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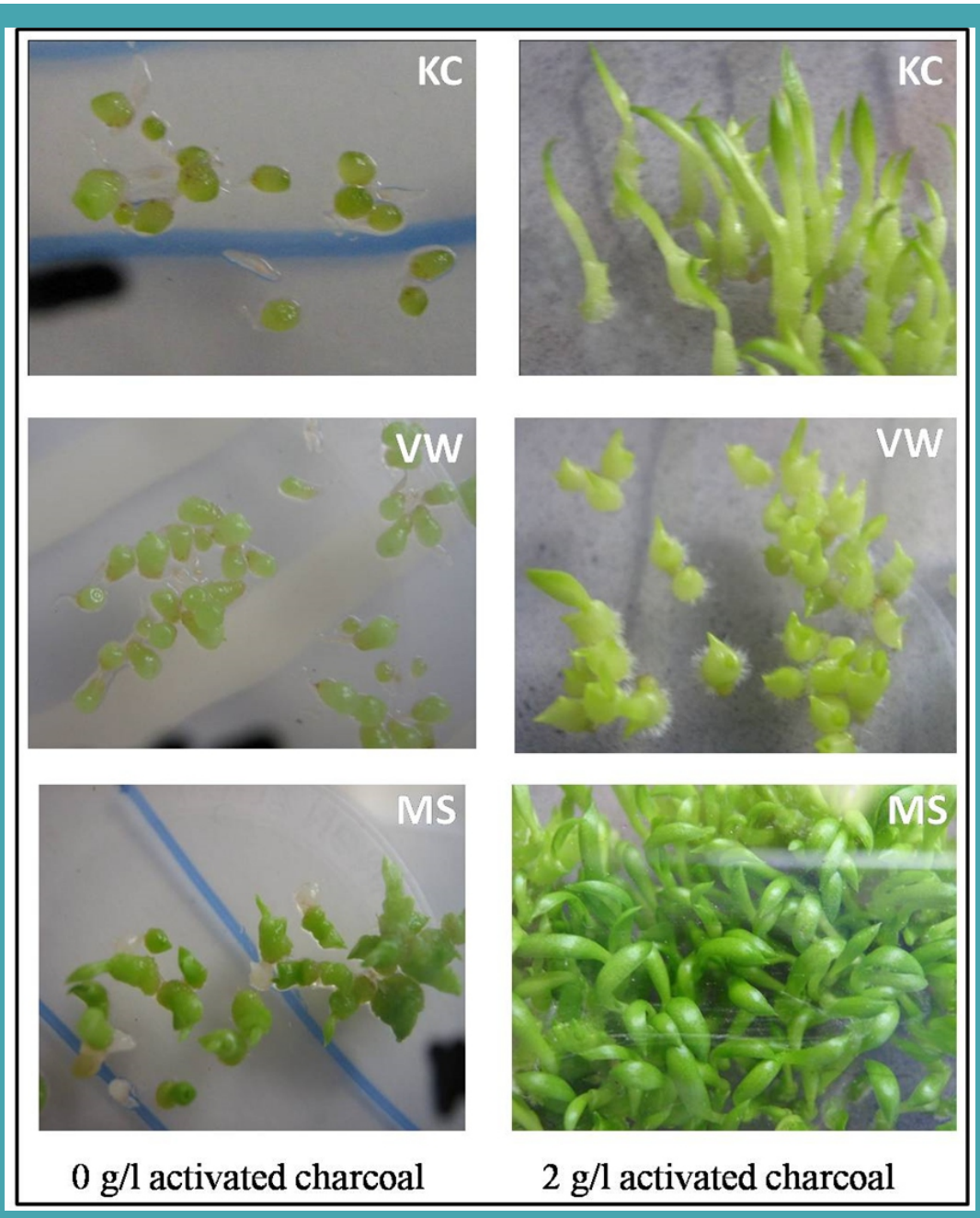

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Keterangan foto cover depan: Pertumbuhan komparatif dan perkembangan *D. taurulinum* (Comparative growth and development of *D. taurulinum*), sesuai dengan makalah pada halaman 49.

