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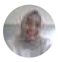

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Composition, abundance, and structure community of larva fish in Ambon Bay

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Abstract. Information on larval fish distribution is essential for fisheries management to provide clues for spawning sites, predict fish populations, and ensure sustainability. Their distribution, in particular in the early stage, is predominantly controlled by the quality of the aquatic environment. However, studies regarding fish larvae are still limited in Ambon Bay. Therefore, this study elucidates the fish larvae composition, abundance and community structure of fish larvae in Ambon Bay. Larval fish sampling was conducted on 2 and 3 September 2019, after the peak of spring tide, by Bongo net horizontally towed. Meanwhile, physicochemical data were collected using Conductivity-Temperature-Depth (CTD) and Niskin bottle. Seventeen families were identified in which Engraulids predominated found in the Inner Ambon Bay (IAB) while Myctophids in the Outer Ambon Bay (OAB). In general, the community structure of fish larvae in both areas was similar during the sampling period. The occurrence of tidal upwelling that changes the physicochemical characteristics of the seawater through deep-water renewal becomes one of the mechanisms to import larva or to draw attention to fish to spawn in the IAB.

Key Words: Ambon Bay, fish larvae, Inner Ambon Bay, Outer Ambon Bay.

Introduction. Fish larvae are the early stage in the life cycle of fishes, even though the period is quite short in the life history of fish, this stage is the most vulnerable (Feng et al 2021). Their distribution provides clues to spawning sites which can predict the recruitment of fish populations and ensure sustainability (Guyah et al 2021), as well as can be used as a bioindicator (Aceves-Medina et al 2019). The assemblages of larval fish are affected by oceanographic features, such as temperature (Sassa et al 2014; King et al 2016; Chermahini et al 2021), transparency, and salinity (Chermahini et al 2021). Other oceanographic features that affected the distribution and abundance of fish larvae are upwelling phenomena (Pattrick & Strydom 2014; Feng et al 2021), depth (Malavolti et al 2018; Feng et al 2021), and flow condition (King et al 2016). In addition to physical and chemical factors, food availability (Sassa et al 2014; Malavolti et al 2018) and seasonal variability (Pattrick & Strydom 2014) play an important role as well in shaping the assemblages of larval fish.

Ambon Bay is divided into a fjord basin (Inner Ambon Bay, IAB) and outer waters (Outer Ambon Bay, OAB) (Salamena et al 2021) which have a maximum depth of about 40 and 600 m, respectively. It is separated by a narrow and shallow sill (800 m wide and 12 m in depth), namely the Sill of Ambon Bay (SAB) (Basit et al 2012). The OAB is connected to the Banda Sea, therefore the seasonal water mass characteristics and dynamics are strongly governed by upwelling and tides in the Banda Sea. Morphology of SAB causes vertical mixing and limited the deep-water exchange at the IAB, especially in the layer below the sill bottom depth. Deepwater exchange and vertical mixing governed by tidal force and seasonal circulation are the key processes and play a significant role in controlling the ecosystem balance in Ambon Bay due to rapid coastal development (Pello et al 2014).

Studies on the early life of fishes in Ambon Bay are limited, the studies were predominantly focused on adult fishes (Latumeten & Latumeten 2021). Hence, to fill the research gap, this study aims to elucidate composition, abundance and community structure of larval fish in Ambon Bay.

Material and Method

Time and study area. The survey was carried out on 2 and 3 September 2019 (2-3 days after the peak of spring tide) during the late easterly monsoonal season in Ambon Bay (Figure 1). There are 10 sampling stations where stations at IAB and OAB were determined to capture the characteristic of marine and estuarine conditions at Ambon Bay, and stations at SAB will capture the transition condition between OAB and IAB.

