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Developing production technique of bloodworm (Chironomidae larvae) in floodplain waters for fish feed

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Chironomid larvae or bloodworm is a natural food that has the nutrients suitable for freshwater fish needs. Bloodworm utilization as fish feed in aquaculture is still limited, due to limited production. Huge floodplain waters are potential to be used as a place of bloodworm production. This study aimed to develop a technique producing bloodworm in floodplain waters which included searching suitable characteristics of sites and substrates for the production of bloodworm. Result of this study showed that the characteristics of the best location for the production of bloodworm were in the waters of the swamp forest covered tree canopy with water depth of 1 to 2 m. Type of a good substrate for the production of bloodworm was coconut fiber. The substrate was placed in a horizontal position at the bottom of hapa. Bloodworm production in this experiment was 0.938 g dry weight/m² or 32.83 g wet weight/m².

Key words: Chironomidae, floodplain, natural fish food, production.

INTRODUCTION

Chironomidae are flying insects such as mosquitoes included in the order diptera. Chironomidae larvae are dominant macroinvertebrates in freshwater (Wetzel, 2001). Study on freshwater Chironomidae in Indonesia is still limited, eg. Kikuchi and Sasa (1990), Wulandari et al. (2005), Krisanti et al. (2011) and Wardiatno and Krisanti (2013). Some larval chironomids are known as bloodworm because they are red in color due to the presence of haemoglobin in their body fluid. The presence of haemoglobin enables bloodworm to take oxygen in low oxygen waters. Haemoglobin are present in larval Tanypodinae, all Chironominae and Propsilocerus and Tokunagayusurika amongst the Orthocladiinae (Armitage et al., 1995).

Bloodworm is a major natural food for many species of freshwater fish (Komatsu et al., 2000; Medeiros and Arthington, 2008, Sulistiyarto, 2010; Broyer and Curlet, 2011). Bloodworm has nutrients that suit the needs of freshwater fish (Thipkonglars et al., 2010) and favored by fish (Gupta and Banerjee, 2009). Bloodworm protein content varies depending on the location and treatment. For example, protein content of cultivated bloodworm is 55.62% (Thipkonglars et al., 2010), and 53.47% (Aslianti et al, 2011).

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However, Rajabipour et al. (2011) report that bloodworm protein content from brackish earth pond is lower (31.51%).

Utilization of bloodworm as fish feed in aquaculture is still very limited because the price bloodworm is high and the bloodworm production highly depends on harvesting from nature. Floodplain of large rivers is potential water as place of the mass production of bloodworm. Floodplain is the aquatic ecosystem along the river and is a flood area of river runoff (Welcomme, 1983). The floodplain areas can potentially be used for bloodworm production sites, as bloodworm naturally can be found in abundance throughout the year in these waters (Sulistiyarto, 2011). Production of bloodworm is affected by location characteristics (De Haas et al., 2006; Wardiatno and Krisanti, 2013) and water quality such as water temperature, pH, Cl, dan NO2-N, conductivity, dissolved oxygen, BOD and COD (Wulandari et al., 2005; Ozkan and Elipek, 2007; Ozkan et al., 2010). The abundance of bloodworm in the floodplain is caused by the abundant availability of food in the form of detritus (De Haas et al., 2006; Solomon et al., 2008). Detritus is mainly derived from forest trees. Several studies show that substrate type also influence bloodworm abundance to colonize (Shieh and Yang, 1999; Saliu and Ovuorue, 2007; Olomukoro and Eloghosa, 2009). Place of substrate seems to influence production of bloodworm. Horizontal position substrate may trap more detritus, be more planted by algae, and be more stable compared to vertical position substrate because the substrate with vertical position was more water current tossed. Moreover, colonization of larval chironomids in artificial substrate is affected by permanence substrate (Saliu and Ovuorue, 2007). Therefore, this study aimed to develop techniques producing bloodworm in the floodplain area included finding characteristics of the location and the substrate for the production. We proposed hypothesis that bloodworm production location type, artificial substrate type, and substrate position may influence the production.