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Pusat Penelitian Biologi - LIPI

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LETHAL DISSOLVED OXYGEN AND BLOOD PROPERTIES OF GREY MULLETS *Mugil cephalus* IN SEAWATER AND FRESHWATER [Oksigen Terlarut Letal dan Gambaran Darah Ikan Belanak *Mugil cephalus* di Air Laut dan Tawar]

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ABSTRAK

Ikan belanak *Mugil cephalus* adalah salah satu spesies ikan *euryhaline* dan merupakan ikan ekonomis penting yang terdistribusi secara luas di dunia. Namun, penelitian tentang pengaruh faktor lingkungan terhadap metabolisme ikan ini masih kurang banyak diteliti meskipun hal tersebut penting untuk mengoptimalkan budidaya. Karena itu, penelitian ini dilakukan untuk mempelajari tingkat oksigen terlarut (DO) letal dan gambaran darah ikan belanak pada kondisi pemeliharaan dengan salinitas berbeda. Dua kelompok penelitian dilakukan untuk mengukur kadar DO letal dan gambaran darah ikan belanak pada kondisi pemeliharaan di air laut (LOS) dan air tawar (LOF). Pengamatan dilakukan menggunakan *closed rectangular chamber* dan sampel darah. Hasil penelitian menunjukkan bahwa kondisi ikan belanak menjadi tidak stabil pada kondisi oksigen terlarut di bawah 2 mg/L (1.3 mg/L pada LOS, 1.6 mg/L pada LOF). Dalam kondisi oksigen terlarut menjadi 1 mg/L, aktivitas ikan menurun dan mengalami kematian pada konsentrasi 0.3 mg/L. Kedua kelompok penelitian yang diamati tidak berbeda nyata ($P>0.05$). Parameter darah seperti Ht, Hb, osmolalitas, kortisol, dan glukosa dalam percobaan LOS memiliki nilai yang lebih tinggi dibandingkan dengan LOF. Percobaan menunjukkan bahwa indikator respon stress pada ikan belanak semakin meningkat dengan menurunnya kandungan oksigen dalam air. Kadar kortisol berada pada nilai 90.7 ng/ml di LOS dan 56.4 ng/ml di LOF, sedangkan kadar glukosa berada pada nilai 169.0 ng/ml di LOS dan 71.5 ng/ml di LOF. Dari penelitian ini dapat disimpulkan bahwa kadar oksigen terlarut bagi ikan belanak dianjurkan tidak berada di bawah 2 mg/L pada kondisi pemeliharaan di air laut maupun air tawar untuk menghindari kematian pada ikan.

Kata kunci: Oksigen, letal, gambaran darah, *Mugil cephalus.*

ABSTRACT

Grey mullets *Mugil cephalus* is one of euryhaline and economically important species widely distributed in the world. However, studies on the effects of environmental factors related to fish metabolism is still less investigated even though it was important to optimize aquaculture. Therefore, present study was conducted to understand the lethal dissolved oxygen (DO) and blood properties of grey mullets under different salinity rearing condition. Two experimental groups were conducted to measure the lethal DO and blood properties of grey mullets in seawater (LOS) and freshwater (LOF). Observation was conducted using closed rectangular chamber and blood samples. The results showed that grey mullets became unstable after DO levels were below 2 mg/L (1.3 mg/L on LOS, 1.6 mg/L on LOF). As the DO levels declined to 1 mg/L, their activities became lower and totally died at 0.3 mg/L. No significant difference found on these lethal DO experiments ($P>0.05$). The blood factors including Ht, Hb, osmolality, cortisol, and glucose in LOS were totally higher than those in LOF. Both experiments showed grey mullets' stress response indicator getting increased by oxygen depletion. The cortisol level was at 90.7 ng/ml in LOS and 56.4 ng/ml in LOF, while glucose level was at 169.0 ng/ml in LOS and 71.5 ng/ml in LOF. In conclusion, DO level of grey mullets *Mugil cephalus* should be maintained not lower than 2 mg/L whether in seawater or freshwater rearing condition to avoid fish mortality.

Key words: Oxygen, lethal, blood property, *Mugil cephalus*.