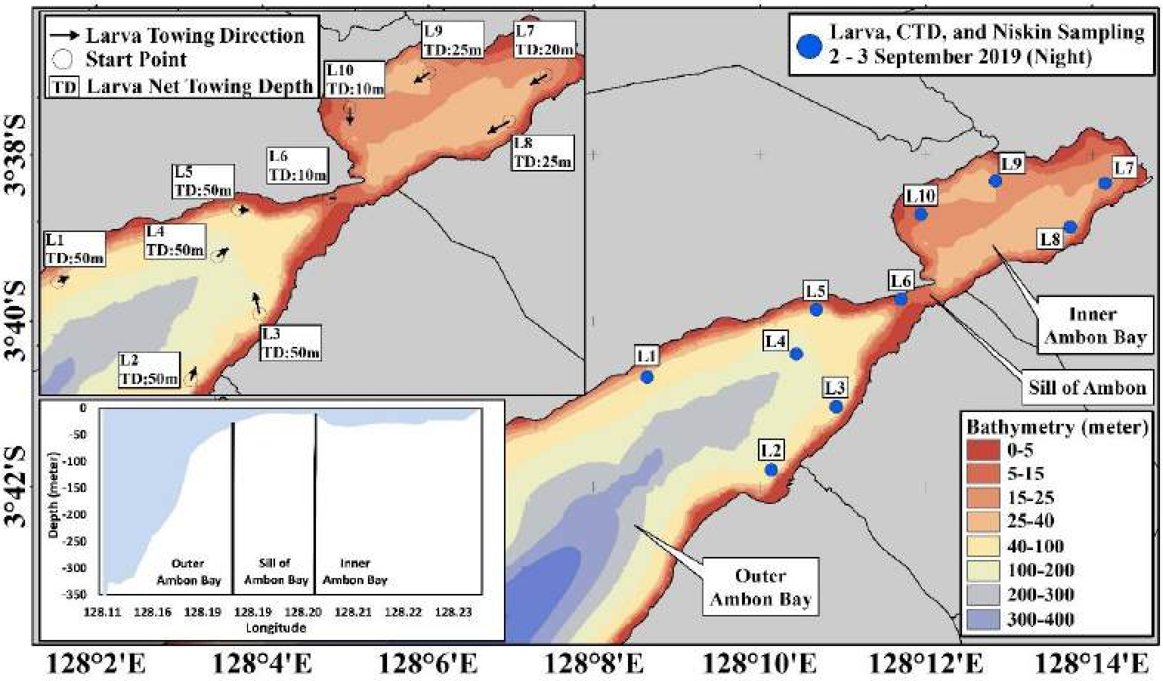

Figure 1. Sampling sites, the longitudinal transect of bathymetry (bottom left), the starting point of larval fish sampling (blank circle), and towing length as well as direction (black arrow) (upper left).

Sampling method. Vertical profile of physical parameters was collected using Compact CTD (ALEC Electronic ASTD 687, parameters: temperature, salinity, chlorophyll-*a*, and turbidity), while water samples for chemical analysis (nitrate, and phosphate) were collected using the Niskin Water Sampler, and larval fish samples were collected using a bongo net (diameter 0.5 meters with the mesh size of 333 µm) in which a flowmeter is attached to its mouth. The date of the survey was chosen to ensure that larvae and eggs in deep layers of OAB have entered IAB during water mass inflow driven by tidal upwelling events.

Larva sampling and analysis. The bongo net was deployed to the max depth of each station (see Figure 1 at upper left) being different and it was towed horizontally for around 5 minutes at the average ship speed of 2 knots with the towing direction set to the opposite direction of the ocean current. Samples were preserved with 4% formaldehyde (in seawater) on board and labeled for laboratory work. In the laboratory, fish eggs and larvae were sorted out from other organisms by observing them under a microscope. The samples were then stored in vials filled with 70% ethanol. Fish larvae were identified morphologically, under a stereomicroscope (Nikon SMZ1270i), to the family level using available literature (Leis & Carson-Ewart 2004; Kendall 2011). The number of myomeres, pigmentation, and the morphology of gut, and head spines were used to identify fish larvae. Larval fish abundance was expressed as larval fish number per cubic meter of seawater (ind m^{-3}).

