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Colonization by Bloodworms (Chironomidae Larvae) using Artificial Substrates in Floodplain Waters: Effect of Exposure Periods and Season

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Abstract: Chironomidae larvae or bloodworms are natural food that has the nutrients suitable for freshwater fish needs. This study aims to determine the effect of exposure periods of artificial substrates and season on increase of bloodworms biomass. The highest quantity of bloodworms biomass in artificial substrates was obtained during the exposure periods of 14-21 days in March (high water phase). Bloodworms biomass were positively correlated with water quality parameters such as nitrate ($r = 0.848$), orthophosphate ($r = 0.805$) and pH ($r = 0.662$) whereas negatively correlated with total suspended solid ($r = -0.642$). Bloodworm production capacity in floodplain waters reached 1.934 tons dry-weight.ha^{-1}.yr^{-1}. The production of bloodworms in floodplain waters using artificial substrate can support the availability of natural food for fish farming.

Key words: bloodworm, floodplain, macroinvertebrate, natural fish food

Introduction

Macroinvertebrates in freshwater are dominated by dipteran larvae (Wetzel, 2001). Chironomidae are flying insects included in the order Diptera and frequently, are the most abundant taxa found in this water bodies. At larval stage, Chironomidae are aquatic macro-invertebrates that live predominantly at the bottom of freshwater, such as rivers, lakes, marshes and ponds. Approximately 93% of macroinvertebrates community in peat swamp waters of Central Kalimantan Indonesia is dominated by Chironomidae larvae (Wulandari *et al.*, 2005).

Floodplain is the aquatic ecosystem marginal to the river and created by main channel's runoff (Welcomme, 1983). In floodplains, Chiro-nomidae larvae can be found in abundance throughout the year (Sulistiyarto, 2011), due to the high availability of food in these environments, especially organic detritus (De Haas *et al.*, 2006; Solomon *et al.*, 2008). Deposits of organic matter in floodplain are

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mainly derived from forest trees.

Chironomidae larvae, also known as bloodworms, are a major natural food for many species of freshwater fish (Komatsu *et al.*, 2000; Medeiros and Arthington, 2008; Sulistiyarto, 2010; Broyer and Curlet, 2011). Fish farmers use bloodworms to feed their fishes because bloodworm has suitable nutrients for freshwater fish, with 55.62% of proteins content (Thipkonglars *et al.*, 2010). Most bloodworm used by fish farmers still comes from natural waters rather than breeding. Fish farmers collect bloodworms by taking the riverbed mud and then separated these organisms from the slurry using a sieve net.

In this study, we tried to collect bloodworms in floodplain waters using artificial substrates. Artificial substrates are devices made of materials for the colonization of aquatic macroinvertebrates (Klemm *et al.*, 1990), and they have been frequently used for sampling macroinvertebrates (Czerniawska-Kusza, 2004; Collier *et al.*, 2009; Collier *et al.*, 2011). This study aims to determine the effect of exposure periods to artificial substrates and season on biomass of bloodworms.

Materials and Methods

Bloodworms sampling was conducted in Rungan River Floodplain, Central Kalimantan, Indonesia in 2014, on the geographical position of 2º 07´ E and 113º 53´ S. Rungan River is a black water river with 86.25 km length and 6 meters depth. A vast area of swamp forest covers alongside of the river. The selected location presented forest canopy cover.

Artificial substrate made from "ijuk" or "kakaban" (sugar palm fibers) was used for collecting bloodworms. Artificial substrate was assembled into a size of 50 × 50 × 5 cm. Ijuk was selected in this study because it has a structure complexity which provide habitat, generate high abundance and diversity of macroinvertebrates (Saliu and Uvourie, 2007; Olomukoro and Eloghosa, 2009; Sulistiyarto *et al.*, 2014) and has water resistant properties (Ishak *et al.*, 2013). Artificial substrates were placed in "hapas" (size 1 × 1 × 1 meter and 1 cm mesh size) which placed in waters by tied on swamp tree at each side (Fig. 1).