INTRODUCTION

Dissolved oxygen (DO) is one of the most vital factors for the fish survival in aquaculture system. DO is a limiting factor for fish metabolism, and it determines growth and activity levels of fish (Brett, 1979). The information of DO level in aquaculture is useful in aquaculture (Lovell, 1998). For many species, low exposure levels of dissolved oxygen can result in mortality (Weithman and Haas, 1984).

Various researches have studied on the oxygen consumption (OC) related to different aspects (Franklin *et al.*, 1995; Chang *et al.*, 2005; Jeong *et al.*, 2007; Tsuzuki *et al.*, 2008; Iwama *et al.*, 1997; Szczepkowski *et al.*, 2011; Duan *et al.*, 2011). From all these researches, the limiting effect of oxygen consumption is considered as important factor (Shepard, 1955). According to that information, other researches had also studied to observe the lethal DO for several fish species (Itazawa, 1959; Sylvester *et al.*, 1975; Kutty dan Mohamed, 1975; Thurston *et al.*, 1981; Schurmann dan Steffensen, 1992; Kim *et al.*, 1995; Matthews dan Berg, 1997).

Grey mullet *Mugil cephalus* is one of euryhaline species which can be developed for aquaculture production. Grey mullet was able to move between marine and freshwater environments of rivers and flooded rice fields (Saleh, 2008). Information about the gonadal development, length-weight relationship, and growth of mullets had already observed

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(Effendie, 1984; Sulistiono *et al.*, 2001; Wahyudewantoro and Haryono, 2013). Therefore, other basic information that should be observed for aquaculture development are oxygen consumption, lethal DO, and blood properties of grey mullets. The aims of this study were to observe the lethal DO and blood properties of grey mullets in seawater and freshwater.

MATERIALS AND METHODS

Grey mullets *Mugil cephalus* were divided and acclimated into seawater and freshwater rearing tanks (Number of fish: 31 fish, TL: 27.3±2.1 cm, BW: 187.9±45.8 g). Grey mullets were collected from Suncheon Bay, Korea. Fish were reared in recirculating tanks before experiments and fed twice a day at 2% of their body weight with commercial feed. Before experiments, no food was given to any experimental fish for 24 hours. Lethal DO of grey mullets in seawater (LOS) and lethal DO of grey mullets in freshwater (LOF) were observed (Table 1).

In order to determine lethal DO, closed rectangular chamber with 14 L volume and magnetic stirrer were used for mixing the experimental water (Figure 1). Five grey mullets were put into closed rectangular chamber and observed their behavioral response due to depletion of DO inside the chamber and time when the fish dying was observed. The oxygen sensor in chamber was connected to personal computer to collect the DO data inside the chamber every 10 minutes, and water temperature inside the rectangular chamber was kept constant at 18°C. In both experiments, corrections for any bacterial consumption were unnecessary as there was a negligible change in oxygen concentration over the hour. During lethal DO experiments, the OC of fish was also measured. The OC was calculated by using formula: $OC = (DO_0 - DO_t) \times (V / (t \times W))$, where OC is the oxygen consumption expressed in miligrams of oxy-

Table 1. Experimental conditions in lethal DO observation

Experiments	Temp. (°C)	Salinity (psu)	Total length (cm)	Body weight (g)	Number of fish (ind)
LOS	18	30	27.7 ± 2.7	182.7 ± 42.8	5
LOF	18	0	27.4 ± 1.1	199.0 ± 28.5	5

Description: LOS: lethal DO experiment of fish reared in seawater, LOF: lethal DO experiment of fish reared in freshwater

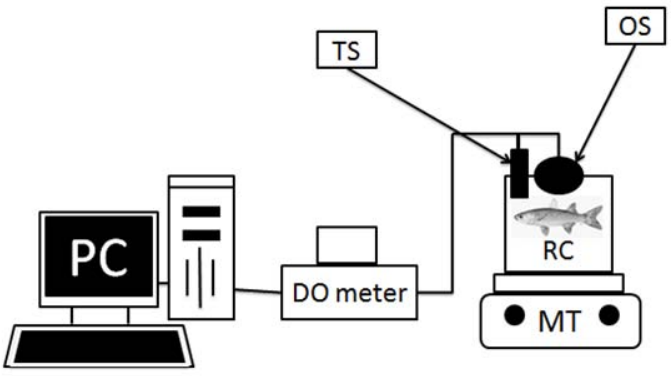

Figure 1. Diagram of lethal DO observation system. MT : magnetic stirrer, OS : oxygen sensor, PC : personal computer, RC : respiratory chamber, TS : temperature sensor.