Physical measurement and analysis. CTD casts were conducted from the surface to the bottom of each station or 50 meters maximum. The recording interval was set to 0.5 measurement/second and casting speed was kept at a maximum of 0.5 m s^{-1}, minimizing the noise during measurement. Five steps of quality control methods were conducted, namely removing negative data, removing outlier data based on the difference of measured data to standard deviation and mean (wild data check), and removing data while the casting speed (during CTD descending or ascending) bigger than 0.5 m s^{-1} (speed data check), averaging the data into 1-meter vertical depth interval (averaging), and screening check by the researcher to determine quality control process. The method was adopted and modified by Valcheva & Palazov (2010) and Sea-Bird Scientific (2017).

Chemical measurement and analysis. Seawater samples were collected after a CTD cast by a 3.5-liter Niskin Bottle. The Niskin bottle was deployed at two depths (surface and bottom) depending on the bathymetry. Water samples (350 mL) were put into labeled black polyethylene bottles. In the laboratory, the water samples were filtered immediately through a 0.45 μm pore size (47 mm) Whatman nitrocellulose membrane filter and using a vacuum pump with a maximum pressure of 0.3 bar and stored at –20°C until the analysis is performed. Samples were analyzed using a UV-visible spectrophotometry method, using Shimadzu type 1700 UV-visible spectrophotometer, to measure nitrate (NO_3) with a wavelength of 543 nm and phosphate (PO_4) with a wavelength of 885 nm (Strickland & Parsons 1972).

Statistical analysis. The data had a skewed distribution, hence, the Mann–Whitney U test was used to test for the presence of significant differences in larval fish abundance between IAB and OAB. The SAB was not included in the analysis, but its role in possibly influencing the fish larvae distribution exchange was explained. The species richness (S) and Simpson's diversity index (1-D) were calculated to identify the diversity of the sites. The Simpson's diversity index is a quantitative measure of the diversity of species in a community and it performs best when differentiating sites (Morris et al 2014). A non-metric multidimensional scaling (NMDS) ordination was used to display the similarities among stations in two-dimensional space. The significance of the cluster group was tested by Permutational Multivariate Analysis of Variance (PERMANOVA).

The analyses were generated using R 4.3.2 (R Core Team 2023), the Vegan 2.6-4 (Oksanen et al 2022), the rstatix 0.7.3 (Kassambara 2023), and the ecodist 2.1.3 (Goslee & Urban 2007) packages and PAST software (4.03).

Results and Discussion

Larval fish assemblage. A total of 117 larval fish that belong to 17 families were found during the study and represented mostly by the pre-flexion phase (Figure 2). The most abundant fish families were Engraulidae followed by Gobiidae and Myctophidae. Gobiids were found in almost every station (7 of 10 sites), while some were only found at a certain station, such as Pomacanthid, Platycephalid, Labrid, Congrid, and Trichiurid at L1 station, Creediid at L3 station, and Ephippidae at L6 station (Figure 3).

The highest fish larvae abundance was found in the L1 station (1.00 ind m^{-3}) followed by the L8 station (0.89 ind m^{-3}), those sites belong to OAB and IAB, respectively. Overall, total abundance in the IAB was a little higher (2.33 ind m^{-3}) compared to the OAB (1.81 ind m^{-3}). But the difference is quite subtle in that the Mann–Whitney U test result showed the difference was insignificant, 95% confidence level.

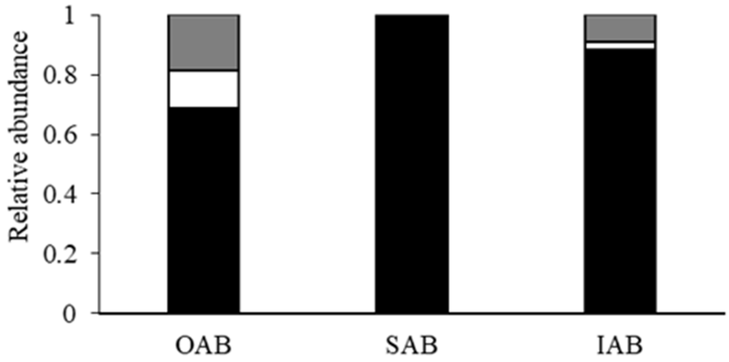

Figure 2. Relative abundance of larval fish stages. The filled black bar represents the pre-flexion stage; the open bar represents the flexion stage; the filled grey bar represents the post-flexion stage.

