest animals necessary. Heffner *et al.* (1996) and Festing *et al.* (2002) provide discussions on the appropriate treatment of samples and experimental units. Investigators are encouraged to consult with a statistician to develop study designs that have the appropriate statistical power to accomplish the research objectives (Nickum *et al.*, 2004).

The CCAC *policy statement on: ethics of animal investigation* (CCAC, 1989) also requires adherence to the following principles:

- animals must be maintained in a manner that provides for their optimal health and well-being, consistent with the demands imposed by the experimental protocol;
- animals must not be subjected to pain and/or distress that is avoidable and that is not required by the nature of the relevent protocol;
- expert opinion must attest to the potential value of studies with all animals, including fishes (e.g., scientific merit for research, see CCAC *policy statement on: the importance of independent scientific merit of animal based research projects* [CCAC, 2000a]; pedagogical value for teaching; and the appropriateness of the method to provide data for testing according to current regulatory requirements);
- if pain or distress is a justified component of

the study, the intensity and duration of pain/distress must be minimized; and

- an animal observed to be experiencing severe, intractable pain and/or distress should immediately be killed using an approved method of euthanasia.

Meeting the principles outlined above requires that fishes be accorded the same degree of care as other animals under the CCAC system. There are two main ethical drivers for CCAC guidelines: to maximize animal well-being, and to minimize pain and/or distress. Any factor that disturbs the normal physiological balance of an animal has an effect on the studies being conducted, and therefore should be avoided or minimized for scientific as well as ethical reasons, unless the factor itself is the subject of investigation.

Fishes comprise a great number of species, each with specific anatomical, physiological and behavioral characteristics. Investigators and animal care staff should therefore acquaint themselves with the characteristics of the species proposed to ensure that appropriate facilities and husbandry procedures are in place prior to obtaining the animals.

4. Responsibilities

Descriptions of the responsibilities of investigators, animal care committees (ACCs) and veterinarians are provided here; however, more detailed information is given throughout these guidelines to assist both investigators and members of ACCs to meet their responsibilities.

4.1 Responsibilities of investigators

4.1.1 Protocols involving the use of fish

Guideline 2:

Projects involving the use of fishes for research, teaching or testing should be described within a protocol. Protocols should be approved by an animal care committee prior to the commencement of the work.

Investigators are responsible for obtaining ACC approval before beginning any animal-based work. For further details concerning the information that should be included in a protocol form to be submitted to an ACC, see CCAC *guidelines on: animal use protocol review* (CCAC, 1997a); and CCAC *policy statement on: terms of reference for animal care committees* (CCAC, 2000b) or most recent revisions. Investigators obtaining fishes from the wild or carrying out field studies should also consult the CCAC *guidelines on: the care and use of wildlife*, Section B 3.1.1.1 Protocols involving the use of wildlife (CCAC, 2003a).

When working outside of Canada, Canadian investigators are subject to the same guidelines that apply to work within Canada, as well as to the relevant legislation, regulations and guidelines pertaining to animal care in the country where the work is conducted. This also applies to collaborative research projects, whether the work is conducted in Canada or elsewhere (see CCAC *policy statement on: animal-based projects involving two or more institutions* [CCAC, 2003b]).

4.1.2 Studies and activities requiring protocols

4.1.2.1 Work requiring protocols and inclusion in animal use inventories

These guidelines provide recommendations for fishes when they are being used by investigators. Fishes should be treated humanely whether or not they are to be included in animal use protocols or inventories.

The following require protocols and inclusion in animal use inventories (i.e. CCAC Animal Use Data Form, see Reporting of Animal Use Data at www.ccac.ca/en/CCAC_Programs/Assessment/AUDFen.htm):

- fishes held live in confinement for any period of time (even hours) for research, display, teaching or testing;

- fishes lethally sampled in the field for research, teaching or non-routine testing purposes;

- fishes caught, sampled or otherwise manipulated and released in the field for research, teaching and testing purposes; and

- genetically modified fishes.

4.1.2.2 Work not requiring protocols or inclusion in animal use inventories

The following will not require protocols or inclusion in animal use inventories:

- fish eggs, embryos or larvae that have not developed beyond exclusive reliance on their own yolk nutrients;
- wild source or hatchery fishes that have not been assigned to research studies, and whose propagation is sufficiently understood to be considered routine;
- fishes being observed in the field that are not being handled or interfered with in any way;
- fishes being counted at installations such as counting fences and traps;
- fishes being lethally sampled under government or other regulatory mandate for established fish inspection procedures, abundance estimates, and other population parameters required for assessing stocks and for routine monitoring of contamination/toxin levels and disease; and
- fishes already killed in the course of established aquaculture industry or commercial fishing purposes.

Guideline 3:

Before working with fishes, investigators, technical staff and post-graduate students must be properly trained and have their competency evaluated.

According to CCAC *guidelines on: institutional animal user training* (CCAC, 1999a), investigators and students should complete the Core Components of the *Recommended Syllabus for an Institutional Animal User Training Program* (CCAC, 1999b) and should have completed the relevant hands-on training to meet the Syllabus requirements on the use of fish as a research animal. "Students" refers to post-graduate students; undergraduate students are expected to be supervised by a properly qualified individual. See the CCAC website (www.ccac.ca/en/CCAC_Programs/CCAC_Programs-ETC.htm) for further information on relevant courses for investigators using fish as a research animal. Animal users should receive refresher training on a five-year basis, and additional training should be given as needed in order to be able to carry out procedures competently.

Guideline 4:

Investigators are responsible for, and must comply with, occupational health and safety regulations regarding the protection of personnel from known or suspected physical and biological hazards.

As with any other laboratory, animal care facilities (including aquatic facilities) should have an occupational health and safety program. All personnel using the facility should be familiar with the requirements of relevant federal, provincial/territorial and municipal legislation. Chapter VIII of the CCAC *Guide to the Care of Experimental Animals* (CCAC, 1993a) provides additional details on occupational health and safety.

Guideline 5:

Investigators should be aware of the potential risks associated with zoonotic agents present in fishes.

A brief review of fish zoonotic agents is provided in Appendix B of this document.

4.2 Responsibilities of the animal care committee

The CCAC *Terms of Reference for Animal Care Committees* (CCAC, 2000b, or most recent version) should be consulted for detailed information on the roles and responsibilities of institutional ACCs. In particular, ACCs are responsible for reviewing all studies conducted by investigators belonging to their institution, whether the work is conducted in-house or elsewhere. ACCs should ensure that appropriate care will be provided for all animals at all stages of their life and under all experimental situations. ACCs are responsible for ensuring that there is appropriate management of the facilities housing the animals. In particular, ACCs should verify that there is a person clearly designated to be in charge of animal care and management of the facilities who should also be a member of the ACC. Additionally, members of the ACC should visit the animal facilities and areas in which animals are used on a regular