gen per hour per kilogram of fish (mg O_2/kg/h). DO_0 and DO_t are dissolved oxygen of initial and after t hours (mg O_2/L). While t is time passed for fish which consumed oxygen (h**).** V is the water volume of respiratory chamber (L), and W is the fish weight (kg).

In addition to measuring the OC and lethal DO, the behavior of the fish was observed during experiments, including their movements in the water and breathing frequency per minute. Behavioral index was used to evaluate the fish activity in each experiment inside the respiratory chamber (Table 2). Blood samples were collected from three of five fish just after the fish died. Fish were anesthetized using 2-phenoxyethanol and blood samples were collected using heparinized syringes, centrifuged (12,000 rpm, 5 min.), and analyzed their blood properties. Hemoglobin (Hb) and hematocrit (Ht) were analyzed to obtain the oxygen binding factor. Meanwhile, Na^+, K^+, Cl^-, Ca, Mg, and osmolality were analyzed to obtain the osmoregulation factor. Furthermore, cortisol, glucose, and total protein were analyzed for the stress factor. Hematocrit (Ht) was analyzed by using micro-hematocrit reader (Micro Hematocrit Reader, Hawksley). Hemoglobin (Hb), Na^+, K^+, Cl^-, Ca, Mg, glucose, and total protein were analyzed by using Chemical Analyzer (Fujifilm Dri-Chem 3500i, Japan). Plasma cortisol was analyzed by Enzyme Immunoassay (EIA) using Cortisol EIA kit (Oxford Biomedical Research, USA). Plasma osmolality was examined with Vapor Pressure Osmometer (Vapro 5520; Wescor Co., USA). All statistical analysis was analyzed by one-way ANOVA, two-way ANOVA, and *t*-test using PASW Statistics 18 software.

RESULTS

Lethal DO in seawater and freshwater showed the same DO of 0.3 mg/L in both environments. The OC of grey mullets was decreased in line with the depletion of DO inside the chamber showing rapid changes from 313.7 mg O_2/kg/h to 0 mg O_2/kg/h within 60 minutes in LOS, while the OC in LOF was decreased from 295.5 mg O_2/kg/h to 0 mg O_2/kg/h within 70 minutes. There was no significant difference between these lethal DO experiments.

Breath frequency in both experiments showed the relationship with the OC and depletion of DO. Those were decreased in line with the declining of OC. Breath frequency was decreased from 116 times per minute to 0 times within 60 minutes in LOS, while it was decreased from 113 times per minute to 0 times within 70 minutes in LOF. These results were indicating the grey mullets consumed more oxygen in seawater environment.

The behaviors of grey mullets during lethal DO were observed based on their swimming activity and breath frequency. In both experiments, the grey mullets showed normal behavior within 20 minutes, and then their behavior became unstable after DO levels were below 2 mg/L (1.3 - 1.6 mg/L). As the DO levels declined to 1 mg/L, their behavioral index was IV to VI, which means their activities became lower and totally died at 0.3 mg/L (Table 3). The grey mullets in LOS and LOF showed the same behavior under the low level of DO, showing unstable behavior, unsteady, hit the chamber, and slower opercular movement due to the stress condition. At the lethal level, the dying fish showing red spots in their body, change of fin color, and severe condition in opercular parts.