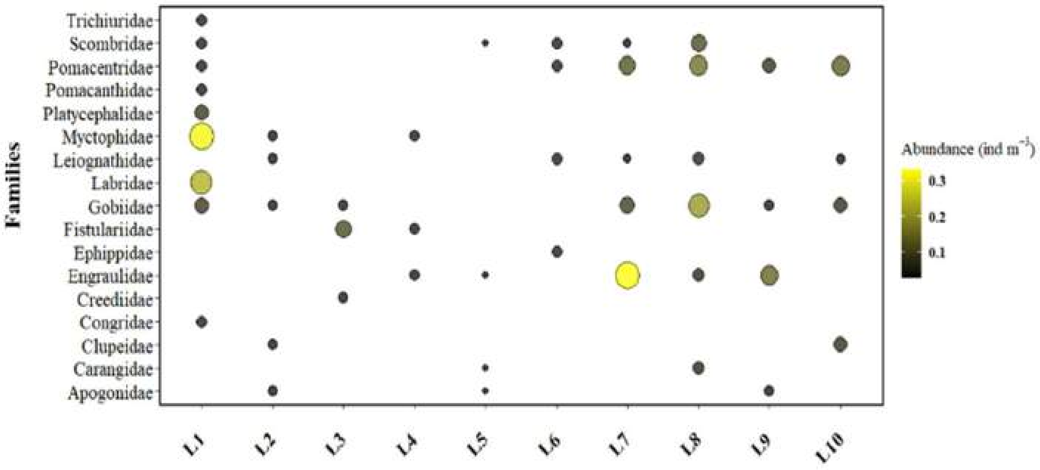

Figure 3. The abundance of fish larvae families in each station during the study.

The diversity index result showed that the L2 station had higher diversity among the stations in which the value of Simpson's diversity was 0.80. Even though the L1 and L8 stations were the highest sites in terms of the total abundance of fish larvae, their diversity index was just below the L2 station. The diversity index of OAB (0.87) was slightly higher than IAB, 0.78. Site clustering based on structure community was depicted by the NMDS biplot graph (Figure 4). The two-dimensional space graph showed that the similarities among stations are overlapping and not compact, except for L3 and L4 stations. The stress value of NMDS was 0.1 indicating the model is quite good in reduced dimensions. Furthermore, the *F-value* from the PERMANOVA result is low as well, 2.1857. Hence, the composition of fish larval among sites indicated a rather homogenous.

Fish larvae generally undergo three phases, pre-flexion, flexion, and post-flexion. The pre-flexion is the first stage, and the movement of fish is restricted during this phase due to the underdeveloped fin system. Therefore, the presence of the pre-flexion larvae in some areas can be used as a clue that the spawning ground is close by, particularly in closed or semi-closed waters, and fish were spawned in the past few days. The fish larvae found in Ambon Bay during the survey are predominantly in the pre-flexion phase. It indicates that Ambon Bay is plausible a spawning ground for some fish families. The ecosystem of Ambon Bay which consists of mangroves, seagrass bed, and coral reefs provides essential habitat for fish larvae. Furthermore, August and September are spawning periods for some fishes, Latumeten & Latumeten (2021) reported that the number of shorthead anchovy (*Encrasicholina heteroloba*) is increasing because of the recruitment process. The OAB has a higher Simpson's diversity index than IAB, this means the OAB is more diverse. Furthermore, the result from community structure analysis shows that the IAB and OAB generally have a similar community.