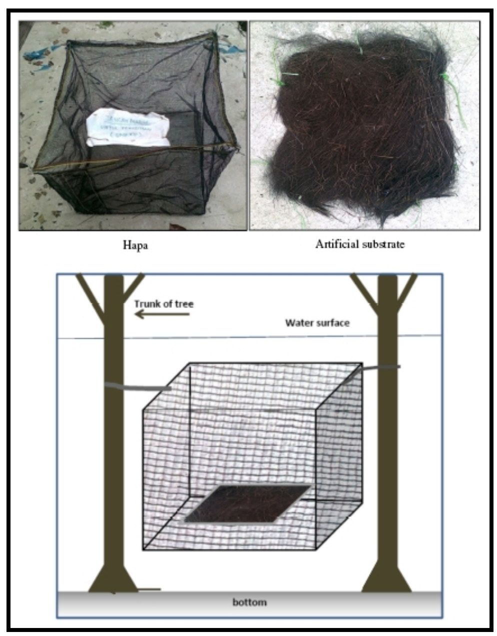

Fig. 1: Artificial substrate and hapa placement in waters.

The effect of exposure period was studied by placing artificial substrates with three replicates into the water by 7, 14, 21, 28, and 35 days. Bloodworms were separated from substrates by washing and sieve sorting, preserved with 10% formalin. Total abundance of bloodworms on each artificial substrate was calculated and body length was measured using a digital microscope. Dry-weight biomass of bloodworms was calculated using body length data according to empirical equation formulated by Towers (1994). Macroinvertebrates found in artificial substrates were identified using Pennak (1978) and Epler (2001).

The effect of season was studied by placing artificial substrates in waters with three replicates in March, May, July, and September. Artificial substrates were collected after 14 days of exposure. Total abundance of bloodworms was calculated, and body length and dry-weight were estimated according to the methods described previously. Water quality parameters were measured in March, May, July and September and included: water temperature, water pH, dissolved oxygen (O_2), total ammonia (NH_3), nitrate (NO_3), orthophosphate (PO_4), and total suspended solids (TSS). All measurements were performed in accordance with Standard Methods (APHA, 1985).

The data of bloodworms biomass were submitted to analysis of variance (ANOVA) and means were compared using Fisher's LSD (Least Significant Different) test. The relationship between water quality parameters and biomass of bloodworms was determined using correlation coefficients. Data analysis was performed according to the methods described by Steel and Torrie (1980) with the aids of Minitab 14 program.

Results

This study recorded 19 taxa of macroinvertebrates that colonized the artificial substrates, which includes 12 of Chironomidae and 7 of other groups. Chironomidae were represented by *Chironomus* sp., *Cricotopus* sp., *Dicrotendipes* sp., *Microspectra* sp., *Polypedilum* sp., *Procladius* sp., *Tanytarsus* sp., *Xenochironomus* sp., *Larsia* sp., *Macropelopia* sp., *Psectrotanypus* sp. and *Clinotanypus* sp.. Other groups were represented by *Bezzia* sp., *Serratella* sp., *Notonecta* sp., *Gompus* sp., *Neureclipsis* sp., *Polycentropus* sp. and *Aelosoma* sp.. Chironomidae contributed to 93.24% of the total abundance of macro-invertebrates.

The abundance of bloodworms collected from experiment of 7, 14, 21, 28 and 35 days of exposure periods is presented in Table 1. Average body length of bloodworms after 7, 14, 21, 28 and 35 days of exposure periods was respectively 2.926 ± 0.765 mm, 3.456 ± 0.923 mm, 3.519 ± 0.899 mm, 3.167 ± 0.824 mm, and 3.128 ± 0.800 mm. Analysis of variance (ANOVA) showed that exposure periods had significant effect on biomass of bloodworms (F

Tab. 1: Mean ± SD of bloodworm's yields after different exposure periods (7, 14, 21, 28, 35 days) using artificial substrate for colonization.