Table 2. Behavioral indices of fish in experiments of lethal DO

Index	Movement	Breath freq/min
I	Active swimming	> 110
II	Moderate swimming	81 - 110
III	Slow swimming	51 - 80
IV	Very slow swimming	31 - 50
V	Lost balance, no movement	1 - 30
VI	Died	0

Table 3. Behavioral indices of fish with time course of lethal DO experiments

Time (minute)	LOS			LOF		
	DO (mg/L)	Behavior	Number of dead fish	DO (mg/L)	Behavior	Number of dead fish
1	7.6	I	0	8.1	I	0
10	4.2	II	0	4.3	II	0
20	2.3	III	0	2.8	II	0
30	1.3	IV	0	1.6	III	0
40	0.9	V	2	1.0	IV	2
50	0.4	V	3	0.6	V	2
60	0.3	VI	5	0.4	V	3
70	-	-	-	0.3	VI	5

Description: LOS and LOF are the same abbreviations as in Table 1

Table 4. Physio-chemical properties of grey mullets' blood before dying in lethal DO experiments

Component	Experiments	
	LOS	LOF
Hematocrit (%)	37.0 ± 6.1	20.7 ± 2.1*
Hemoglobin (g/dL)	7.4 ± 0.4	4.6 ± 0.1***
Na^+ (mEq/L)	181.0 ± 9.9	139.0 ± 2.8**
K^+ (mEq/L)	3.6 ± 0.5	3.6 ± 0.5
Cl^- (mEq/L)	162.5 ± 12.0	129.0 ± 4.2**
Ca (mg/dL)	13.3 ± 1.1	10.5 ± 0.0**
Mg (mg/dL)	4.9 ± 1.5	1.7 ± 0.1**
Osmolality (mOsm/kg)	455.0 ± 12.7	361.0 ± 50.9*
Cortisol (ng/dL)	90.7 ± 11.3	56.4 ± 28.5*
Glucose (mg/dL)	169.0 ± 39.6	71.5 ± 17.7**
Total protein (g/dL)	2.5 ± 0.2	2.6 ± 0.1

Description: LOS and LOF are the same abbreviations as in Table 1. Values are the mean ± SD (n = 3). The mean values with different superscript are significantly different (*: $P<0.05$, **: $P<0.01$, ***: $P<0.001$, *t*-test)

As shown in Table 4, the results of blood properties investigation showed that most of parameter on grey mullets in LOS was significantly higher than LOF. The blood factors including Ht, Hb, Na^+, Cl^-, Ca, Mg, osmolality, cortisol, and glucose in LOS were significantly higher than those in LOF. However, the values of K^+ and total protein in LOS were not significantly different than LOF ($P<0.05$).

DISCUSSION

Based on the obtained results, no significant difference between grey mullets reared in seawater and grey mullets reared in freshwater according to their lethal DO. There was less information which reported about the lethal DO of grey mullets and oxygen requirements for adult grey mullets. In other study, it was reported that mullets 1arvae apparently can not survive in DO below 4 mg/L (Sylvester *et*

al., 1975). Mallya (2007) stated that oxygen deficiency in fish will cause asphyxiation and mortality. It depends on the species and their rate of adaptation. Swimming behavior was also affected by hypoxia due to reduction in fish activity when oxygen decreased (Dalla Via *et al*., 1998). Further observations were required to determine the lethal oxygen level for grey mullets in larger scale aquaculture farm.

In this study, when DO less than 1 mg/L, grey mullets started suffering stress and then die either in seawater or freshwater. These results were similar with Kutty and Mohamed (1975) which reported that *Rhinomugil corsula* lose equilibrium at 0.8 mg/L in freshwater at 30^{o}C. Another study (Itazawa, 1959) also reported that *Mugil cephalus* showed symptoms of dyspnoea at oxygen concentration of 1 mg/L in 12 -19^{o}C. Therefore, it could be concluded that grey mullets require high oxygen level for their normal activity. Based on observations, grey mullet activity was normal when oxygen level is above 2 mg/L. At severe levels, oxygen deficiency will affect to growth and survival of fish (Smith and Able, 2003). Meanwhile, hypoxia will affect to their metabolism if the oxygen concentration was above the lethal oxygen levels (Lefrancois and Claireaux, 2003).