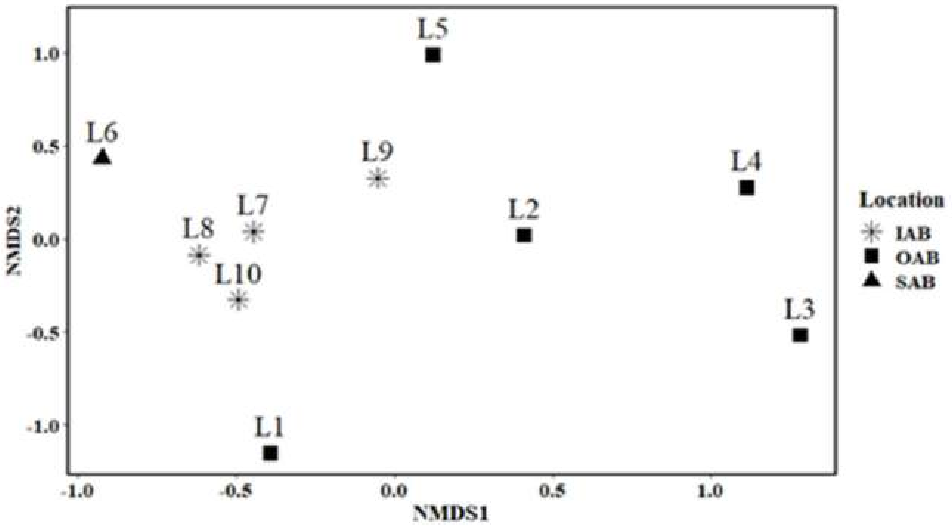

Figure 4. A two-dimensional space graph of NMDS displayed the similarities among sites.

Physicochemical conditions in Ambon Bay. Temperature at all stations of Ambon Bay was found around 25.1-25.6°C at upper layer and 24.5-24.9°C at deep layer. However, salinity was found around 32.8-34.0 pps at upper layer and 33.8-34.0 pps at deep layer. Average temperature**Error! Reference source not found.Error! Reference source not found.** at upper layer of OAB was found slightly colder and increased toward SAB and IAB except at L7 station. Meanwhile the temperature at the deep layer of OAB was warmer than at SAB and IAB (Figure 5). For salinity, the value was higher at OAB (upper and deep layer) than at IAB with slight changes in the deep layer and significant changes in upper layer. Colder temperature at Ambon Bay during the east monsoon season in Ambon Bay was also reported by Saputra & Lekalette (2016), where the average temperature during the east monsoon was 25.95^{o}C at surface layer and 25.37°C at deep layer. Saltier deep layer water (34 pps) at IAB was found throughout the year, while low salinity upper layer water was found around 25 pps during east monsoon or rain season (June-August) and 33 pps during west monsoon or dry season (December-February) (Putri et al 2008).

All stations are located **Error! Reference source not found.**near the river, but the effect of freshwater input on salinity was different at each location. Freshwater input reduced salinity at all stations in IAB (average of 33.3 pps) due to water mass exchange limitation caused by semi-close basin morphology and reduced significantly at the L10 station (32.8 pps) because of anti-clockwise water circulation pattern in IAB that make all freshwater will accumulate at L10 station before flushing out to OAB (Noya et al 2016; Putra & Pratomo 2019) (Figure 5). The effect of freshwater input at upper layer of OAB is not significant due to saline water input from Banda Sea. Meanwhile in the deep layer, salinity value is higher in OAB (average of 34.1 pps) and slightly reduced in SAB and IAB (average of 33.9 pps) because of intense mixing with freshwater.

Temperature and salinity condition at Ambon Bay influenced by upwelling phenomena in the Banda Sea that happened from June to September, when cold and saline water from the deep layer of the Banda Sea, flows to Ambon Bay and make the temperature (from upper to deep layer) become colder, and the deep layer salinity becomes higher at OAB (Saputra & Lekalette 2016; Wyrtki 1961). The signature of upwelling event can be seen at deep layer temperature at L2 and L3 stations (24.55°C at 49-meter depth) which had a similar value to the deep layer temperature at L7 and L8 stations (24.5°C at 29-meter depth). **Error! Reference source not found.**Deep cold and saline water had less influence on the upper layer of IAB due to surface heating in semi-close basins which made surface temperature warmer. This heating effect is less influential in OAB because the vertical mixing process keeps the upper layer still cold. Cold and saline water in the deep layer of IAB is caused by a water mass inflow mechanism driven by previous spring-neap tidal upwelling events which occurred more frequently during the spring tide phase, that push deep water from OAB to IAB through narrow and shallow SAB and triggers deep-water renewal in IAB (Saputra & Lekalette 2016; Salamena et al 2021). During the inflow process especially at a steep slope of SAB, some part of the water mass from the deep layer of OAB will be reflected (overturning circulation) and mixed with the upper layer water mass of OAB and SAB, which make the temperature and salinity colder and saltier. Deep water inflow occurs in a pulse pattern within a spring-neap sequence (about 2 weeks) and causes deep water renewal of IAB at approximately 80% volume of IAB (Salamena et al 2021). This intense water mass renewal is important in water mass and material exchange (including larva and egg) between two bays and plays a role in the change of physicochemical condition in a short time scale.