MATERIALS AND METHODS

The study was conducted in Rungan River floodplain, Central Kalimantan, Indonesia, on the geographical position of 113°53'LS and 2°07’E for 4 months (March to July, 2013). The research applied experimental methods with a completely randomized design. Experiments included:

1. Experiment 1: To determine the characteristics of the appropriate location. Experiments were four treatment with different placement of the substrate in the waters: covered canopy forest with a water depth of < 1 m, open water with water depth < 1 m, covered canopy forest with a water depth of 1-2 m, open water with a depth of 1-2 m. Substrates using forest tree leaves (100 g) were placed in net (hapa) with a mesh size of 10 mm (Calisto et al., 2007).

2. Experiment 2: To determine the type of a suitable substrate. Experiments using the substrate of four treatments were using sugar palm fiber of Elaeis guineensis, coconut fiber, polymeric plastic rope, and swamp grass. Substrates were sized 50 x 50 x 3 cm and placed horizontally in a hapa (100 x 100 x 100 cm) with 10 mm net mesh size. The experiments were conducted in waters selected based on the results of Experiment 1.

3. Experiment 3: To determine the position of the substrate in hapa with four treatments, that is, the vertical position, the horizontal position at the bottom of hapa, horizontal position at surface hapa, and diagonal position with 45°. This experiment used a substrate selected based on the results of Experiment 2. Each experiment was carried out for 8 days. On Day 8, the substrate was collected from the site and taken to the laboratory for analysis. The substrate was rinsed with water and filtered using a filter macrObenthos and then bloodworm was collected. Biomass of bloodworm was measured for each substrate. Biomass of bloodworm was determined by drying the samples at 105°C for 24 h, and weighed after cooling in a desiccator. Water quality parameters were observed at the beginning and end of each experiment which included: water depth, water temperature, water pH, ammonia, nitrate, orthophosphate, dissolved oxygen, total suspended solid (TSS) and total dissolved solid (TDS). The significance effect of characteristic location, substrates type and substrates position on biomass of bloodworm was analyzed statistically using the ANOVA test and followed by Least Significant Different (LSD) test.

RESULTS

Water quality

Ranges of water quality in site of experiments were as followed: water temperature, pH, ammonia, nitrate, orthophosphate, dissolved oxygen (DO), total suspended solid (TSS), and total dissolved solid (TDS) were 27.9 – 30.2°C, 5.44 – 6.38, 0.538 – 0.678 mg L⁻¹, 0.444 – 0.815 mg L⁻¹, 0.042 – 0.090 mg L⁻¹, 1.91 – 2.62 mg L⁻¹, 46.0 – 90.0 mg L⁻¹, and 17.0 – 27.0 mg L⁻¹. The water contained peat so that there was low pH. Water quality in average of experiment performed area was presented in Table 1.

Experimental results

Floodplain waters can be divided into two groups, namely the covered forest canopy area and the without covered forest canopy. Placement of the substrate in the form of leaves on the waters covered forest trees and without covered forest canopy provided different bloodworm abundances. Biomass of bloodworm in Experiment 1 ranged from 0.0049 to 0.1362 g dry weight/substrate (Table 2). Figure 1 showed the average biomass of bloodworm each treatment. Based on ANOVA test, the characteristics of the location was significant to affect bloodworm abundances ($F_{value} = 35.66 > F_{0.01} (3.11)$). LSD test ($p = 0.05$) indicated that the location of covered canopy with a water depth of 1 to 2 m produced the highest biomass of bloodworm, followed by a location
Table 1. Average value of water quality in floodplain of experiment performed area.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Average value of observation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment 1</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>29.0 ± 0.8</td>
</tr>
<tr>
<td>pH</td>
<td>6.29 ± 0.10</td>
</tr>
<tr>
<td>Ammonia (mg L⁻¹)</td>
<td>0.644 ± 0.034</td>
</tr>
<tr>
<td>Nitrate (mg L⁻¹)</td>
<td>0.778 ± 0.037</td>
</tr>
<tr>
<td>Orthophosphate (mg L⁻¹)</td>
<td>0.086 ± 0.004</td>
</tr>
<tr>
<td>Dissolved oxygen (mg L⁻¹)</td>
<td>2.00 ± 0.13</td>
</tr>
<tr>
<td>Total suspended solid (mg L⁻¹)</td>
<td>48.0 ± 1.8</td>
</tr>
<tr>
<td>Total dissolved solid (mg L⁻¹)</td>
<td>18.0 ± 1.3</td>
</tr>
</tbody>
</table>

Figure 1. Bloodworm biomass harvested from substrate with different depth and forest cover (Experiment 1).

with a water depth of < 1 m either covered canopy or without covered canopy. Locations without a canopy with a water depth of 1 to 2 m provided the lowest bloodworm biomass (Table 2). Thus bloodworm production may be optimal when performed in the forest canopy waters with a water depth of 1 to 2 m.