The blood properties from lethal DO experiments revealed the significant differences between grey mullets in LOS and LOF. The blood factors including Ht, Hb, osmolality, cortisol, and glucose in LOS were significantly higher than LOF. Those differences were caused by different environmental condition which made the grey mullets reared in freshwater were lower on those blood factors. Kucuk *et al.* (2013) reported that salinity influences sodium, potassium, chloride, glucose, total protein, triglycerides as well, while total protein and glucose in plasma are related to fish metabolism. According to the depletion of DO, these results showed high levels of cortisol and glucose. Fish have adapted to cope with the oxygen deficiency mainly through cardiovascular and respiratory adjustments (Randall, 1982). This condition will trigger to increase the stress. Porchas *et al.* (2009) reported that cortisol and glucose were the stressor parameters to indicate the stress response of fish. Likewise, Zaki *et al.* (2009) indicated that the blood glucose level was affected by the rate of carbohydrate metabolism under hypoxia and stress conditions. According to the information from this study, grey mullet should be maintained above its lethal oxygen level to optimize their growth and survival on aquaculture farm.

CONCLUSION

In conclusion, DO level of grey mullets *Mugil cephalus* should not lower than 2 mg/L whether in seawater or freshwater to avoid the mortality during fish rearing. Grey mullet should be reared in optimum DO level to get their optimum growth and avoid the stress in seawater or freshwater environment. We point out that further detailed studies were needed to apply aquaculture management of grey mullets on large scale farming system.

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Pedoman Penulisan Naskah Berita Biologi

Berita Biologi adalah jurnal yang menerbitkan artikel kemajuan penelitian di bidang biologi dan ilmu-ilmu terkait di Indonesia. Berita Biologi memuat karya tulis ilmiah asli berupa makalah hasil penelitian, komunikasi pendek dan tinjauan kembali yang belum pernah diterbitkan atau tidak sedang dikirim ke media lain. Masalah yang diliput, diharuskan menampilkan aspek atau informasi baru.

Tipe naskah

1. **Makalah lengkap hasil penelitian (*original paper*)**
   Naskah merupakan hasil penelitian sendiri yang mengangkat topik yang *up-todate*. Tidak lebih dari 15 halaman termasuk tabel dan gambar. Pencantuman lampiran seperlunya, namun redaksi berhak mengurangi atau meniadakan lampiran.
2. **Komunikasi pendek (*short communication*)**
   Komuniasi pendek merupakan makalah hasil penelitian yang ingin dipublikasikan secara cepat karena hasil termuan yang menarik, spesifik dan baru, agar dapat segera diketahui oleh umum. Artikel yang ditulis tidak lebih dari 10 halaman. Hasil dan pembahasan boleh digabung.
3. **Tinjauan kembali (*review*)**
   Tinjauan kembali merupakan rangkuman tinjauan ilmiah yang sistematis-kritis secara ringkas namun mendalam terhadap topik penelitian tertentu. Hal yang ditinjau meliputi segala sesuatu yang relevan terhadap topik tinjauan yang memberikan gambaran '*state of the art*', meliputi temuan awal, kemajuan hingga issue terkini, termasuk perdebatan dan kesenjangan yang ada dalam topik yang dibahas. Tinjauan ulang ini harus merangkum minimal 30 artikel.