Exposure periods	Abundance ($indv.substrate^{-1}$)	Body length (mm)	Dry-weight biomass ($g.substrate^{-1}$)
7 days	387±39	2.926±0.765	0.1484±0.004
14 days	6470±1792	3.456±0.923	4.0380±1.6468
21 days	5660±550	3.519±0.899	3.6424±0.7365
28 days	3236±296	3.167±0.824	1.5577±0.3202
35 days	3145±519	3.128±0.800	1.4316±0.1310

value = 11.8518). LSD test (p = 0.01) indicated that exposure periods of 14 and 21 days generated the highest biomass of bloodworms, the exposure periods of 28 and 35 days presented lower value of biomass, and the exposure periods of 7 days yielded the lowest. Thus the exposure periods of 14-21 days are the optimal periods for collecting bloodworms.

Bloodworms' yields in March, May, July and September 2014 after 14 days exposure period are presented in Table 2. Analysis of variance (ANOVA) showed that season (months) had significant effect on biomass of bloodworms (F value = 73.7382). LSD test (p = 0.01) showed that bloodworms sampled in March yielded the highest biomass, bloodworms in May and September presented lower biomass, and in July they presented the lowest values of biomass.

Tab. 2: Mean ± SD bloodworms' yields in different months (March, May, July, and September 2014) using artificial substrate for colonization.

Parameter	March	May	July	September
Abundance ($indv.m^{-2}$)	29568±4504	10291±1893	6533±1469	12064±2846
Body length (mm)	3.331±0.787	3.419±0.920	3.399±0.786	3.214±0.839
Dry-weight biomass ($g.m^{-2}$)	16.0845±1.2918	6.0328±0.9710	3.7821±0.8524	7.4491±1.1722

Production capacity of bloodworms in floodplain waters using artificial substrate can be estimated based on the results of this study (Tab. 3). Bloodworms Production at 14^{th} day is average value of the biomass from samples taken in March, May, July and September 2014, which takes into account variations of production at low and high water phase. Assuming each month can be obtained 2 times collecting bloodworm, so that production per month is twice the value of the production at 14th day and production in a year is 12 times the production per month.

Tab. 3: Bloodworm production estimation in floodplain waters

Detail	Value
Bloodworm production at 14th day (g dry weight.m^{-2})	8.06
Bloodworm production in a month (g dry weight.m^{-2})	16.12
Bloodworm production in a year (kg dry weight.ha^{-1})	1934.40

Water quality conditions in the study area are presented in Table 4. Water of study area was brown-colored and acidic with pH ranging from 4.39-5.02, that indicating characteristics of blackwater. Water quality conditions of floodplain waters were strongly influenced by the seasonal conditions in the study area, since that two phases, high water and low water, can be distinguished. The high water phase occurs during the rainy season when the water level increases, and the low water phase occurs during the dry season, when water level decreases and the floodplain becomes shallow. High water phase occurs in January, February, March, April, May, November, and December, and low water phase in June, July, August, September, and October (Sulistiyarto, 2007). In this study, March and May exhibited a high water phase with 2.68 meters mean value of water depth, while in July and September, exhibited low water phase with 1.34 meters mean value of water depth.

Biomass of bloodworms showed positive correlation with nitrate ($r = 0.848$, p-value = 0.152), orthophosphate ($r = 0.805$, p-value = 0.195) and pH ($r = 0.662$, p-value = 0.376) whereas negative correlation with total suspended solid (TSS) ($r = -0.642$, p-value = 0.358).

Discussion

Twelve taxa of Chironomidae colonized the artificial substrates used in this study. Sulistiyarto (2011) found only 7 species of Chironomidae at the bottom of same waters. Artificial substrate proved to be efficient for collecting bloodworms because they are installed in a shallower position then benthic substrate, so that the dissolved oxygen is higher, and habitat structure is more complex because it consists of sugar palm fibers. The complexity of the habitat provides protection against predators and provide more living space (Schneider and Winemiller, 2008).

Tab. 4: Mean ± SD of Temporal variation on water quality parameters in the study area.