The results of Experiment 2 indicated that this type of substrate affected the production of bloodworm. Biomass of bloodworm on Experiment 2 ranged between 0.0976 to 0.2423 g dry weight/substrate (Table 3). Bloodworm biomass observations in different substrates are presented in Figure 2. ANOVA test showed that the type of substrate provided a significant effect ($F_{value} = 103.45 > F_{0.01}(3.11)$). Moreover, LSD test ($p = 0.05$) indicated that the substrate of coconut fiber provided the highest biomass of bloodworm, followed by treatment of the fibers, and the lowest biomass of bloodworm was in use of plastic and swamp grass. The use of plastic materials and swamp grass as substrates was not statistically different (Table 3).

The results of the Experiment 3 showed that the position of the laying of the substrate in hapa affected the production of bloodworm. In Experiment 3, there was use of coconut fiber substrate according to the results of the Experiment 2. Bloodworm biomass observations at different positions are presented in Figure 3. Biomass of bloodworm on Experiment 3 ranged between 0.1907 to 0.2398 g dry weight/substrate (Table 4). Based on
Table 2. Bloodworm biomass measurement and LSD test result in Experiment 1.

<table>
<thead>
<tr>
<th>Characteristic site (treatment)</th>
<th>Biomass (g dry weight/substrate)</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canopy covered (Depth &lt; 1m)</td>
<td>0.0309</td>
<td>0.0446</td>
<td>0.0418</td>
<td>0.0391&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Without canopy (Depth &lt; 1m)</td>
<td>0.0278</td>
<td>0.0406</td>
<td>0.0314</td>
<td>0.0333&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Canopy covered (Depth: 1-2 m)</td>
<td>0.0879</td>
<td>0.1362</td>
<td>0.1145</td>
<td>0.1129&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Without canopy (Depth: 1-2m)</td>
<td>0.0056</td>
<td>0.0049</td>
<td>0.0110</td>
<td>0.0072&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Note: LSD value = 0.0200.

Table 3. Bloodworm biomass measurement and LSD test result in Experiment 2.

<table>
<thead>
<tr>
<th>Substrate position treatment</th>
<th>Biomass (g dry weight/substrate)</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ijuk (Palm fibre)</td>
<td>0.1956</td>
<td>0.1782</td>
<td>0.2116</td>
<td>0.1951&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Coconut fibre</td>
<td>0.2423</td>
<td>0.2305</td>
<td>0.2218</td>
<td>0.2315&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Plastic</td>
<td>0.0991</td>
<td>0.1109</td>
<td>0.0976</td>
<td>0.1025&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Swamp grass</td>
<td>0.1027</td>
<td>0.1132</td>
<td>0.0995</td>
<td>0.1051&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Note: LSD value = 0.0168.

ANOVA test the position of the placement was significant effect ($F_{value} = 10.91 > F_{0.01(3,11)}$). Moreover, LSD test ($p = 0.05$) obtained the highest bloodworm biomass in a horizontal position of substrate in the bottom of hapa continued vertical and diagonal position (45°) of substrates. Vertical and diagonal positions of substrates were not statistically different. Horizontal position of the substrate on the surface of hapa provided the lowest biomass (Table 4). Enhancement of bloodworm production techniques was capable of producing approximately 0.9380 g dry weight/m$^2$ or 32.83 g wet weight/m$^2$. Bloodworm lengths ranged from 4.99 to 17.35 mm.
**DISCUSSION**

Distribution of chironomid larvae in waters is influenced by environmental factors such as location (Collier, 1993; Lobinske et al., 2002), season (Higuti and Takeda, 2002), water quality (Ozkan and Elipek, 2007), the availability of food (Oliveira et al., 2003; De Haas et al., 2006) and the substrate (Shieh and Yang, 1999; Saliu and Ovuorie, 2007; Epele et al., 2012). Therefore, chironomid production efforts need to pay attention to the environmental factors in order to obtain optimal results. Floodplain waters in Central Kalimantan in general are acidic with pH ranging from 2.6 to 6.7 (Kurasaki et al., 2000), because there are the content of peat. Dissolved oxygen is generally low. The condition can still be a place to live larvae of Chironomidae because these species have a high tolerance to various environmental conditions.