Struktur naskah

1. **Bahasa**
   Bahasa yang digunakan adalah bahasa Indonesia atau Inggris yang baik dan benar.
2. **Judul**
   Judul harus singkat, jelas dan mencerminkan isi naskah diikuti oleh nama dan alamat surat menyurat penulis. Nama penulis untuk korespondensi diberi tanda amplop cetak atas (*superscript*).
3. **Abstrak**
   Abstrak dibuat dalam dua bahasa, bahasa Indonesia dan Inggris. Abstrak memuat secara singkat tentang latar belakang, tujuan, metode, hasil yang signifikan, kesimpulan dan implikasi hasil penelitian. Abstrak berisi maksimum 200 kata, spasi tunggal. Di bawah abstrak dicantumkan kata kunci yang terdiri atas maksimum enam kata, dimana kata pertama adalah yang terpenting. Abstrak dalam bahasa Inggris merupakan terjemahan dari bahasa Indonesia. Editor berhak untuk mengedit abstrak demi alasan kejelasan isi abstrak.
4. **Pendahuluan**
   Pendahuluan berisi latar belakang, permasalahan dan tujuan penelitian. Sebutkan juga studi terdahulu yang pernah dilakukan.
5. **Bahan dan cara kerja**
   Pada bagian ini boleh dibuat sub-judul yang sesuai dengan tahapan penelitian. Metoda harus dipaparkan dengan jelas sesuai dengan standar topik penelitian dan dapat diulang oleh peneliti lain. Apabila metoda yang digunakan adalah metoda yang sudah baku cukup ditulis sitasi dan apabila ada modifikasi harus dituliskan dengan jelas bagian mana dan apa yang dimodifikasi.
6. **Hasil**
   Sebutkan hasil-hasil utama yang diperoleh berdasarkan metoda yang digunakan. Apabila ingin mengacu pada tabel/grafik/diagram atau gambar uraikan hasil yang terpenting dan jangan menggunakan kalimat 'Lihat Tabel 1'. Apabila menggunakan nilai rata-rata harus menyebutkan standar deviasi.
7. **Pembahasan**
   Jangan mengulang isi hasil. Pembahasan mengungkap alasan didapatkannya hasil dan apa arti atau makna dari hasil yang didapat tersebut. Bila memungkinkan, bandingkan hasil penelitian ini dengan membuat perbandingan dengan studi terdahulu (bila ada).
8. **Kesimpulan**
   Menyimpulkan hasil penelitian, sesuai dengan tujuan penelitian, dan penelitian berikut yang bisa dilakukan.
9. **Ucapan terima kasih**
10. **Daftar pustaka**
    Tidak diperkenankan untuk mensitasi artikel yang tidak melalui proses peer review. Apabila harus menyitir dari "Laporan" atau "komunikasi personal" dituliskan 'unpublished' dan tidak perlu ditampilkan di daftar pustaka. Daftar pustaka harus berisi informasi yang *up to date* yang sebagian besar berasal dari *original papers*. Penulisan terbitan berkala ilmiah (nama jurnal) tidak disingkat.

Format naskah

1. Naskah diketik dengan menggunakan program Word Processor, huruf New Times Roman ukuran 12, spasi ganda kecuali Abstrak. Batas kiri -kanan atas-bawah masing-masing 2,5 cm. Maksimum isi naskah 15 halaman termasuk ilustrasi dan tabel.
2. Penulisan bilangan pecahan dengan koma mengikuti bahasa yang ditulis menggunakan dua angka desimal di belakang koma. Apabila menggunakan bahasa Indonesia, angka desimal menggunakan koma (,) dan titik (.) bila menggunakan bahasa Inggris. Contoh: Panjang buku adalah 2,5cm. Lenght of the book is 2.5 cm. Penulisan angka 1-9 ditulis dalam kata kecuali bila bilangan satuan ukur, sedangkan angka 10 dan seterusnya ditulis dengan angka. Contoh lima orang siswa, panjang buku 5 cm.
3. Penulisan satuan mengikuti aturan *international system of units*.
4. Nama takson dan kategori taksonomi merujuk kepada aturan standar termasuk yang diakui. Untuk tumbuhan *International Code of Botanical Nomenclature* (ICBN), untuk hewan *International Code of Zoological Nomenclature* (ICZN), untuk jamur *International Code of Nomenclature for Algae, Fungi and Plant* (ICFAFP), *International Code of Nomenclature of Bacteria* (ICNB), dan untuk organisme yang lain merujuk pada kesepakatan Internasional. Penulisan nama takson lengkap dengan nama author hanya dilakukan pada bagian deskripsi takson, misalnya pada naskah taksonomi. Sedangkan penulisan nama takson untuk bidang lainnya tidak perlu menggunakan nama author.
5. Tata nama di bidang genetika dan kimia merujuk kepada aturan baku terbaru yang berlaku.
6. Ilustrasi dapat berupa foto (hitam putih atau berwarna) atau gambar tangan (*line drawing*).
7. Tabel
   Tabel diberi judul yang singkat dan jelas dalam bahasa Indonesia dan Inggris, sehingga Tabel dapat berdiri sendiri. Tabel diberi nomor urut sesuai dengan keterangan dalam teks. Keterangan Tabel diletakkan di bawah Tabel. Tabel tidak dibuat tertutup dengan garis vertikal, hanya menggunakan garis horisontal yang memisahkan judul dan batas bawah.
8. Gambar
   Gambar bisa berupa foto, grafik, diagram dan peta. Judul ditulis secara singkat dan jelas. Keterangan yang menyertai gambar harus dapat berdiri sendiri, ditulis dalam bahasa Indonesia dan Inggris. Gambar dikirim dalam bentuk .jpeg dengan resolusi minimal 300 dpi dan terpisah dari badan tulisan atau dalam file yang berbeda.
9. Daftar Pustaka
   Sitasi dalam naskah adalah nama penulis dan tahun. Bila penulis lebih dari satu menggunakan kata 'dan' atau *et al.* Contoh: (Kramer, 1983), (Hamzah dan Yusuf, 1995), (Premachandra *et al.,* 1992). Bila naskah ditulis dalam bahasa Inggris yang menggunakan sitasi 2 orang penulis maka digunakan kata 'and'. Contoh: (Hamzah and Yusuf, 1995).