The turbidity value (Figure 5) showed that the vertical mean concentration of material in the water column (upper to deep layer) of IAB is higher (0.60 FTU) than in OAB (0.32 FTU), except at L8 station. Ambon bay coastal and riverside area are densely populated areas where there are 6 rivers which flow to IAB and 15 rivers flow to OAB (Saputra & Lekalette 2016). Despite the relatively smaller number of rivers contributing sediment input to the basin, the IAB exhibits a higher turbidity value owing to its semi-closed basin morphology, which restricts water mass exchange. In addition, local rainfall predominantly occurs, with the rare occurrence of widespread precipitation extending to the entire island of Ambon. Occasionally, the rainfall only occurs in the catchment area, however, an increase in sediment input from the river results in temporary high turbidity in specific areas of the bay such as L8 station (Figure 5). This condition only persists for a limited duration as the water exchange between two basins subsequently flushes out the turbid water. The increase in reclamation along the coastal area and development on the island further contribute to higher seawater turbidity in Ambon Bay.

The range of nitrate concentration in the upper was 0.006 to 0.265 mg L^{-1} and in the area near the bottom was 0.013 to 0.170 mg L^{-1}. L3 was the station with the highest concentration (0.265 mg L^{-1}) and L2 was the station with the lowest (0.027 mg L^{-1}) (Figure 6). There was an undetectable concentration in the L6 station (upper). While the range of phosphate concentration in the upper was 0.006 to 0.070 mg L^{-1} while the area near the bottom was 0.017 to 0.133 mg L^{-1}. The highest phosphate concentration was at the L9 station (0.133 mg L^{-1}), and the lowest concentration was at the L4 station (0.002 mg L^{-1}) (Figure 6).

The concentration of phosphate and nitrate in Ambon Bay on both upper and deep layer has exceeded the quality standard set by APHA (1992). The high content of phosphate and nitrate is also compared to the data in Ambon Bay (Pello et al 2014), the value is not significantly different from the results of this study, although there are differences in the concentration values. The high concentration of phosphate and nitrate indicates that there has been pollution in Ambon Bay. The average concentration of phosphate and nitrate in Ambon Bay is high, so it is a major concern considering that these nutrients are needed by phytoplankton to grow in aquatic ecosystems (Suteja et al 2021).

The chlorophyll-*a* concentration (Figure 5) showed unique spatial distribution where high concentration at western part of upper layer of OAB (L1 and L2 station; mean value of 2.2 ppb) and decreased toward SAB (1.3 ppb) and increased again in IAB until reach 2.0 ppb at eastern part (L7 station). In the deep layer, concentration of chlorophyll-*a* was higher at OAB (average of 0.7 ppb) than at IAB (average of 0.5 ppb). A unique feature was found and SAB where upper layer concentration was 1.3 ppb and at deep layer is 1.6 ppb.