Based on this research, a good location to place bloodworm production in floodplain waters was water with a depth of 1 to 2 m with a tree canopy cover of forest. The open water of the floodplain without a canopy was not suitable for bloodworm production sites. Open water was moved/agitated than forested area because of the wind influence. According to Lothrop and Mulla (1996) Chironomidae oviposition habitat preferred quieter than agitated habitat. Moreover, Chironomidae preferred habitat with a small stream about 0.1 to 1.8 ms⁻¹ (Collier, 1993). Waters canopied forest trees obtained supply of falling leaves of the forest. Organic material falling from the forest tree to the water will be a source of food bloodworm (Callisto et al., 2007). Organic matters or detritus are the bloodworm main food (Sanseverino and Nessimian, 2008). According Lorion and Kennedy (2009), the abundance of macrobenthos is influenced by forests.
Therefore, deforestation results in decreased macrobenthos abundance. Bloodworm was more abundant in water with a depth of 1 to 2 m compared in more shallow water. It seemed due to water too shallow and unstable caused by high fluctuations of water depth in the floodplain. Moreover, the stability of the substrate affects the abundance of Chironomidae (Epele et al., 2012).

Types of substrates preferred by bloodworm are a substrate with trapping food, having microhabitat space and stable substrate (Saliu and Ovuorie, 2007; Epele et al., 2012). Of four types of substrates tested in this study, coconut fiber provided the best results followed by the fibers. While the plastic material and swamp grass provided the lowest abundance of bloodworm. Coconut fiber had the most complex structures in comparison to other materials. The complex structures were able to capture the organic material in the water and providing good microhabitat for bloodworm. Coconut fiber attached cyanobacteria can be degraded into organic matter (Arunachalam and Rajasekaran, 2009) so it can be a source of food for bloodworm. Bloodworm abundance in fibers was quite high, although it was lower than in coconut fiber. Fibers are a good substrate because it can capture the organic material as well as a medium for the growth of algae (Saliu and Ovuorie, 2006). Fibers are also a substrate which has a high stability (Ishak et al., 2013). Plastic substrates provided a low abundance of bloodworm as slippery surface making it more difficult to capture organic materials. Correia and Strixino (2005) also report that plastic substrates provide a low abundance compared to other substrates. Marsh grass less favored by bloodworm seemed due to the grass too quickly rotten, and according to Shaftel et al. (2011), grass litters are a poor food resource for macroinvertebrates. Laying of substrates in a horizontal position on the bottom of hapa provided the highest bloodworm colony. Horizontal position obtained better lighting compared to vertical or diagonal positions. The light affected the growth of periphyton which were material to feed bloodworm. The position substrates on the surface were not favored by bloodworm because of high temperature fluctuations, as well as the movements of water were greater than the deeper waters.

According Lundstrom et al. (2010) productions of Chironomidae in wetlands are generally low due to unstable waters. Floodplain waters have high fluctuations such as changes in water depth of water can be even partially dry. Development of bloodworm production techniques in the floodplain can be to produce bloodworm biomass as to culture in a closed container or controlled, either in length or weight of biomass. According to Shafruddin et al. (2006) bloodworm culture using chicken manure can produce biomass around 24.40 to 88.44 g/m², whereas in this study produced bloodworm was 32.83 g/m². It was that quantity of bloodworm production in this study included level of bloodworm culture using fertilizer. Therefore, benefit of method used in this study was no fertilizer added.

Conclusions

Bloodworm production techniques in floodplain waters were achieved through the study of characteristics of locations and substrates used. Characteristics of the best location for the production of bloodworm in the floodplain was water swamp forest tree canopy with a water depth of 1 to 2 m. Type of a good substrate for the production of bloodworm was coconut fibers. The substrate was placed horizontally at the bottom of hapa. Bloodworm production in this experiment was approximately 0.938 or 32.83 g wet weight/m². Further research is still needed for the developing production techniques of bloodworm in floodplain waters which include: efforts to increase production through the addition of food or nutrients, the impact of season on the production, and the optimal harvesting systems.

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Conflict of Interests

The authors have not declared any conflict of interests.

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