   a. Jurnal
      Nama jurnal ditulis lengkap.
      Premachandra GS, H Saneko, K Fujita and S Ogata. 1992. Leaf Water Relations, Osmotic Adjustment, Cell Membrane Stability, Epicu-tilar Wax Load and Growth as Affected by Increasing Water Deficits in Sorghum. *Journal of Experimental Botany* **43,** 1559-1576.

b. Buku
   Kramer PJ. 1983. *Plant Water Relationship*, 76. Edisi ke-(bila ada). Academic, New York.
c. Prosiding atau hasil Simposium/Seminar/Lokakarya.
   Hamzah MS dan SA Yusuf. 1995. Pengamatan Beberapa Aspek Biologi Sotong Buluh *(Sepioteuthis lessoniana)* di Sekitar Perairan Pantai Wokam Bagian Barat, Kepulauan Aru, Maluku Tenggara. *Prosiding Seminar Nasional Biologi XI,* Ujung Pandang 20-21 Juli 1993. M Hasan, A Mattimu, JG Nelwan dan M Litaay (Penyunting), 769-777. Perhimpunan Biologi Indonesia.
d. Makalah sebagai bagian dari buku
   Leegood RC and DA Walker. 1993. Chloroplast and Protoplast. In: *Photosynthesis and Production in a Changing Environment.* DO Hall, JMO Scurlock, HR Bohlar Nordenkampf, RC Leegood and SP Long (Eds), 268-282. Champman and Hall. London.
e. Thesis dan skripsi.
   Keim AP. 2011. Monograph of the genus *Orania* Zipp. (Arecaceae; Oraniinae). University of Reading, Reading. [PhD. Thesis].
f. Artikel online.
   Artikel yang diunduh secara online mengikuti format yang berlaku misalnya untuk jurnal, buku atau thesis, serta dituliskan alamat situs sumber dan waktu mengunduh. Tidak diperkenankan untuk mensitasi artikel yang tidak melalui proses *peer review* atau artikel dari laman web yang tidak bisa dipertangung jawabkan kebenarannya seperti wikipedia.
   Forest Watch Indonesia[FWI]. 2009. Potret keadaan hutan Indonesia periode 2000-2009. *http://www.fwi.or.id*. (Diunduh 7 Desember 2012).

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Setiap penulis yang mengajukan naskahnya ke redaksi Berita Biologi akan diminta untuk menandatangani lembar persetujuan yang berisi hak alih terbit naskah termasuk hak untuk memperbanyak artikel dalam berbagai bentuk kepada penerbit Berita Biologi. Sedangkan penulis tetap berhak untuk menyebarkan edisi cetak dan elektronik untuk kepentingan penelitian dan pendidikan. Formulir itu juga berisi pernyataan keaslian naskah, yang menyebutkan bahwa naskah adalah hasil penelitian asli, belum pernah dan sedang diterbitkan di tempat lain.

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Untuk setiap penelitian yang melibatkan hewan sebagai obyek penelitian, maka setiap naskah yang diajukan wajib disertai dengan '*ethical clearance approval*' terkait *animal welfare* yang dikeluarkan oleh badan atau pihak berwenang.

Lembar ilustrasi sampul
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