The concentration and appearance of chlorophyll-*a* depend on various factors such as temperature, sunlight penetration for the photosynthesis process, water clarity (turbidity), the concentration of nutrients (nitrate or phosphate), upwelling phenomena, and supply from adjacent waters (Pitcher et al 1992; Henriksen et al 2002; Richardson et al 2002). Cold upwelled water with low turbidity, high nutrient concentration and adequate sunlight makes chlorophyll-*a* can growth well especially at OAB where chlorophyll-*a* concentration can be found until deep layer. Meanwhile at IAB, higher turbidity value especially at deep layer make high chlorophyll-*a* concentration only found at upper layer and reduce significantly at deep layer due to limitation of sunlight penetration that limited the photosynthesis process. High concentration of chlorophyll-*a* at SAB is caused by deep water renewal process induced by internal tidal upwelling that supply the chlorophyll-*a* from OAB to IAB continuously through SAB.

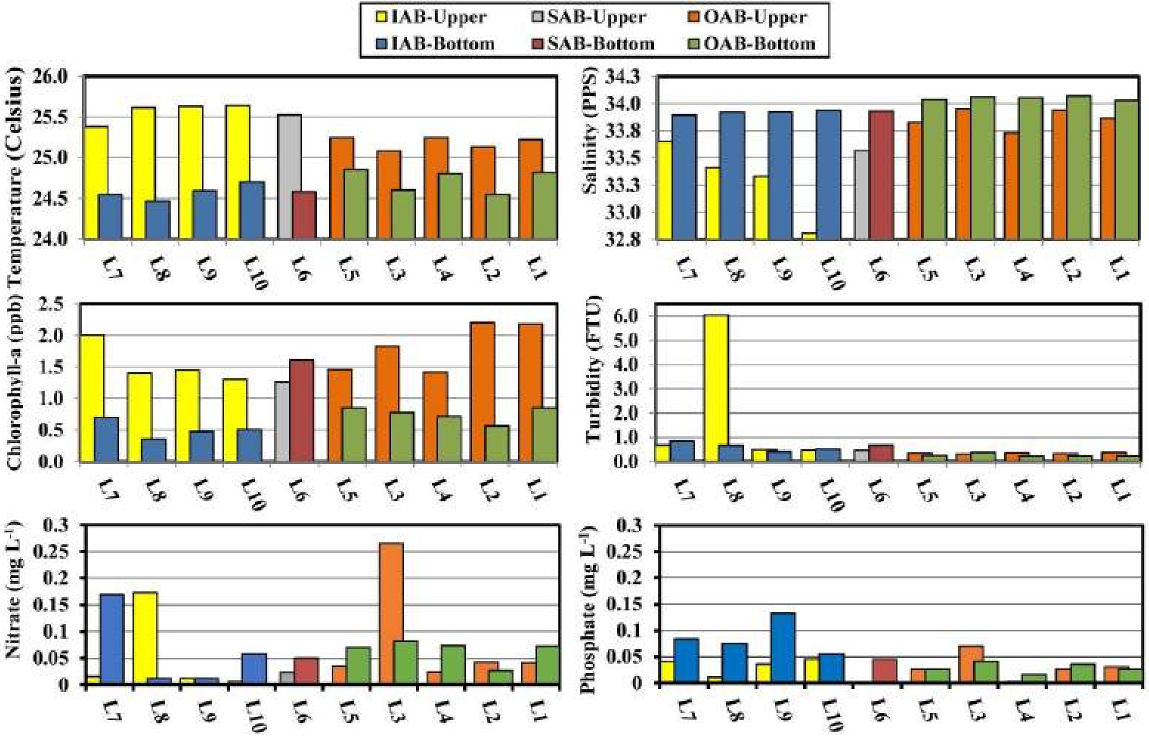

Figure 5. Upper layer and bottom layer of temperature, salinity, chlorophyll, and turbidity of Ambon Bay. Concentration (mg L^{-1}) of PO_4-P and NO_3-N on the upper and the bottom layers of Ambon Bay.

Conclusions. The inner Ambon Bay has a higher abundance of fish larvae, but a smaller number of families compared to the outer. However, the community structure is relatively homogenous. This study has a limitation on larval fish identification and time-series data. Thus, identification until the species level is needed in future studies. Furthermore, the measurement of the ocean current and oceanographic parameters should be conducted in time series to elucidate the larvae-environment relationship better.

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Conflict of interest. The authors declare that there is no conflict of interest.

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