Parameter	March	May	July	Sept
Water depth (cm)	313.50±5.92	223.25±4.03	136.50±5.00	130.75±6.40
Temperature (^{0}C)	27.05±0.21	27.30±1.27	26.70± 0.85	27.70±0.28
pH	5.02±0.08	4.58±0.05	4.77±0.11	4.39±0.05
Dissolved oxygen (mg.L^{-1})	1.95±0.21	1.80± 0.14	2.20±0.14	2.55±0.21
Ammonia (mg.L^{-1})	1.043±0.032	0.653±0.016	1.024±0.105	0.925±0.104
Nitrate (mg.L^{-1})	1.167±0.030	0.522±0.034	0.767±0.030	0.709±0.028
Orthophosphate (mg.L^{-1})	0.090±0.006	0.049±0.003	0.065±0.005	0.074±0.004
Total suspended solid (mg.L^{-1})	47.0±5.7	50.0±15.6	92.0±8.5	54.0±2.8

The highest numbers of bloodworm were recorded during the 14-day and 21 day exposure periods. Bloodworms require sufficient time to colonize artificial substrates. Shieh and Yang (1999) found that the bloodworms have colonized the artificial substrate only after 3-day of exposure. Moreover, exposure periods of 7 days presented predominantly small bloodworms, consequently lower biomass obtained. Total population increased significantly after 21-day exposure periods. Olomukoro and Eloghosa (2009) showed that the highest number of bloodworm individuals in artificial substrates (semen brick) was obtained during the exposure period of 21 days. On a longer exposure period (28 and 35 days), many bloodworms have transformed to pupae or emerge as fly, thus reducing the population in the substrate. The larval period and the emergence of bloodworms required from 14 to 20 days (Kumar and Ramesh, 2012). Furthermore, the accumulation of detritus in artificial substrate with increasing exposure time may influence the rate of colonization, because detritus is known to be the most commonly recorded food type ingested by bloodworms (Sanseverino and Nessimian, 2008).

Seasonal variation in abiotic variables has influenced the collection yields of bloodworm on artificial substrates. Bloodworms were more abundant in the artificial substrate at high water phase than the low water phase. Bloodworm abundances are influenced by season (Higuti and Takeda, 2002; Shimabukuro and Henry, 2011) and water quality conditions (Ozkan and Camur-Elipek, 2007). In high water phase (which occurred in March) it has been verified higher nutrient levels (nitrate and orthophosphate) in the water and higher pH values. These conditions possible contributed for a more productive environment. Miracle *et al*. (2006) found that high nutrient levels are correlated with increasing abundance of macroinvertebrates. In addition, Shimabukuro and Henry (2011) observed that bloodworms were more abundant during the rainy season. Furthermore, suspended solids such as mud increased during low water phase. This condition may inhibit bloodworms colonization in the artificial substrate.

Bloodworm production with artificial substrate is very different from bloodworm production in bottom sediment of floodplains. Artificial substrate enables higher production. Bloodworm abundance in the artificial substrate reached 10291-29568 individual.m^{-2} during high water phase, and 6533 - 12064 individual.m^{-2} during low water phase (Tab. 2), while in the sediment, at the same location only 2842 individual.m^{-2} during high water phase and 1625 individuals.m^{-2} in low water phase (Sulistiyarto, 2011). The structural complexity of artificial substrate generates high abundance of bloodworm (Saliu and Uvourie, 2007, Olomukoro and Eloghosa, 2009; Sulistiyarto *et al*., 2014). Artificial substrate made from sugar palm fibers may be effective to trap detritus in the water which is a food source for bloodworms. High abundance of bloodworms was results from the combined influence of food source availability and refuge from predation provided by structurally complex habitats.

In relation to biomass production, Ahiska (2009) verified about 1.25 g.m^{-2} monthly mean value of bloodworm biomass from artificial lake in Ankara and Lundstrom *et al*. (2010) obtained an annual bloodworm production in wetlands ranging from 20 to 63 g.m^{-2}. However bloodworm production using artificial substrates seems to be lower than the production that uses chicken manure as fertilizers which yielded 28 g.m^{-2} in about 50 days (Shaw and Mark, 1980). Based on this study, we conclude that the production of bloodworms in floodplain waters using artificial substrate (sugar palm fibers) can support the availability of natural food for fish farming.

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